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# Impact of the loss of caveolin-1 on lung mass and cholesterol metabolism in mice with and without the lysosomal cholesterol transporter, Niemann–Pick type C1

Dorothy I. Mundy [a], Adam M. Lopez [a], Kenneth S. Posey [a], Jen-Chieh Chuang [a], Charina M. Ramirez [b], Philipp E. Scherer [a], Stephen D. Turley [a,*]

[a] Department of Internal Medicine, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX, USA
[b] Department of Pediatrics, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX, USA

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ABSTRACT

Caveolin-1 (Cav-1) is a major structural protein in caveolae in the plasma membranes of many cell types, particularly endothelial cells and adipocytes. Loss of Cav-1 function has been implicated in multiple diseases affecting the cardiopulmonary and central nervous systems, as well as in specific aspects of sterol and lipid metabolism in the liver and intestine. Lungs contain an exceptionally high level of Cav-1. Parameters of cholesterol metabolism in the lung were measured, initially in Cav-1-deficient mice (*Cav-1*$^{-/-}$), and subsequently in *Cav-1*$^{-/-}$ mice that also lacked the lysosomal cholesterol transporter Niemann–Pick C1 (Npc1) (*Cav-1*$^{-/-}$:*Npc1*$^{-/-}$). In 50-day-old *Cav-1*$^{-/-}$ mice fed a low- or high-cholesterol chow diet, the total cholesterol concentration (mg/g) in the lungs was marginally lower than in the *Cav-1*$^{+/+}$ controls, but due to an expansion in their lung mass exceeding 30%, whole-lung cholesterol content (mg/organ) was moderately elevated. Lung mass (g) in the *Cav-1*$^{-/-}$:*Npc1*$^{-/-}$ mice (0.356 ± 0.022) markedly exceeded that in their *Cav-1*$^{+/+}$:*Npc1*$^{+/+}$ controls (0.137 ± 0.009), as well as in their *Cav-1*$^{-/-}$:*Npc1*$^{+/+}$ (0.191 ± 0.013) and *Cav-1*$^{+/+}$:*Npc1*$^{-/-}$ (0.213 ± 0.022) littermates. The corresponding lung total cholesterol contents (mg/organ) in mice of these genotypes were 6.74 ± 0.17, 0.71 ± 0.05, 0.96 ± 0.05 and 3.12 ± 0.43, respectively, with the extra cholesterol in the *Cav-1*$^{-/-}$:*Npc1*$^{-/-}$ and *Cav-1*$^{+/+}$:*Npc1*$^{-/-}$ mice being nearly all unesterified (UC). The exacerbation of the Npc1 lung phenotype and increase in the UC level in the *Cav-1*$^{-/-}$:*Npc1*$^{-/-}$ mice imply a regulatory role of Cav-1 in pulmonary cholesterol metabolism when lysosomal sterol transport is disrupted.



## 1. Introduction

Research using genetically manipulated mouse models has identified several key proteins that are involved in regulating lung cholesterol homeostasis. These include ATP binding cassette transporter G1 (ABCG1), ATP binding cassette transporter A1 (ABCA1), and sterol-27 hydroxylase (CYP27A1) [1–4]. The loss of any of these proteins results in significant changes in lung cholesterol concentration, particularly in older ABCG1-deficient mice where the level of both unesterified and esterified cholesterol rises appreciably [3]. In ABCA1-deficient mice, lung cholesterol concentration rises modestly, reflecting an increase mainly in the esterified fraction [2,4], whereas in mice lacking CYP27A1 lung total cholesterol concentration declines marginally [1]. Other proteins are involved in cholesterol trafficking in all tissues including the lungs. For example, within the lysosomal compartment of every cell, three proteins, lipoprotein acid lipase (LAL), Niemann–Pick C1 (Npc1) and Npc2, play a critical role in the processing of cholesterol contained in lipoproteins taken up largely via receptor-mediated endocytosis [5,6]. In LAL deficiency there is a pronounced increase in tissue cholesteryl ester content whereas loss of function of either Npc1 or Npc2 results in a continuing and highly detrimental expansion of tissue unesterified cholesterol content in all organs. LAL, Npc1 and Npc2 deficiency each results in a distinctive lung phenotype [7–11].

Another protein that could conceivably play a role in regulating cholesterol homeostasis in the lungs is Caveolin-1 (Cav-1), a structural protein in caveolae within plasma membranes that is highly expressed in pulmonary tissue principally in endothelial cells and type I pneumocytes [12,13]. Caveolae are critically important in facilitating

*Abbreviations:* ABCA1, ATP binding cassette transporter A1; ABCG1, ATP binding cassette transporter G1; bw, body weight; Cav-1, Caveolin-1; EC, esterified cholesterol; CYP27A1, sterol-27 hydroxylase; FVB, Friend Leukemia Virus; LAL, lysosomal acid lipase; Npc1, Niemann–Pick C1; Npc2, Niemann–Pick C2; UC, unesterified cholesterol

* Corresponding author at: Dept of Internal Medicine, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390-9151, USA. Tel.: +1 214 648 3446; fax: +1 214 648 9761.

*E-mail addresses:* dorothy.mundy@utsouthwestern.edu (D.I. Mundy), adam.lopez@utsouthwestern.edu (A.M. Lopez), kenneth.posey@utsouthwestern.edu (K.S. Posey), jen-chieh.chuang@UTSouthwestern.edu (J.-C. Chuang), charina.ramirez@utsouthwestern.edu (C.M. Ramirez), philipp.scherer@utsouthwestern.edu (P.E. Scherer), stephen.turley@utsouthwestern.edu (S.D. Turley).

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various processes including endocytosis, transcytosis, and signal transduction [14,15]. Investigations using Cav-1 knockout mice were first reported in 2001. Initially, four of these models were independently generated, all using a mixture of different mouse strains [16–19]. Since then, Cav-1-deficient mice on a single background, either C57BL/6, FVB or BALB/c, have been developed [20–22]. There is a plethora of published data describing the impact that abnormalities in Cav-1 expression have on several major organ systems. This includes multiple changes to cardiopulmonary pathology and function [17,18,23–25], neurologic disorders [26,27], defective insulin-regulated lipogenesis and lipid droplet formation [22,28–31], and decreased intestinal fat absorption [32]. Lung enlargement is caused by hypercellularity and a thickening of the alveolar septa [13,16,33]. Importantly, re-expression of Cav-1 in the endothelium reverses the vascular, cardiac, and pulmonary defects evident in *Cav-1*$^{-/-}$ mice [34]. While loss of Cav-1 is mostly detrimental in some organ systems, in a mouse atherosclerosis model, the elimination of Cav-1 reduced disease severity [35,36]. Another positive impact is protection from diet-induced obesity [37].

Other publications have focused on the effects of Cav-1 deficiency on particular aspects of hepatic and intestinal cholesterol metabolism as well as plasma lipoprotein composition [20,31,38–40]. One study found that in *Cav-1*$^{-/-}$ mice there was a marginal rise in hepatic cholesterol levels, a reduction in very low density lipoprotein secretion, and an increase in HDL levels with a greater enrichment of esterified cholesterol in the HDL [20]. More recently, detailed studies using multiple approaches including three different *Cav-1*$^{-/-}$ mouse models revealed a role of Cav-1 in bile acid signaling, synthesis, and trafficking [31]. The hypothesis being tested here is whether the types of regulatory influences that Cav-1 is now thought to exert on specific aspects of sterol metabolism within the liver are unique to that organ, or whether this protein plays a role in regulating cholesterol homeostasis in multiple tissues. Of particular interest are the lungs which manifest Cav-1 protein expression levels far exceeding those in the liver. Despite the extensive literature detailing the lung phenotype in Cav-1-deficient mice, there are no published data on any aspect of cholesterol metabolism in this model. In the present studies we used mice lacking Cav-1 only, or both Cav-1 and Npc1. Our rationale in using this particular double knockout, in addition to mice deficient in Cav-1 only, was that the loss of Npc1 alone results in a marked lung phenotype characterized in part by decisive changes in cholesterol metabolism resulting from defective lysosomal sterol transport. Given the role of caveolae in cellular cholesterol movement, and the disruption of intracellular sterol transport caused by deficiency of Npc1, it seemed a reasonable premise that in lung tissue deficient in both the main structural protein of caveolae and Npc1, there might be more overt changes in cholesterol metabolism than seen with either Cav-1 or Npc1 deficiency alone. The data show that while Cav-1 deficiency alone did not alter cholesterol metabolism in the lungs, it exacerbated the Npc1 lung phenotype with an accompanying expansion of the tissue level of unesterified cholesterol.

## 2. Materials and methods

### 2.1. Animals and diets

Male Cav-1 deficient (*Cav-1*$^{-/-}$) mice [17], on a pure FVB background [21], were bred to wild-type (*Cav-1*$^{+/+}$) females (also FVB) to establish a colony of *Cav-1*$^{+/-}$ breeding stock. These were used to generate all the *Cav-1*$^{-/-}$ and matching *Cav-1*$^{+/+}$ control mice used in these studies. Depending on the experiment, these mice were studied at 24, 50, 100 or 195 days of age. To generate *Cav-1*$^{-/-}$:*Npc1*$^{-/-}$ mice, we first crossed *Cav-1*$^{-/-}$ males (FVB) with *Npc1*$^{+/-}$ females (BALB/c). Offspring that were heterozygous for both Cav-1 and Npc1 (*Cav-1*$^{+/-}$:*Npc1*$^{+/-}$) were in turn used to produce mice of the four genotypes needed for study (*Cav-1*$^{+/+}$:*Npc1*$^{+/+}$, *Cav-1*$^{-/-}$:*Npc1*$^{+/+}$, *Cav-1*$^{+/+}$:*Npc1*$^{-/-}$, and *Cav-1*$^{-/-}$:*Npc1*$^{-/-}$). From a large number of litters we obtained just 5 double-knockouts, and 4 to 8 siblings of each of the other three genotypes needed for comparison to the *Cav-1*$^{-/-}$:*Npc1*$^{-/-}$ pups. All litters were weaned at 21 days onto a cereal-based rodent chow diet (Teklad 7001 Madison, WI). This formulation had an inherent cholesterol and crude fat content of 0.02 and 4% (wt/wt), respectively. In one study involving cholesterol-fed mice, the level of cholesterol in the diet was raised to 0.5% (wt/wt). All mice were group-housed in rooms with alternating 12-h periods of dark and light, and were studied in the fed state toward the end of the dark phase. Pups were genotyped at weaning by automated real-time quantitative PCR (Transnetyx, Inc., Cordova, TN). All experiments were approved by the Institutional Animal Care and Use Committee of the University of Texas Southwestern Medical Center.

**Table 1**
Primer sequences used for analyzing mouse mRNA expression in lung.

| Gene | Gene name | Accession # | Primer sequence (5′–3′) |
|---|---|---|---|
| *Cyclophilin* | Cyclophilin | M60456 | F: TGGAGAGCACCAAGACAGACA<br>R: TGCCGGAGTCGACAATGAT |
| *Abcg1* | ATP-binding cassette G1 | NM_009593 | F: GCTGTGCGTTTTGTGCTGTT<br>R: TGCAGCTCCAATCAGTAGTCCTAA |
| *Soat1*(*Acat1*) | Sterol O-acyltransferase 1 | NM_009230 | F: AGTATGCCCTCGCCATCTG<br>R: CCGACTGTCGTTAACAATGAAGT |
| *CD11C* | CD11c antigen; Integrin alpha X | NM_021334 | F: CTTCATTCTGAAGGGCAACCT<br>R: CACTCAGGAGCAACACCTTTTT |
| *CD68* | CD68 antigen | NM_009853 | F: CCTCCACCCTCGCCTAGTC<br>R: TTGGGTATAGGATTCGGATTTGA |
| *TNFa* | Tumor necrosis factor alpha | NM_013693 | F: CTGAGGTCAATCTGCCCAAGTAC<br>R: CTTCACAGAGCAATGACTCCAAAG |
| *Hmgcr* | Hydroxymethylglutaryl coenzyme A reductase | NM_008255 | F: CTTGTGGAATGCCTTGTGATTG<br>R: AGCCGAAGCAGCACATGAT |
| *Hmgcs* | Hydroxymethylglutaryl coenzyme A synthase | NM_145942 | F: GCCGTGAACTGGGTCGAA<br>R: GCATATATAGCAATGTCTCCTGCAA |
| *Ldlr* | Low density lipoprotein receptor | NM_010700 | F: GAGGAACTGGCCGCTGAA<br>R: GTGCTGGATGGGGAGGTCT |
| *SPA*(*Sfpa1*) | Surfactant associated protein A1 | NM_023134 | F: TCCAGGGTTTCCAGCTTACCT<br>R: GACAGCATGGATCCTTGCAAG |
| *SPD*(*Sfpd*) | Surfactant associated protein D | NM_009160 | F: GGACTCAAGGGGGACAGAG<br>R: AGCTTTCTGATAGTGGGAGAAGG |
| *Casp3* | Caspase 3 | NM_009810 | F: CATAAGAGCACTGGAATGTCATCTC<br>R: CCCATGAATGTCTCTCTGAGGTT |
| *Casp8* | Caspase 8 | NM_009812 | F: CCTGAGGGAAAGATGTCCTCAA<br>R: GTCGTCTTTATTGCTCACGTCATAG |