Caveolin-1 Is Necessary for Hepatic Oxidative Lipid Metabolism: Evidence for Crosstalk between Caveolin-1 and Bile Acid Signaling


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SUMMARY

Caveolae and caveolin-1 (CAV1) have been linked to several cellular functions. However, a model explaining their roles in mammalian tissues in vivo is lacking. Unbiased expression profiling in several tissues and cell types identified lipid metabolism as the main target affected by CAV1 deficiency. CAV1−/− mice exhibited impaired hepatic peroxisome proliferator-activated receptor α (PPARα)-dependent oxidative fatty acid metabolism and ketogenesis. Similar results were recapitulated in CAV1-deficient AML12 hepatocytes, suggesting at least a partial cell-autonomous role of hepatocyte CAV1 in metabolic adaptation to fasting. Finally, our experiments suggest that the hepatic phenotypes observed in CAV1−/− mice involve impaired PPARα ligand signaling and attenuated bile acid and FXRα signaling. These results demonstrate the significance of CAV1 in (1) hepatic lipid homeostasis and (2) nuclear hormone receptor (PPARα, FXRα, and SHP) and bile acid signaling.

INTRODUCTION

Caveolae are plasma membrane microdomains enriched in cholesterol and sphingolipids. They are extremely abundant in specific cell types, including endothelial cells and adipocytes, but not in cells such as hepatocytes (Calvo et al., 2001; Pilch and Liu, 2011). Caveolin-1 (CAV1) is an integral membrane protein and, in association with PTRF/Cavin1, is the main structural protein of caveolae in nonmuscle cells. Although caveolae and CAV1 have been implicated in lipid regulation, mechanosensation, signaling, and endocytosis (Drab et al., 2001; Garg and Agarwal, 2008; Hayashi et al., 2009; Liu et al., 2008; Parton and Simons, 2007; Razani et al., 2002), a universal model that explains the specific roles of caveolae and CAV1 in metabolism in vivo is lacking.

Liver lipid metabolism can be physiologically challenged during fasting and obesity when it becomes essential for systemic energy homeostasis (Desvergne et al., 2006). In this context, peroxisome proliferator-activated receptor α (PPARα) regulates fatty acid oxidation (FAO) and ketogenesis. During fasting, hepatic PPARα-dependent lipid metabolism depends on fatty acid synthase (FAS) and diet-derived lipid metabolites that work as endogenous PPARα ligands (Chakravarthy et al., 2009; Chakravarthy et al., 2005). Moreover, bile acids (BAs) and BA activation of FXRα are direct regulators of PPARα signaling (Pineda Torra et al., 2003). The excessive accumulation of BAs also provides protection against liver steatosis, but it impairs FAO in mice (Watanabe et al., 2004). It has been proposed that CAV1 is important in the maintenance of hepatic lipid homeostasis (Fernández et al., 2006; Fernández-Rojo et al., 2012; Martin and Parton, 2006; Parton and Simons, 2007). Recently, several reports have described the involvement of CAV1 in mitochondrial regulation (Asterholm et al., 2012; Bosch et al., 2011). Asterholm et al. (2012) postulated that the metabolic phenotype of tissues from CAV1−/− mice, including the liver, is mainly caused by the adipocyte-CAV1 deficiency. However, the lack of data showing the rescue of wild-type (WT) phenotypes by
the re-expression of CAV1 in adipocytes from CAV1+/+ mice means that some of the phenotypes observed in tissues from CAV1+/−/− mice, including the liver, are totally or partially caused by cell-specific CAV1 deficiency. Therefore, the mechanisms by which CAV1 modulates hepatic lipid metabolism are still poorly understood.

In this study, we used a wide range of approaches, including microarray analysis, electron microscopy, biochemistry, indirect calorimetry, three different CAV1+/−/− mouse models, and two different CAV1-deficient AML12 hepatocyte cell models to show (1) that CAV1 participates in multiple facets of lipid metabolism that are essential for the maintenance of lipid homeostasis, (2) the universal and cell-autonomous role of CAV1 in maintaining hepatocyte PPARα signaling during fasting, and (3) CAV1-dependent BA and FXRα signaling during fasting.

RESULTS

Lipid Metabolism Is the Major Cell and Tissue Process Modulated by CAV1

Comparative genome-wide expression analysis in mouse embryonic fibroblast (MEFs) primary cultures, livers, and visceral adipose tissue from CAV1+/−/− and CAV1+/+ mice (Drab et al., 2001) provided an unbiased approach for investigating pathways directly or indirectly affected by CAV1 deficiency. We observed statistically significant alterations in 195, 421, and 288 genes in CAV1+/−/− MEFs, livers, and adipose tissue, respectively, representing different sets of genes. A remarkably small number of modulated genes were common to two tissues or to all three tissues (see Table S1). The group of genes modulated in all three tissues, predicted to represent key universal proteins linked to CAV1 expression, included signaling proteins such as K-Ras, sorting nexin 5, the sumoylation enzyme Ube21, and liprinβ1, a phosphatase scaffolding protein.

Enrichment of differentially expressed genes in several pathways and functions by ingenuity pathways analysis (IPA) revealed that the only common cellular process affected by CAV1 deficiency in adipose tissue, livers, and MEFs was a down-regulation of lipid metabolism (Figure S1), demonstrating the importance of CAV1 for the maintenance of lipid homeostasis. Interestingly, the highest gene enrichment in lipid metabolism, both the function and the pathway, was observed in CAV1+/−/− livers (Figure 1A).

CAV1 in Fasting Hepatic Lipid Metabolism

Genome-wide expression profiling and gene enrichment analysis suggested that CAV1 deficiency altered the expression of genes involved in hepatic peroxisomal and mitochondrial FAO, bile synthesis, ketogenesis, lipid droplet formation, minor fatty acid, and amino acid metabolism (Figure 1A).

In the mouse liver, these metabolic pathways are under the control of the nuclear receptor PPARα (Mandard et al., 2004). The expression of hepatic PPARα and its target genes is induced 6–8 hr after food deprivation and peaks at approximately 12 hr of starvation (Yang et al., 2006). At this time of fasting, the transcript levels of hepatic PPARα and its target genes, Cpt1α, Cpt1β, and PGC-1α, and the protein levels of PPARα and PGC-1α were significantly reduced in CAV1+/−/− mice in comparison to CAV1+/+ mice (Figures 1B–1D). Furthermore, decreased expression of hepatic Bdh1 (Figure 1E) and reduced plasma levels of the ketone body β-hydroxybutyrate (BOH) (Figure 1F) also suggest that CAV1 deficiency impaired hepatic ketogenesis. Moreover, CAV1+/−/− MEFs oxidized less palmitate than CAV1+/+ MEFs (Figure 1G).

Defective Fatty Acid Oxidation and Ketogenesis Is Independent of the Lipodystrophic Phenotype Seen in CAV1+/−/− Mice

Because fatty acids function as endogenous ligands for the activation of PPARα (Chakravarthy et al., 2009; Chakravarthy et al., 2005; Jump, 2011; Schroeder et al., 2008), we investigated whether lipodystrophy in CAV1+/−/− mice (Drab et al., 2001; Razani et al., 2002) might reduce available endogenous fatty-acid-derived PPARα ligands and be responsible for the impairment of PPARα signaling and ketogenesis. Neither supplementation with oleic acid (OA), one of the main fatty acid components of triacylglycerols (TAG) stored in hepatocyte lipid droplets (LDs) (Fujimoto et al., 2006), nor with arachidonic acid, the main precursor for endogenous PPARα ligands (Jump, 2011), recovered plasma BOH levels when compared to untreated CAV1+/−/− and CAV1+/+ mice (Figure S2A).

Moreover, high-fat diet (HFD)-fed CAV1+/−/− mice, which still have available fatty acids for FAO and ketogenesis in the form of high levels of plasma lipids and cytoplasmic LDs in hepatocytes (Asterholm et al., 2012; Fernández-Rojo et al., 2012), also showed lower levels of hepatic PPARα messenger RNA (mRNA) and the PPARα target genes Cpt1α and MCAD than HFD-fed CAV1+/+ mice (Figure S2B). HFD-fed CAV1+/−/− mice also exhibited decreased expression of the ketogenesis-related genes Bdh1 and FGF21 as compared to HFD-fed CAV1+/+ mice (Figure S2C). Finally, chow-fed CAV1+/−/− mice showed reduced plasma BOH in comparison to chow-fed CAV1+/+ mice, which was unchanged by HFD in the latter (Figure S2D).

In addition, microarray analysis (Figure S2E) and reduced protein levels of PPARα and PGC1α in CAV1+/−/− adipose tissue (Figure S2F), as well as reduced expression of PPARα, Cpt1α, and MCAD in CAV1+/−/− white adipose tissue explants in comparison to CAV1+/+ explants (Figure S2G), supported the hypothesis that CAV1 deficiency also deregulated adipocyte mitochondrial function, PPARα signaling, and the expression of FAO-related genes, despite the availability of fatty acids. Hence, these results suggest that, independent of fatty acid availability, CAV1 deficiency impairs PPARα signaling in metabolic tissues such as liver and white adipose tissue.

Cell-Autonomous Modulation of Hepatocyte Fatty Acid Catabolism and Mitochondrial Metabolic Adaptation by CAV1

We examined the cell-autonomous role of caveolae and CAV1 in metabolic adaptation to fasting and hepatic mitochondrial function during carbohydrate and lipid metabolism. We developed stable CAV1 knockdown (CAV1-kd) (Fernández-Rojo et al., 2012) and PTRF/Cavin1 knockdown (PTRF/Cavin1-kd) AML12 hepatocyte cell lines (Figure 2A and the Extended
Experimental Procedures). Mimicking physiological conditions in mice, AML12 hepatocyte cell lines were maintained in fed-like (25 mM glucose) or fasting-like (10 mM glucose/100 μM OA) culture conditions for 24 hr before we examined mitochondrial metabolic adaptation, oxidative phosphorylation—also referred as mitochondrial respiration and presented as oxygen consumption ratio—and cellular glycolysis—presented by the extracellular acidification ratio—in living cells by using an XF extracellular metabolic flux analyzer. Under normal fed-like conditions, CAV1 deficiency was associated with a trend toward a compensatory increase in glycolytic flux (Figure 2C) without consequences for the ratios of mitochondrial respiration ratios (Figure 2D). In agreement with previous data (Fernández-Rojo et al., 2012), these results suggested that CAV1 deficiency favors anaerobic glycolysis over oxidative phosphorylation because of carbohydrate catabolism. Switching to fasting-like conditions in WT AML12 hepatocytes was associated with a general conservation of nutrients, which was reflected by a trend to decreased nonoxidative (glycolysis) (Figures 2B and 2C) and oxidative metabolism (Figures 2B and 2D). However, CAV1 deficiency resulted in metabolic deregulation when switching to fasting-like conditions, highlighted by an increase in oxidative metabolism (Figures 2B and 2D) and higher glycolytic flux (Figures 2B and 2C). On the other hand, PTRF/Cavin1-kd cells showed no metabolic adaptation to fasting-like conditions, but their mitochondrial respiration did not differ from WT cells (Figures 2B and 2D). These results suggested that CAV1 deficiency impairs hepatocyte mitochondrial metabolism towards a compensatory increase in glycolytic flux (Figure 2C) without consequences for the ratios of mitochondrial respiration (Figures 2D). To emphasize the significance of CAV1 in hepatic lipid metabolism and metabolic diseases, we tested whether the specific PPARα agonist wy14643 rescued PPARα-dependent pathways in fasted 9- to 12-month-old CAV1+/+ and CAV1−/− mice (old CAV1+/+ and CAV1−/− mice; Figures S3H–S3J). Hence, our results demonstrated universal and genetic-background-independent regulation of hepatic oxidative lipid metabolism, including PPARα-dependent metabolism and ketogenesis by CAV1.

Universal Role of CAV1 in Liver Oxidative Lipid Metabolism during Fasting

The deleterious effects of CAV1 deficiency on liver regeneration and higher ratios of systemic carbohydrate metabolism in fed mice (Asterholm et al., 2012; Fernández et al., 2006; Fernández-Rojo et al., 2012; Mayoral et al., 2007) depend on the genetic background. In contrast, by studying Batb/Cav1−/− mice (Fernández-Rojo et al., 2012) and Jak2/Cav1−/− mice obtained from the Jackson Laboratory (Razani et al., 2002), we observed decreased hepatic PPARα protein levels, reduced MCAD and PDK4 expression, and a low concentration of plasma BOH in 24 hr-fasted Batb/Cav1−/− mice (Figures S3A–S3C). Similar results were obtained in 24 hr-fasted Jak2/Cav1−/− mice, which showed reduced MCAD and Bdh1 expression and a low concentration of plasma BOH (Figures S3D and S3E). Furthermore, similar results were obtained by comparing 24 hr-fasted PTRF/Cavin1−/− and PTRF/Cavin1+/+ littermates. PTRF/Cavin1−/− mice lack caveolae in all their tissues (Bastiani et al., 2009; Liu et al., 2008) but expressed 14% and 18% of WT CAV1 cells in fed and fasted PTRF/Cavin1−/− livers, respectively (Figure S3F). Similar to the three CAV1−/− mouse strains, 24 hr-fasted PTRF/Cavin1−/− mice showed reduced levels of total and phosphorylated PPARα protein in liver homogenates (Figure S3G), correlating with the defective expression of PPARα-dependent genes involved in FAO and ketogenesis and lower levels of plasma BOH (Figures S3H–S3J). Hence, our results demonstrated universal and genetic-background-independent regulation of hepatic oxidative lipid metabolism, including PPARα-dependent metabolism and ketogenesis by CAV1.

The Absence of CAV1 Confers Resistance to Wy14643-Induced Activation of PPARα Signaling during Fasting

In order to recover PPARα signaling, old CAV1+/+ and CAV1−/− mice were starved for the last 24 hr of the 7-day wy14643 treatment. Wy14643 treatment increased total and active hepatic PPARα protein levels in old CAV1+/+, but not in CAV1−/−, mice (Burns and Vanden Heuvel, 2007) (Figures 3B and 3C). Wy14643 treatment recovered the hepatic expression of the PPARα target gene MCAD, but not Bdh1, in 24 hr-fasted CAV1−/− mice (Figure 3A). Accordingly, wy14643 did not stimulate ketogenesis in old CAV1−/− mice (Figure 3D). Interestingly, plasma ketone body concentration in untreated old CAV1−/− mice was significantly lower than it was in young CAV1−/− mice (Figures 3D–3D1), suggesting that CAV1 deficiency might confer more dramatic metabolic consequences during aging. Similar to young mice (Fernández-Rojo et al., 2012), we observed decreased hepatic PPARα protein levels, reduced MCAD and PDK4 expression, and a low concentration of plasma BOH in 24 hr-fasted Batb/Cav1−/− mice (Figures S3A–S3C). Similar results were obtained in 24 hr-fasted Jak2/Cav1−/− mice, which showed reduced MCAD and Bdh1 expression and a low concentration of plasma BOH (Figures S3D and S3E). Furthermore, similar results were obtained by comparing 24 hr-fasted PTRF/Cavin1−/− and PTRF/Cavin1+/+ littermates. PTRF/Cavin1−/− mice lack caveolae in all their tissues (Bastiani et al., 2009; Liu et al., 2008) but expressed 14% and 18% of WT CAV1 cells in fed and fasted PTRF/Cavin1−/− livers, respectively (Figure S3F). Similar to the three CAV1−/− mouse strains, 24 hr-fasted PTRF/Cavin1−/− mice showed reduced levels of total and phosphorylated PPARα protein in liver homogenates (Figure S3G), correlating with the defective expression of PPARα-dependent genes involved in FAO and ketogenesis and lower levels of plasma BOH (Figures S3H–S3J). Hence, our results demonstrated universal and genetic-background-independent regulation of hepatic oxidative lipid metabolism, including PPARα-dependent metabolism and ketogenesis by CAV1.

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2012), old CAV1−/− also showed impaired fasting-induced steatosis when compared to old CAV1+/+ mice (Figure 3F). In accordance with the wy14643-induced overactivation of PPARα signaling and FAO together with increased mobilization of LD-TAG, ADRP mRNA and protein levels, as well as the number of LDs, were reduced in wy14643-treated old CAV1+/+ mice. However, neither ADRP mRNA and protein levels nor the number of LDs were reduced in livers from wy14643-treated old CAV1−/− mice (Figures 3A, 3B, and 3F). Furthermore, small interfering RNA (siRNA) against CAV1 in AML12 hepatocytes inhibited the stimulation of PPARα and PPARγ target genes in response to wy14643 recapitulating the phenotype seen in CAV1−/− mice (Figure 3E) and supporting the cell-autonomous role of CAV1 in hepatocyte PPARα signaling. Intriguingly, and unlike in fasted CAV1−/− mice, wy14643-stimulated PPARα target genes in fed ad libitum CAV1−/− mice. Wy14643 induced MCAD and ADRP, and their expression levels were similar to or higher than those seen in CAV1+/+ mice (Figure 3G), whereas Bdh1 transcript levels were unaffected in either mouse strains, probably because the activation of ketogenesis is
unecessary in light of the availability of glucose in the peripheral tissues.

**CAV1 Deficiency Impairs the BA-FXRα Signaling Axis in Hepatocytes**

BAs, through the activation of FXRα, are essential regulators of PPARα (Pineda Torra et al., 2003). Also, BAs regulate CAV1 expression in esophageal epithelial cells (Prade et al., 2012). Hence, we investigated the potential crosstalk between BA signaling and CAV1 by examining the BA- and FXRα-dependent induction of the expression of short heterodimer partner (SHP, a nuclear receptor), which is a critical regulator of hepatic cholesterol and BA homeostasis (Goodwin et al., 2000; Wang et al., 2002). CAV1-kd AML12 hepatocytes showed a dramatic loss of BA-induced SHP expression (Figure 4A) relative to BA-dependent induction of SHP in WT cells. Moreover, the induction of SHP expression in response to the synthetic FXRα agonist GW4064 was also attenuated in CAV1-kd AML12 hepatocytes relative to WT cells (Figure 4B). Consistent with experiments in AML12 hepatocytes and in correlation with impaired hepatic PPARα signaling, 24 hr-fastened CAV1−/− mice displayed decreased hepatic SHP expression (Figure 4C), providing evidence for physiological crosstalk between CAV1 and hepatocyte BA signaling in vivo. No difference in hepatic FXRα expression between CAV1−/− and CAV1+/+ mice was observed (Figure S4A). However, further evidence supporting attenuated BA signaling in vivo was gleaned from the hepatic expression profiling analysis, which revealed significant upregulation of the estrogen receptor α (Figure S4B), an SHP-repressed gene (Seol et al., 1998).

Furthermore, in CAV1−/− mice, the expression of hepatic cholesterol 7α-hydroxylase (Cyp7a1), whose product catalyzes the rate-limiting step in the synthesis of BAs, was significantly higher than it was in CAV1+/+ mice (Figure 4D). In mice, Cyp7a1 expression is mainly suppressed by the activation of intestinal FXRα, enterocyte expression and secretion of FGF15, and, to a lesser degree, the hepatic FXRα-SHP axis (Goodwin et al., 2000; Kong et al., 2012). Hence, the deregulation of Cyp7a1 will be indicative of a defective intestinal FXRα-FGF15 signaling axis.

Finally, the volume of the accumulated BA in the gallbladders of fasted CAV1−/− mice was lower than that of CAV1+/+ mice (Figure S4C), suggesting that CAV1 might also be important for intracellular trafficking and secretion into the canaliculi of the BAs. Interestingly, in comparison to fasted CAV1+/+ mice, serum BA levels were also reduced in 24 hr-fastened CAV1−/− mice (Figure S4D). In summary, our data suggest that CAV1 modulates BA signaling, synthesis, and trafficking.

**DISCUSSION**

The genome-wide expression profile shown in this study supports the notion that CAV1 participates in multiple facets of lipid metabolism. Specifically, our data highlight the metabolic significance of the systemic expression of CAV1 for hepatic lipid metabolism. CAV1 maintains liver oxidative lipid metabolism and ketogenesis during fasting and high-fat feeding independently of the genetic background and the availability of fatty acids. Alternatively, our data suggest that CAV1 might control oxidative lipid metabolism in other cell types, such as fibroblasts and adipocytes.

Moreover, two different and independent CAV1-deficient AML12 hepatocyte models supported the cell-autonomous role of hepatocyte CAV1 in hepatic metabolic adaptation to fasting and energy homeostasis. This conclusion is consistent with previous studies overexpressing CAV1 or Caveolin-3 in vivo in rodent livers (Frank et al., 2001; Otsu et al., 2010). These data are fundamental for understanding the implications of CAV1 in liver and systemic mammalian lipid metabolism and energy homeostasis. Furthermore, the data argue against recent models that suggest that all the phenotypes observed in CAV1−/− mice are due to adipocyte CAV1 deficiency (Asterholm et al., 2012). Other studies have demonstrated that cholesterol accumulation in the mitochondria underlies mitochondrial dysfunction (Bosch et al., 2011). However, selective diet-dependent activation of hepatic PPARα signaling in CAV1−/− mice by wy14643 treatment argues against mitochondrial cholesterol overload being responsible for the defective metabolic adaptation to starvation in CAV1−/− mice. In a similar manner, given that BA and FXRα signaling operates independently of mitochondrial function, it would be unlikely that mitochondrial cholesterol overloading attenuates SHP expression.

Evidence showing that CAV1 deficiency impairs BA and FXRα signaling, which is necessary for PPARα expression, suggested crosstalk between CAV1 and nuclear receptor signaling (i.e., FXRα, SHP, and PPARα). Indeed, the hallmark of attenuated BA signaling (i.e., significantly reduced SHP expression) and elevated expression of liver Cyp7a1, whose regulation depends on the activation of the intestinal FXRα-FGF15 signaling axis (Kong et al., 2012), suggested that liver lipid metabolism might...
Figure 4. CAV1 Deficiency Impairs Hepatic BA and FXRα Signaling

(A and B) SHP expression in 40 μM BA-treated (A) and 3 μM GW404-treated (B) AML12 hepatocytes.

(C and D) Liver SHP (C) and Cyp7a1 (D) expression in 24 hr-fasted CAV1+/+ and CAV1-/- mice.

(E) Hypothetical model of the deregulation of PPARα-signaling in CAV1-/- livers.

- In addition to the defective Diet/FAS-derived PPARα-ligands storage compartmentalization CAV1 deficiency impairs the function Synthetic PPARα ligands (wy14643) when fasting.

- Defects in BA/FXRα signalling and in the trafficking of BA towards the canaliculus/gallbladder may increase intracellular BA concentration (green dots) in CAV1-/- hepatocytes. Detergent properties of BA may impair the biochemical properties, and therefore the function of Diet/FAS-derived and synthetic PPARα-ligands.

The data represent the mean ± SEM. The statistical significance was assessed with a Student’s t test. *p < 0.05, **p < 0.01, ***p < 0.001.
be regulated by CAV1-dependent modulation of nuclear receptors in the liver and other tissues, such as the small intestine. We hypothesize that the lack of CAV1 might modify the lipid and protein organization and/or composition of the hepatocyte plasma membrane, which is required for BA-dependent SHP expression. In addition, our results show dramatically attenuated GW4064-dependent activation of FXRα in CAV1-kd hepatocytes showing additional downstream effects on this signaling pathway. Furthermore, CAV1 reduced gallbladder bile acid volume in CAV1−/− mice, suggesting that CAV1 also regulates BA secretion into the canaliculi. Despite low gallbladder bile volume, circulating serum bile levels are not increased in fasted CAV1−/− mice. Instead, they tend to be lower than those of CAV1+/+ mice, suggesting that BA might accumulate in hepatocytes.

On the basis of these data, we hypothesize that, when hepatic lipid metabolism is challenged in CAV1−/− mice, such as during fasting and HFD feeding, PPARα signaling is compromised because CAV1 deficiency leads to the hepatic accumulation of BAs (Figure 4E). BA accumulation, coupled with BA detergent properties, is consistent with resistance to developed hepatosteatosis and defective PPARα signaling (Watanabe et al., 2004). Despite the availability of FAS and diet “new fat”-derived liver PPARα ligands by fasted and HFD-fed CAV1−/− mice, BA accumulation might perturb the storage and/or compartmentalization, trafficking, and/or function of endogenous PPARα ligands and impair PPARα signaling. In a similar manner, hepatocyte BA accumulation in fasted CAV1−/− mice may explain fasting-specific impaired function of the synthetic PPARα agonist wy14643. These defects would be the combination of the deleterious effects that a lack of CAV1 has on (1) the regulation of the hepatocyte endocytic network (Parton and Simons, 2007; Woudenberg et al., 2010), (2) the mobilization of arachidonic acid (Astudillo et al., 2011), and (3) BA and FXRα signaling. Interestingly, wy14643-dependent regulation of hepatic expression of ADRP in ad libitum fed, but not fasted, CAV1−/− mice suggested that defective steatosis in CAV1−/− mice (Asterholm et al., 2012; Fernández et al., 2006; Fernández-Rojo et al., 2012) might also be caused by the disruption of PPARα ligand function in hepatocytes during metabolic challenges.

This study provides a framework for understanding how CAV1 modulates lipid metabolism in a tissue-specific manner and underscores the significance of the genetic background for the development of global and tissue-specific mouse models. Moreover, the significance of our study is underscored by the central position of the liver in the regulation of lipid and glucose homeostasis and emphasizes the relevance of hepatocyte CAV1 for maintaining hepatic mitochondrial function in the context of liver and systemic lipid homeostasis.

**EXPERIMENTAL PROCEDURES**

**Animals and Reagents**

K/O CAV1+/+ and K/O CAV1−/− mice were obtained as described in Fernández et al. (2006). Liver sample collection and K/O CAV1−/− mice and K/O CAV1−/− mice were generated as described in Fernández-Rojo et al. (2012). PTFR/Cavin1+/+ mice were generated as described in Liu et al. (2008). For experiments in K/O CAV1−/− mice and PTFR/Cavin1−/− mice, we used their matching CAV1+/+ littermates. Mice were kept under a controlled humidity and lighting schedule with a 12 hr dark period. All animals received care in compliance with institutional guidelines regulated by the Australian government. HFD feeding was performed as described in Fernández-Rojo et al. (2012). For fasting experiments, food withdrawal was initiated at 6 a.m. after animal house lights were switched on. When applicable, CAV1−/− mice were provided with 500 μl of 4 mM of OA and arachidonic acid by intraperitoneal injection. Mice 10–18 weeks old were fasted for up to 24 hr prior to experimentation. Wy14643 was obtained from Sigma-Aldrich (C7081). CAV1 antibody was obtained from BD Biosciences (#610060), and ADRP was obtained from Progen Biotechnik (#GP40). PPARα (Cayman Chemical), PGC1α (Santa Cruz Biotechnology, H-300), PTFR/Cavin1 antibody as described in Bastiani et al. (2009), and mouse β-actin antibody were from Chemicon (#MAB1501).

**BAs and GW4064 FXRα Agonist Treatment of AML12 Hepatocytes**

Prior to RNA purification, WT, CAV1-kd, and PTFR/Cavin1-kd AML12 hepatocytes were treated with 40 μM of cholic acid and chenodeoxycholic acid or with 3 μM GW4064 in 10% heat inactivated serum supreme (HSS)-supplemented Dulbecco’s modified Eagle’s serum/F12 medium for 24 hr.

**Bile Collection from Gallbladders**

Prior to resection, gallbladder-stored bile of 12 hr- and 24 hr-fasted CAV1+/+ and CAV1−/− mice was harvested with syringes with 30G needles. We quantified the harvested bile using micropipettes.

**ACCESSION NUMBERS**

The microarray data for MEFs, adipose tissue, and liver tissue of CAV1−/− mice have been deposited in the Gene Expression Omnibus under accession number GSE19045.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Extended Experimental Procedures, four figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2013.06.017.

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