Lack of mitochondria-generated acetyl-CoA by pyruvate dehydrogenase complex downregulates gene expression in the hepatic de novo lipogenic pathway

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Mahmood S, Birkaya B, Rideout TC, Patel MS. Lack of mitochondria-generated acetyl-CoA by pyruvate dehydrogenase complex downregulates gene expression in the hepatic de novo lipogenic pathway. Am J Physiol Endocrinol Metab 311:E117–E127, 2016. First published May 10, 2016; doi:10.1152/ajpendo.00064.2016.—During the absorptive state, the liver stores excess glucose as glycogen and synthesizes fatty acids for triglyceride synthesis for export as very low density lipoproteins. For de novo synthesis of fatty acids from glucose, the mitochondrial pyruvate dehydrogenase complex (PDC) is the gatekeeper for the generation of acetyl-CoA from glucose-derived pyruvate. Here, we tested the hypothesis that limiting the supply of PDC-generated acetyl-CoA from glucose would have an impact on expression of key genes in the lipogenic pathway. In the present study, although the postnatal growth of liver-specific PDC-deficient (L-PDCKO) male mice was largely unaltered, the mice developed hyperinsulinemia with lower blood glucose levels in the fed state. Serum and liver lipid triglyceride and cholesterol levels were unaltered, and modifications to the cytosolic AcCoA pool in the fed and fasted states on the expression of key metabolic genes. Hence, the major contributors to cytosolic AcCoA pool in the fed and fasted states are not known. In the cytosolic compartment, AcCoA is generated by several reactions. The major pathway is mitochondrial PDC, which in turn is converted to citrate and then transported into the cytosol, where citrate is reconverted to AcCoA by ATP-citrate lyase (ACL; Fig. 1) (29). The cytosolic pool of AcCoA serves as the precursor for synthesis of lipids and acetylation of specific cytosolic and nuclear proteins (2, 12).

The major contributors to the cytosolic AcCoA pool are the mitochondria-generated AcCoA from the catabolism of carbohydrates, FAs, and amino acid carbon skeletons (Fig. 1). In addition to the PDC reaction, there are other pathways by which AcCoA is generated in liver mitochondria. β-Oxidation of long-chain FAs as well as catabolism of amino acids and activation of acetate contribute to the AcCoA pool in the mitochondria. The contributions of these mitochondrial reactions to the cytosolic AcCoA pool in the fed and fasted states are not fully known. In the cytosolic compartment, AcCoA is generated by several reactions. The major pathway is mitochondria-derived citrate, which is cleaved by ACL (Fig. 1). Formation of AcCoA from acetate by acetyl-CoA synthetase-1 and conversion of mitochondria-derived acetyl-carnitine to AcCoA are other sources for AcCoA in the cytosol (Fig. 1).

The sources of lipids in the liver are 1) dietary lipids derived through chylomicon remnants, 2) de novo synthesis of FAs and cholesterol from excess of dietary carbohydrates and amino acids, and 3) free FAs transported from adipose tissues after lipolysis of stored TG. Relative contributions of these sources in hepatic lipid metabolism and their deposition have been investigated using several animal models in which a specific gene product was ablated or overexpressed. For example, hepatic de novo synthesis of FAs was markedly diminished in mice with liver-specific deficiency of acetyl-CoA carboxylase 1 (ACC1KO) (27), whereas liver-specific overexpression of mature SREBP-1c, a regulator of the FA biosynthetic pathway, resulted in upregulation of expression of key genes in the lipogenic pathway (23). In studies employing deletion mouse models for ACC1 (27) or fatty acid synthase (FAS) (5), there was, as expected, inhibition of FA synthesis due to the lack of a given enzyme activity in the pathway and not due to a lack of AcCoA availability as the substrate for FA synthesis. Furthermore, Wellem et al. (42) showed that RNAi knockdown of Acly, which generates cytosolic AcCoA from citrate, caused a reduction in global histone acetylation, decreasing the expression of several metabolic genes. Hence, variable effects are observed on the hepatic lipogenic pathway, depending on the sites being altered in the pathway.

We generated a line of mice with a conditional null mutation using the Cre-loxP system in the Pdhal gene [encoding the α-subunit of the pyruvate dehydrogenase component (PDH) of
PDC] localized on chromosome X (18). Generation of the systemic null mutation in male mice proved to be lethal at an early embryonic stage. However, tissue-specific (except the brain) deletion of the Pdh1 gene permitted both pre- and postnatal development and growth of male mice for metabolic studies (6, 30, 38, 39). Earlier, we generated liver-specific deletion of the Pdh1 gene in male mice (L-PDCKO) to investigate its impact on lipid biosynthesis from glucose in the liver (6). As expected, there was no incorporation in vitro of the [14C]glucose carbon into FAs by liver (6). Hence, it is of interest to investigate whether the absence of FA synthesis in the liver from L-PDCKO mice was due solely to the lack of availability of the precursor AcCoA for FA synthesis or to other possible factors contributing to this outcome in the liver.

Given the major role of AcCoA generated from citrate formed from glucose carbon in the liver in acetylation of nucleocytoplasmic proteins for transcriptional regulation (2, 7, 13), we tested the hypothesis that limiting the generation of cytosolic AcCoA from glucose carbon in livers from L-PDCKO mice would have a significant impact on the expression of key genes in the lipid and glucose metabolic pathways as well as on expression of key upstream regulatory genes. The results presented here show that in the absence of hepatic mitochondrial PDC activity, which normally serves as the provider of the cytosolic AcCoA derived from glucose carbons during the fed state, there is downregulation of expression of key metabolic genes and several upstream regulatory genes involved in hepatic lipogenesis. Although surprisingly the total cellular AcCoA pool remained unaltered in livers of L-PDCKO mice, modified acetylation of proteins in the nuclear compartment most likely contributed to altered hepatic gene expression in L-PDCKO mice. These findings suggest a unique role of PDC-generated AcCoA in modulating the expression of genes that regulate its own utilization for hepatic lipid synthesis.

METHODS

Mouse model and animal care. Animal colony maintenance and all experiments were performed in accordance with the Guide for the Use and Care of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of the University at Buffalo. Mice harboring the Pdh1flox8 allele(s) were generated as described previously (18, 30). This mouse colony had a 129J genetic background. In our previously published paper (6), we bred PDH-floxed females with a 129J genetic background with liver-specific Cre transgenic mice (C57BL/6J genetic background), creating the progeny with a mixed genetic background (129J/B6). To overcome any effects of a mixed genotype on metabolic phenotype of L-PDCKO mice, the 129J PDH-floxed females were back-crossed with wild-type males (B6 genetic background) for 10 generations, as reported previously (30). The progeny of the last breeding were intrabred to derive a PDH-floxed colony with a B6 genetic background. In the present study, to generate deletion of exon 8 in the Pdh1 gene (L-PDCKO) in liver, homozygous Pdh1flox8flox8 females with a B6 genetic background (30) were bred with males from the C57BL/6-TgN9AlbCre 21Mgn transgenic line from The Jackson Laboratory, which carried an autosomally integrated Cre gene with liver-specific albumin promoter (33). To generate control mice (L-PDCCCT), wild-type B6 females were bred with transgenic liver-specific Cre B6 males. Only Cre-positive male progeny were used as the control. All animals had free access to a standard rodent diet and water.

Tail DNA from ~15-day-old progeny were isolated using a kit (OmniprepTM I; Geno technology) and genotyped (6). Mice were weaned on postnatal day 21 on a standard rodent diet and water ad libitum. Body weights were taken once/wk. Food intake was recorded in single-caged mice from days 49 to 56. Sixty-day-old mice in the fed state were deeply anesthetized, blood was collected by cardiac puncture, and serum was separated by centrifugation and stored at ~20°C. Livers were quickly removed, frozen in liquid nitrogen, and stored at ~80°C.

Insulin, glucose, and acetyl-CoA assays. Serum insulin levels were measured using radioimmun-assay kits (Millipore). Blood glucose levels were determined using a glucometer (Abbott). Liver AcCoA content was assayed using an acetyl-CoA assay kit (Sigma-Aldrich). Pulverized liver (~200 mg) was deproteinized in 1 N perchloric acid and centrifuged, and supernatants were neutralized with KHCO3 and centrifuged, and supernatants were neutralized with KHCO3. Aliquots (50 μl) were assayed using the standards provided by the manufacturer.

Lipid analyses. Serum total cholesterol was measured by an enzymatic kit (BioAssay Systems) according to the manufacturers’ instructions. Serum TG was quantified by a commercial kit (Abcam). Hepatic TG was extracted by homogenization in aqueous Triton-X buffer (2%) and measured using a commercial kit (Abcam). For analysis of hepatic FAs, approximately 0.5 g of pulverized liver was spiked with heptadecanoic acid (C17:0) as an internal standard. Total lipids were isolated from liver tissue with a modified Dole mixture, followed by extraction with heptane, and saponified (36, 41). FA extracts were methylated with methanolic boron trifluoride (Sigma Aldrich). FA methyl esters were separated using a Supelcowax 10 column (30 m × 0.25 mm with 0.25-m film thickness; Supelco) in a Shimadzu GC-17A gas chromatograph fitted with a flame ionization detector. Relative hepatic FA content was calculated by using individual FA peak area relative to the total areas and expressed as the percentage of total FAs.
Hepatic cholesterol was extracted and analyzed as reported previously (36). Pulverized liver (~500 mg) was spiked with α-cholestane as internal standard and saponified, extracted with petroleum diethyl ether, and dried under nitrogen gas. Sterol fractions were analyzed on the same gas chromatography system using a SAC-5 capillary column.

Quantitative real-time PCR for gene expression. Liver and epididymal adipose tissue (~100 mg) were homogenized in TRIzol reagent, and total RNA was extracted as per the manufacturer’s instructions (Life Technologies). Total RNA (~1 µg) was reverse transcribed into cDNA using an iScript cDNA kit (Bio-Rad). RT-PCR reactions were performed using appropriately diluted cDNA in triplicate with 18S rRNA serving as an internal control, and gene expression levels were quantified using a CFX96 Touch RT-PCR detection system (Bio-Rad) according to the manufacturer’s recommendation. Relative quantification of amplified DNA was performed using the 2^-ΔΔCt method (26, 30). Primers used for gene expression analysis are presented in Table 1.

Subcellular fractionation of liver and Western blotting. Liver homogenates were fractionated according to the procedure described earlier (8). Briefly, liver (~100 mg) was homogenized in buffered sucrose containing a protease inhibitor cocktail (Sigma-Aldrich), kept on ice for 30 min, and centrifuged at 800 g for 15 min. Following differential centrifugation, nuclear, mitochondrial, and cytosolic frac-

Table 1. Primers for gene expression analyses

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<th>Reverse Sequence (5′→3′)</th>
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ChREBP, carbohydrate response element-binding protein; SREBP-1c, sterol regulatory element-binding protein-1c; peroxisome proliferator-activated receptor-γ coactivator-1α; Foxo1, forkhead box O1; LXRα, liver X receptor-α; Sirt6, sirtuin 6; Gck, glucokinase; Pklr, liver-pyruvate kinase; Acly, carnitine palmitoyltransferase 1a; Ppara, peroxisome proliferator-activated receptor-α; Hmger, hydroxy-methylglutaryl-CoA reductase; Acat1 and -2, acetyl-CoA acetyltransferase 1 and 2, respectively; AceCS1 and -2, acetyl-CoA synthetase 1 and 2, respectively.

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tions were prepared and stored at −80°C. Nuclei fractions were washed in the same buffer and resuspended in nuclei resuspension buffer containing a protease inhibitor cocktail (Sigma-Aldrich). The nuclei were sonicated for 15 s, the lysate was centrifuged at 9,000 g for 30 min at 4°C, and the supernatant was stored at −80°C. Mitochondrial fractions were solubilized in resuspension buffer containing protease inhibitor cocktail (Sigma-Aldrich) and sonicated for 15 s and stored −80°C. For whole cell lysate preparation, liver tissues were homogenized in RIPA buffer (Abcam) containing protease inhibitor cocktail, agitated for 2 h at 4°C, and centrifuged at 13,000 g for 20 min. The supernatant was saved and stored at −80°C. The protein content of all tissue preparations was determined using Bio-Rad protein assay. Proteins were separated and immunodetected using the Western blotting technique as described (6). Equal amounts of protein (50 μg) were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred on the nitrocellulose membrane, and detected using specified antibodies as listed: anti-acetyl-CoA carboxylase 1 (04–322; EMD Millipore), SREBP1 antibody (NB600-582; Novus Biologicals), anti-acetyl-CoA synthetase (ab133664; Abcam), anti-histone H3 (acetyl K9) antibody (ab10812; Abcam), SIRT6 antibody (12496; Cell Signaling Technology), and anti-PDH antibody (6) to detect pyruvate dehydrogenase component of PDC. β-Actin used as a loading control was detected using β-actin (D6A8) antibody (8457; Cell Signaling Technology), and gels were stained with Ponceau S stain, followed by densitometry analysis (for nuclear protein analysis only). Protein bands were visualized using an Enhanced Chemiluminescence kit (Perkin-Elmer) and analyzed using Bio-Rad ChemiDoc MP image analyzer.

Data analysis. Results are presented as means ± SE of eight animals unless otherwise indicated. Differences between the means of the L-PDCT and L-PDCKO groups were performed using Students’ t-test. For postnatal growth of mice, body weight data were analyzed using the analysis of variance (one-way ANOVA), followed by the Holm-Sidak method for two groups of mice at different age periods. Significance was assigned when the P value was ≤0.05.

RESULTS

Analysis of liver-specific PDC-deficient mice. Progeny from the breeding of Pdha1-targeted homozygous females with liver-specific Cre males were found to be normal in average litter size with no embryonic lethality. In the present study, only 2 mo-old male progeny were analyzed unless otherwise indicated. Genomic DNA analysis of liver (L), skeletal muscle (SM), heart (H) and adipose tissue (AT) from 2-mo-old control (L-PDCCT) mice by PCR showed a 700-bp band corresponding to the wild-type allele (Pdha1wt) (Fig. 2A). Tissue DNA analysis of L-PDCKO mice detected the 800-bp Pdha1Δex8 allele in skeletal muscle, heart, and adipose tissue, whereas liver showed the 400-bp Pdha1Δex8 deleted allele, indicating that the Pdha1 gene was deleted in the liver only (Fig. 2A). As expected, a 240-bp Cre allele was present in all tissues analyzed (Fig. 2A), indicating that the Cre transgene was ubiquitously present in all of the tissues. Liver Pdha1 mRNA analysis by qRT-PCR using one primer in the region of the deleted exon 8 showed the level at 1.3% in L-PDCKO mice compared with control (L-PDCT) mice (Fig. 2B). Western blot analyses showed the complete absence of the α- and β-subunits of PDH in livers of three L-PDCKO mice (L1, L2, and L3) compared with three L-PDCT mice (Fig. 2C). As expected, two PDH subunits were present in other tissues analyzed in mice (Fig. 2C). Previously, it was shown that in the absence of the α-subunit of PDH, the β-subunit of PDH was found to be absent due to its instability (17).

![Fig. 2. Genetic and protein analyses of control (L-PDCT) and liver-specific pyruvate dehydrogenase complex-deficient (L-PDCKO) mice. A: PCR amplification of 3 different alleles in liver (L), skeletal muscle (SM), heart (H), and adipose tissue (AT). B: quantitative RT-PCR (qRT-PCR) analysis of Pdha1 mRNA in L-PDCKO liver (black bar) compared with L-PDCTC liver (open bar). Results are means ± SE (n = 6). *P < 0.05. C: Western blot analyses of PDH proteins (both the 41-kDa α- and 36-kDa β-subunits) in tissue lysates from L-PDCT and L-PDCKO mice.](http://ajpendo.physiology.org/Downloadedfrom http://ajpendo.physiology.org/ by October 21, 2017)
not de novo FA synthesis. It should be noted that there are differences in body weights, blood glucose levels, and serum insulin levels in L-PDCKO mice used in the present study and that of L-PDCKO mice reported previously (6). These phenotypic differences are due to differences in the genetic background of these two strains of mice. Mice used in the present study had a B6 genetic background, whereas mice used in our previously reported paper (6) had a mixed (129J/B6) genetic background. Earlier, we also reported some differences for H9252-cell structure and function between two pancreatic H9252-cell-specific PDCKO mouse strains (30, 39). So it is not surprising that we have observed some variations in the levels of serum insulin and blood glucose of L-PDCKO mice (B6 genetic background) in the present study compared with that of the L-PDCKO mice (129J/B6 mixed genetic background), as reported previously (6).

**Hepatic gene expression analyses in L-PDCKO mice.** Using quantitative RT-PCR analysis, we carried out gene expression analyses of several key enzymes as well as their upstream regulatory transcriptional factors in the liver of L-PDCKO mice. Significant reductions in the expression of key lipogenic genes (40% Acly and 60% Acc1 for FA synthesis, 60% hydroxy-methylglutaryl-CoA reductase (Hmgcr) for cholesterol biosynthesis, and 62% glucose-6-phosphate dehydrogenase (G6pd2) for both of these pathways] were observed in livers of L-PDCKO mice compared with L-PDCCT mice (Fig. 4A). Furthermore, for three key enzymes involved in FA oxidation, namely fatty acid translocase (FAT; a.k.a. Cd36), carnitine-palmitoyl-CoA transferase-1 (Cpt1a), and Acc2, their mRNA levels were significantly reduced in L-PDCKO livers by 56, 51, and 29%, respectively (Fig. 4A). Interestingly, for two key enzymes in the glycolytic pathway, the level of hepatic glucokinase (Gck) mRNA was not significantly altered in L-PDCKO mice; whereas the mRNA level of liver-pyruvate kinase (Pklr) was significantly decreased (65%) in L-PDCKO mice (Fig. 4A). In the gluconeogenic pathway, the level of PEP-carboxykinase (Pck1) mRNA was significantly decreased (62%), whereas the level of glucose-6-phospha-

**Fig. 3.** Body weights, food intake, blood glucose levels, serum insulin levels, and liver lipid analyses in L-PDCCCT and L-PDCKO male mice in the fed state. A: body weights of mice from days 10 to 120. Body weights were recorded every 7 days. B: food intake of mice (single caged) was determined for 1 wk from days 49 to 56. C: tail blood glucose levels in randomly fed mice were recorded on day 60. D and E: serum insulin and serum triglyceride (TG) levels of mice were measured on day 60. F–H: levels of liver cholesterol and TG and fatty acid composition of TG of mice on day 60. Results are expressed as means ± SE (n = 8–12). *P < 0.05.
tase \[G6Pase (G6pc)\] mRNA was not significantly affected (Fig. 4A).

Hepatic lipid metabolism is controlled by transcription factors such as liver X receptors (LXRs), sterol regulatory element-binding protein (SREBP)-1c, SREBP2, and carbohydrate response element-binding protein (ChREBP) that regulate the expression of critical enzymes involved in the lipogenic and glycolytic pathways (11). Furthermore, several other key transcription factors and coactivators such as peroxisome proliferator-activated receptor (PPAR)\(/\)coactivator-1 (PGC-1) (14, 22, 31, 32). Interestingly, mRNA levels of all these transcription factor modifiers were decreased significantly in the liver from L-PDCKO mice compared with L-PDCT mice [%reduction: 52 LXRs (Nr1h3), 61 SREBP1c (Srebfl), 40 SREBP2 (Sreb2), 53 ChREBP (Mlipl), 65 Pgc-1a (Ppargc1a), 64 Ppara, 76 Foxo1, and 63Sir2 (6)]; Fig. 4A).

The mRNA levels of the cytosolic acetyl-CoA synthetase gene (AceCS1) and the mitochondrial acetyl-CoA synthetase gene (AceCS2) were determined. The mRNA levels of AceCS1 were significantly decreased (45%) in livers from L-PDCKO mice, whereas the mRNA levels AceCS2 were similar between the two groups of mice (Fig. 4B).

Early we reported a compensatory increase in FA biosynthesis in epididymal adipose tissue from male L-PDHKO mice (6). In epididymal adipose tissue the level of Acc1 mRNA was significantly increased (145%), whereas the level of Hmgcr mRNA was decreased by 60%, and the mRNA level of G6pd2 remained unaltered in L-PDCKO mice, whereas the mRNA levels of G6pd2 were also similar between the two groups of mice (Fig. 4C). The mRNA levels of acetyl-CoA:acetyltransferase 1 (Acat1) and acetyl-CoA:acetyltransferase 2 (Acat2) were also similar between the two groups of mice (Fig. 4C).
SREBP1c, ChREBP, LXRα, and Sirt6 were not altered in adipose tissue from L-PDHKO mice, but the mRNA levels of Pgc-1α, PPARα, and Foxo1 were significantly increased by 132, 124, and 95%, respectively, in L-PDHKO mice (Fig. 4E). Interestingly, the expression of SREBP2 was decreased by 41% in adipose tissue from L-PDHKO mice (Fig. 4E), which is consistent with the observed reduction in Hmgcr mRNA levels (Fig. 4D).

**Protein analyses in livers from L-PDCKO mice.** In light of alterations in expression of several genes in livers of L-PDCKO mice, we investigated the amounts of four hepatic proteins. The level of cytosolic ACC1 was significantly decreased (36%) in livers of L-PDCKO mice (Fig. 5A). The levels of SREBP-1c were significantly decreased in both the nuclear (33%) (Fig. 5B) and the cytosolic (43%) (Fig. 5C) fractions of L-PDCKO liver. Also, the protein level of AceCS1 that converts acetate to AcCoA in the cytosol was decreased (42%) in L-PDCKO livers (Fig. 5D). In contrast, the nuclear level of SIRT6 protein was significantly increased (57%) in L-PDCKO livers (Fig. 5E), although its mRNA level in L-PDCKO livers was not significantly altered (Fig. 4B). The increase in SIRT6 protein levels is most likely due to its stabilization, as there was no change in Sirt6 gene transcription (Fig. 5E) (21).

**AcCoA content and protein acetylation in livers from L-PDCKO mice.** We reasoned that PDC deficiency in the liver could impact the steady-state levels of AcCoA in ad libitum-fed L-PDCKO mice. Surprisingly, there was no significant difference in total liver AcCoA content between L-PDCC and L-PDCKO livers (Fig. 6A). However, measuring the total hepatic amount of AcCoA does not allow the discrimination of any changes in the subcellular AcCoA pools in mitochondria and nucleocytosolic compartments. Since histone acetylation has been shown to be associated with changes in the cellular AcCoA levels (2, 42), we investigated possible changes in these pools by measuring their impact on the level of protein.

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**Fig. 5. Western blot analyses of liver proteins from L-PDCC and L-PDCKO mice.** A: acetyl-CoA carboxylase 1 (ACC1) in liver cytosol. B: sterol regulatory element-binding protein (SREBP-1) in liver nuclei. C: SREBP-1 in liver cytosol. D: acetyl-CoA synthetase gene (AceCS1) in liver cytosol. E: sirtuin 6 (SIRT6) in liver nuclei. Top (blots A–E): Western blots for listed proteins. Bottom (graphs A–E): quantitation of Western blot proteins by densitometry analysis. Results were normalized with β-actin and expressed as means ± SE (n = 6). *P < 0.05. LKO, L-PDCKO.
acetylation in liver nuclear fraction from L-PDCKO mice. Interestingly, there was a marked increase (2.1-fold) in histone H3 acetyl K9 protein (H3K9) in liver nuclei from mice compared with L-PDCCT mice (Fig. 6, B and C). When the SDS-PAGE-separated nuclear proteins were immunodetected using antiacetylated lysine antibodies, at least three major protein bands were significantly altered in L-PDCKO mice, namely 1) a 2.1-fold increase in the ~22-kDa band representing histone H3K9, 2) a 24% reduction in the ~35-kDa protein band, and 3) a 44% reduction in the ~45-kDa band (Fig. 6, D and E).

**DISCUSSION**

During the absorptive period, the liver utilizes glucose as the primary substrate for the synthesis of glycogen and fatty acids/cholesterol. During this period, dephosphorylation/activation of PDC by insulin-mediated activation of PDH phosphatase allows the formation of AcCoA from pyruvate in the mitochondria (3, 19). The transfer of the acetyl moiety as citrate to the cytosol and regeneration of AcCoA from citrate serve as the major sources for lipid biosynthesis and protein acetylation in the nucleocytoplasmic compartments (Fig. 7). The null mutation in the Pdhb1 gene resulting in the complete loss of hepatic PDC activity eliminates the availability of glucose-derived AcCoA for FA synthesis in the cytosol (6). The results of the present study extends this finding showing downregulation of several key genes involved in lipid synthesis and glucose metabolism in livers of L-PDCKO mice. This is a novel finding for glucose-derived AcCoA, which is not only the precursor for lipid biosynthesis but also serves as a modulator of gene expression of several key enzymes in de novo lipogenesis as well as their upstream regulators.

Since PDC-generated AcCoA is the primary source of cytosolic citrate in the fed state, mitochondria-generated citrate derived from pyruvate metabolism via PDC cannot be the source for the cytosolic AcCoA pool in the liver of L-PDCKO mice (Fig. 7). However, there are other pathways such as β-oxidation of long-chain FAs, amino acid catabolism, and acetate activation by which AcCoA is generated in liver mitochondria. The acetyl moiety of mitochondrial AcCoA is transported out into the cytosol in the forms of citrate and acetyl-carnitine. As shown, when the contribution of the mitochondria-generated AcCoA via the deletion of PDC was completely eliminated in the liver, there was no effect on the level of total AcCoA in the liver of L-PDCKO mice, suggesting either increased generation by the alternate pathways or/and reduced utilization of AcCoA for lipid biosynthesis, resulting in the maintenance of the steady-state levels of AcCoA in the liver of L-PDCKO mice. The latter possibly appears to be a major contributor to this outcome. The levels of mRNA of FAT and Cpt1a were reduced in livers of L-PDCKO mice (Fig. 4A), suggesting that FA oxidation is not a source of the cytosolic AcCoA in the fed state. Another source of mitochondrial AcCoA is acetyl-CoA synthetase 2 (AceCS2) utilizing acetate as the substrate, whose mRNA levels were not altered in livers of L-PDCKO livers (Fig. 4C). The availability of acetate in the

**Fig. 6. Hepatic acetyl-CoA (AcCoA) content and analyses of acetylated proteins in liver nuclei from L-PDCT (control) and L-PDCKO (LKO) mice.** A: hepatic AcCoA content in mice. B: Western blot analysis of histone H3 acetyl K9 protein (H3K9) in liver nuclei from mice. C: densitometry analysis of histone H3 acetyl K9 protein detected in B. D: Western blot analysis of acetyl-lysine proteins in liver nuclei. E: densitometry analysis of 22-, 35-, and 45-kDa acetyl-lysine proteins bands detected in D. Protein loading was analyzed by staining of gels with Ponceau S stain, followed by densitometry analysis (results not shown). Results are expressed as means ± SE (n = 6–8). *P < 0.05.
mitochondria of L-PDCKO mice is not known, and hence, its contribution to the cytosolic AccCoA pool cannot be evaluated. Formation of AccCoA from acetate by acetyl-CoA synthetase-1 and conversion of acetyl-carnitine are other potential sources of cytosolic AcCoA. Interestingly, the level of AceCS1 mRNA was markedly decreased in livers of L-PDCKO mice (Fig. 4C), suggesting a possible decrease in its contribution to the cytosolic AccCoA pool. It is possible that amino acid catabolism could serve as an alternate source of mitochondrial AccCoA in livers of L-PDCKO mice. This, however, remains to be further investigated.

Liver X receptors (LXRs), SREBPs, and ChREBP are key transcription factors controlling glycolysis and lipid biosynthesis (20, 24, 32, 37). SREBP-1c is involved in transcriptional regulation of several genes in de novo FA synthesis in fatty acids, whereas SREBP-2 regulates cholesterol synthesis in the liver (9, 14, 20). In liver, LXRs stimulate the expression of both SREBP-1c and ChREBP by binding to their cognate LXRE in the liver appears to be a key regulatory event controlling glycolytic and lipogenic responses, although the mechanism(s) by which this is accomplished in liver from L-PDCKO mice remains to be investigated. One possibility is that the hyperinsulinemic state of these mice causes enhanced lipogenesis in epididymal adipose tissue. It should be noted that the gene expression profile of the lipogenic pathway in adipose tissue was selective (an increase in the expression of Pgc-1α and Pgc-2 genes only and not of the LXR, SREBP1c, or ChREBP genes), suggesting that other mechanism(s) contribute to this outcome.

In a study in which the use of cytosolic AccCoA for FA biosynthesis was restricted by liver-specific deletion of Acc1, the mRNA expression of several genes (Acly, Acc2) involved in de novo FA synthesis was significantly increased in livers from L-ACC1KO mice (27). These investigators suggested that reduction in FA biosynthesis in the liver triggered increased expression of the lipogenic genes. In contrast, the levels of these genes remained unaffected in the epididymal adipose tissue from L-ACC1KO mice (27). In another study using the liver-specific ACC1 null mutation, expression of the hepatic ACC2 gene was enhanced to compensate for the formation of malonyl-CoA and lipogenic capacity (15). Mice with Acc2 deletion demonstrated a significant upregulation of lipogenic enzymes [Acly, Acc1, FAS (Fasn), and Hmgcr] and their upstream transcription factor genes (such as SREBP1, SREBP2, and ChREBP) (1). Interestingly, no significant effect was observed in the expression of other regulatory genes such as SREBP1c, Pgc-1α, and Pgc-1β in double-KO (deletion of Acc1 and Acc2) mice (7).

In yet another study, liver-specific deletion of fatty acid synthase (L-FASKO), allowing the conversion of cytosolic AccCoA to malonyl-CoA by ACC1 but restricting the use of malonyl-CoA for FA synthesis, resulted in reduction in SREBP-1c expression in chow-fed L-FASKO mice with low circulating insulin levels (5). The levels of mRNAs of Gck, Pck1, PPARα, Pgc-1α, Cpt1a, and LXRα were not altered in livers from chow-fed L-FASKO mice (5). In contrast, our L-PDCKO mice with ablation of AccCoA formation from pyruvate exhibited completely opposite effects on expression of lipogenic genes in the liver. The mRNA expression of several lipogenic genes (Acly, Acc1, Acc2, Hmgcr, and G6pd2) and their upstream regulators (LXR, SREBP-1c, and ChREBP as well as Pgc-1α, PPARα, and Foxo1) were significantly downregulated in livers of L-PDCKO mice (Fig. 4, A and B). These results suggest that complete inhibition in the ability to generate AccCoA from glucose carbons as the precursor for lipogenesis in the liver triggers a downregulation in the expression of upstream regulators of hepatic lipogenic genes.

PGC-1α enhances gluconeogenesis as well as the uptake of FAs and their β-oxidation by coactivating hepatic transcription factors such as PPARα and FOXO1 (25, 32, 43). Insulin indirectly regulates PGC-1α by lowering cyclic AMP levels and also by inactivating FOXO1 (14, 16, 34). In livers of L-PDCKO mice, the expression of coactivator PGC-1α and its transcription factors (e.g., PPARα and FOXO1) was significantly reduced. Expression of their target genes such as Pck1 (gluconeogenesis), Cpt1a (FA oxidation), and Fat (FA uptake) was also significantly reduced in livers from L-PDCKO mice (Fig. 3A). Given the hyperinsulinemic state of L-PDCKO mice, the expression of several genes involved in lipogenesis and glucose metabolism was downregulated in the liver as indicated by the downregulation in the expression of their upstream regulators.
mice in the fed state, this is an expected outcome for down-regulation of key gene expression in gluconeogenesis and FA oxidation in the livers of L-PDCKO mice.

A link between intracellular AcCoA levels and protein acetylation was shown in budding yeast-metabolizing glucose (2, 12, 40). Studies in yeast showed that increased availability of AcCoA due to Acc1 gene deletion was sufficient to enhance histone acetylation (13, 45). Similarly, activation of ACC1 by inhibition of AMPK in yeast resulted in decreased histone acetylation (44). In budding yeast, AcCoA production by the PDC in the mitochondria was indirectly altered by deletion of mitochondrial pyruvate carrier-1 (MPC1), resulting in accumulation of acetate in the cytosol, triggering upregulation of the cytosolic AceCS1 for AcCoA production, increased histone acetylation, and repression of autophagy genes (10). When mitochondrial AcCoA generation was eliminated by combined deletion of ACH1 (acetyl-CoA hydrolase/acetyl-CoA-CoA-transferase-1) and MPC1 in yeast, an upregulation of the Acc2p to synthesize AcCoA in the nucleocytoplasmic pathway was reported. In the present study, elimination of PDC activity in liver mitochondria resulted in a significant decrease in cytosolic AceCS1 gene expression but no change in AceCS2 gene expression, suggesting that AceCS1 is not able to compensate for reduced AcCoA supply due to PDC deficiency.

Protein acetylation of intermediary metabolic enzymes is highly prevalent and plays a major role in metabolic regulation (45). For example, liver-specific double-Acc1 and -Acc2 knockout mice had no effect on the mitochondrial and cytosolic AcCoA levels in the liver. However, an increase in the acetylation of proteins in the nucleocytoplasmic space and hypoacetylation of mitochondrial proteins was observed in these mice (7). Interestingly, many enzymes in glucose metabolism and FA synthesis were altered in livers of double (Acc1/Acc2) KO mice (7). We have observed modifications in acetylation status of several proteins in the nuclear compartment (Fig. 6, B–E). Since lipogenic gene expression is downregulated in the liver of PDCKO mice, altered acetylation profile of specific transcription involved in the regulation of lipogenic genes appears to be a contributing factor for this outcome.

In summary, AcCoA generated in liver mitochondria from glucose-derived pyruvate not only is necessary as the building block for de novo lipid biosynthesis in mice during the fed state (6) but also serves as a modulator of gene expression involved in de novo lipid biosynthesis, most likely via acetylation of histones and other specific proteins in the nuclear compartment (present study). It is suggested that altered acetylation of a key transcriptional regulatory sensor(s) (yet unidentified) plays a key role in influencing the altered metabolic state of the liver of L-PDCKO mice. Furthermore, alternate pathways for the generation of AcCoA in the liver appear not to be sufficient to compensate for gene expression in L-PDCKO mice during the fed state. These findings have important implications for regulation of hepatic fatty acid synthesis in obesity, type 2 diabetes, and nonalcoholic fatty liver disease.

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DISCLOSURES

The authors declare that there are no conflicts of interest, financial or otherwise, for this research.

AUTHOR CONTRIBUTIONS


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