Role of Caveolin-1 in the Regulation of Lipoprotein Metabolism†

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Abbreviations: ABCA1, ATP-Binding Cassette transporter A1; apoA-I, apolipoprotein A-I; CE, cholesteryl ester; eNOS, endothelial nitric oxide synthase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SR-BI, scavenger receptor class B type I; VLDL, very low-density lipoprotein.
Abstract

Lipoprotein metabolism plays an important role in the development of several human diseases, including coronary artery disease and the metabolic syndrome. A good comprehension of the factors that regulate the metabolism of the various lipoproteins is therefore a key to a better understanding of the variables associated with the development of these diseases. Among the players identified are regulators such as caveolins and caveolae. Caveolae are small plasma membrane invaginations that are observed in terminally differentiated cells. Their most important protein marker, caveolin-1, has been shown to play a key role in the regulation of several cellular signaling pathways and in the regulation of plasma lipoprotein metabolism. In the present paper, we have examined the role of caveolin-1 in lipoprotein metabolism using caveolin-1 deficient [Cav-1(-/-)] mice. Our data show that, while Cav-1(-/-) mice show increased plasma triglyceride levels, they also display reduced hepatic VLDL secretion. Additionally, we also found that a caveolin-1 deficiency is associated with an increase in HDL and these HDL particles are enriched in cholesteryl ester in Cav-1(-/-) mice, when compared with HDL obtained from wild-type mice. Finally, our data suggest that a caveolin-1 deficiency prevents the transcytosis of LDL across endothelial cells and therefore, that caveolin-1 may be implicated in the regulation of plasma LDL levels. Taken together, our studies suggest that caveolin-1 plays an important role in the regulation of lipoprotein metabolism by controlling their plasma levels as well as their lipid composition. Thus, caveolin-1 may also play an important in the development of atherosclerosis.
**Introduction**

Caveolae are 50-100 nm cell surface plasma membrane invaginations that are found at the surface of terminally differentiated cells (31). They play important roles in the regulation of endocytosis, transcytosis, and cell signaling as well as cholesterol homeostasis. Caveolae are defined as plasma membrane domains, characterized by particularly high cholesterol and sphingolipid contents. The later properties are shared by caveolae and lipid raft. In fact, caveolae are a subset of lipid raft but differ from the latter in that they contain the protein caveolin. Several isoforms of caveolin have been identified (5). Caveolin-1 is the most common isoform, and its function has been extensively studied (31). The role of this protein has been more clearly characterized since the development of caveolin-1-deficient (Cav-1(-/-)) mice (8, 34). In particular, the role of caveolin-1 in breast and prostate cancer has been well documented (29, 39).

Caveolin-1 has also been suggested to play an important role in the regulation of plasma lipoprotein metabolism (11, 14). Cav-1(-/-) mice were shown to present increased plasma triglyceride levels compared to WT mice (33). Caveolin-1 has also been suggested to play a role in the regulation of HDL metabolism. In particular, our studies have shown that caveolin-1 can act as a negative regulator of SR-BI mediated HDL-CE uptake (17). These data are in agreement with the work of Matveev et al (24). Other studies, however, have indicated that caveolin-1 may not affect this pathway (42). Further work is therefore required to evaluate this point. Studies by Fielding et al have also proposed an important role for caveolae/caveolin-1 in the regulation of cellular cholesterol efflux to HDL. In this model, caveolae may be the primary site where cholesterol efflux occurs. However, we and others have been unsuccessful in finding corroborating evidences (12, 13, 19, 24, 42, 43).
In the present study, we have examined the role of caveolin-1 in the regulation of lipoprotein metabolism. For this purpose, we have used the well-characterized caveolin-1 deficient mouse model (34). Our data suggest a complex role for caveolin-1 in the regulation of lipoprotein and lipid metabolisms.
Experimental Procedures

Materials. Antibodies and their sources were as follows: anti-caveolin-1 IgG (mAb 2297; gift of Dr. Roberto Campos-Gonzalez, BD Biosciences, San Diego, CA) (36); rabbit anti-SR-BI and anti-ABCA1 (Novus Biological, Littleton, CO); rabbit anti-CD36, (Cayman Chemical, Ann Arbor, Mi); mouse anti-LRP clone 8B8 (Biodesign International, Saco, ME); guinea pig pAb anti-ADRP (Research Diagnostics, Inc., Flanders, NJ). All other reagents were analytical grade.

Animals. Caveolin-1 deficient (Cav-1(-/-)) mice have previously been described (34). All animals used in these studies were backcrossed at least 6 times in the C57Bl/6J genetic background and were genotyped by PCR, as previously described (34). Mice were kept on a 12-h light/dark cycle and, on a normal chow diet (LabDiet, Richmond, IN). All animal protocols used in this study were pre-approved by the Albert Einstein College of Medicine Institute for Animal Studies and Thomas Jefferson University. Experiments were performed in 3-month old male mice unless otherwise indicated.

Plasma Lipoprotein Analysis. Blood samples were collected into EDTA-containing tubes following a laparotomy and subsequent clipping of the descending aorta. Plasma (200 µL) was isolated and loaded onto one Superose 6 column (analytical grade, GE Healthcare Bio-Sciences Corp., Piscataway, NJ) to achieve a total bed volume of approximately 24 mL and void volume of 8 mL. Plasma was passed over the columns at a flow rate of 0.25 mL/min, and 0.5 mL fractions were collected. Total cholesterol and triglyceride content of each fraction was determined and plotted against elution volume [Wako Chemicals USA (Inc., Richmond, VA) and Sigma-Aldrich (St. Louis, MO) colorimetric kits, respectively].

Measurement of hepatic VLDL Production. The measurement of hepatic VLDL production was carried out after blocking VLDL catabolism with Triton WR-1339, an inhibitor of plasma TG
lipolysis (30). Baseline plasma TG levels were first determined. Mice were then injected with 15% Triton WR-1339 (Sigma-Aldrich, St. Louis, MO) in 0.9% NaCl solution (0.5g/kg body weight). Finally, blood samples were collected after injection and plasma TG levels were assayed.

**Liver Lipid Content Determination.** Livers from 3-month old WT and Cav-1(-/-) mice were collected after a 4-hour fasting period and snap-frozen in liquid N\textsubscript{2}. After solubilization of the tissue, tissue lipids were extracted by the method of Bligh and Dyer (2). TG and cholesterol concentrations were determined using colorimetric assays for cholesterol and TG [Wako Chemicals USA (Inc., Richmond, VA) and Sigma-Aldrich (St. Louis, MO) colorimetric kits, respectively].

**Western blot analysis of liver proteins.** Livers were harvested from 3-month old WT and Cav-1(-/-) mice and solubilized in lysis buffer, as previously described (17). Equivalent amounts of protein were then separated by SDS-PAGE and transferred to nitrocellulose. The expression levels of caveolin-1, ABCA1, LRP, CD36, and ADRP were assessed using specific antibodies.

**In Vitro $^{125}$I-LDL uptake by aortic rings.** Aortic segments from three wild-type and three Cav-1(-/-) mice (3-month old animals) were generated and cut into 2-4-mm segments and placed into 24-well plates. Three segments were used per time point and were incubated with 5 µCi of $^{125}$I-LDL (Biomedical Technologies, Inc., Stoughton, MA) in 0.5 mL of aerated serum-free DMEM for 15 min at 37°C. One set of aortic segments from both genotypes was also incubated at 4°C for 15 min. All samples were then washed four times in 0.2 M acetic acid and 0.5 M NaCl buffer, pH 2.5, to remove any $^{125}$I-LDL that remained attached to the cell surface. The amount of radioactivity was determined for each set of aortic segments individually using an LKB 1282
CompuGamma scintillation counter. The samples were then dried and weighed. The final value for each sample was obtained by dividing the cpm value by the dry weight.

*In Vivo LDL clearance and tissue uptake.* 100 µl of $^{125}$I-LDL (15 µCi diluted in a solution of 0.9% NaCl) was introduced into mice (3-month old Cav-1(-/-) and wild-type females) via tail vein injection. Blood samples were then collected from the tail at 2 and 45 min, and 2, 5, 7 and 24 h post-injection. Plasma was then isolated by centrifuging the blood at 6,000 rpm for 6 min at 4°C. The amount of TCA-precipitable radioactivity present in 10 µl of plasma was determined using a gamma-counter. The rate of clearance for each mouse was determined by using the number of cpm obtained from the 2-min time point as the starting point, i.e. 100%.

*Discontinuous Gradient Density Ultracentrifugation and Sample Analysis.* Plasma samples were subjected to discontinuous gradient density ultracentrifugation, as described (27). After ultracentrifugation, fractions (1 mL) were collected from the top to the bottom, yielding a total of 11 fractions. The densities of the fractions were determined and their lipid content was determined using colorimetric reagent [Wako Chemicals USA (Inc., Richmond, VA) and Sigma-Aldrich (St. Louis, MO) colorimetric kits].

*Statistical analyses.* Values are reported as the mean ± SEM. Comparisons between control and Cav-1(-/-) mice were performed using the Student $t$ test when appropriate.
Results

Lipoprotein metabolism in caveolin-1 deficient mice.

We examined plasma cholesterol, triglyceride, and lipoprotein distribution in WT and Cav-1(-/-) mice (Tables I and II, Figure 1). We found that plasma triglyceride and cholesterol levels were significantly increased in 3-month old Cav-1(-/-) mice (Table I). In addition, this increase in plasma triglyceride was due to an increase in plasma VLDL/CM levels (Figure 1A) in all cases. Moreover, very similar results were obtained in older animals (1-year old, see Figure 1B). We also observed that plasma HDL levels in 3-month old mice were higher in Cav-1(-/-) mice than in WT.

Effect of caveolin-1 deficiency on hepatic TGs production.

To further examine the regulation of plasma TG in Cav-1(-/-) mice, we examined the rate of liver VLDL-TG production in WT and Cav-1(-/-) mice. This experiment was performed by injecting mice with Triton WR-1339 (Figure 2). This compound has the property of inhibiting TG hydrolysis (3) and the accumulation of TG in the plasma of the injected mice will reflect the rate of VLDL production (mice are fasted for 4 hours before injection to prevent chylomicron secretion) (3). In WT and Cav-1(-/-) mice, the concentration of plasma TG rises linearly for 4 hrs after the initial injection of the compound. Interestingly, despite higher plasma TG levels at the time of injection, Cav-1(-/-) mice displayed reduced plasma TG levels 4 hours after injection. These data suggest that Cav-1(-/-) mice present reduced VLDL production compared with WT animals.
Caveolin-1 deficiency is associated with reduced hepatic triglyceride but increased hepatic cholesterol content.

The liver plays an important role in the regulation of lipoprotein and lipid metabolisms. As a consequence, liver lipid metabolism is often altered in cases of dyslipoproteinemia. Therefore, we decided to examine liver TG and cholesterol content. We found that liver TG and total cholesterol content were altered in caveolin-1 deficient mice. However, while liver TG content was reduced in Cav-1(-/-) mice, liver total cholesterol content was increased (Figure 3). These findings suggest that liver TG (decreased by 35%) and cholesterol (increased by 30%) metabolism are affected differently in Cav-1(-/-) mice. In addition, these data are also consistent with a role for caveolin-1 in the regulation of cellular TG and cholesterol metabolism.

Expression of key hepatic proteins involved in lipoprotein metabolism in Cav-1(-/-) and WT mice.

An important regulator of plasma cholesterol levels is the ATP-Binding Cassette transporter A1 (ABCA1). A deficiency in ABCA1 (in animal models or natural mutations found in Tangier’s disease) leads to markedly reduced plasma HDL cholesterol levels. We show that liver ABCA1 protein levels were increased in Cav1(-/-) mice (Figure 4). This modification in ABCA1 protein levels may be associated with the increase in liver cholesterol content (Figure 3) since previous studies have shown a positive correlation between cellular cholesterol levels and ABCA1 protein expression levels (21).

We have also examined other proteins involved in the regulation of TG and apoB-containing lipoproteins. Earlier studies have shown that caveolin-1 deficient mouse embryonic fibroblasts cannot accumulate large amounts of TG (6). Two main proteins are responsible for the formation
of a lipid droplet within a cell and therefore play an important role in the accumulation of cellular TG. ADRP (or adipophilin) is expressed in most tissues, including the liver (4, 22). Perilipin is the adipose tissue specific isoform. We now show that, in caveolin-1 deficient mice, liver ADRP protein levels are 50% reduced compared to wild-type animals (Figure 4). This finding is in agreement with previous studies showing a correlation between ADRP protein levels and triglyceride levels in hepatocytes (23). This reduction is consistent with the reduced liver TG content observed in Cav-1(-/-) mice. Conversely, we also find that the levels of two proteins involved in the uptake of remnant lipoproteins are increased. These proteins are the LDL-related protein receptor (LRP) and CD36 (Figure 4). Interestingly, these two proteins have been localized into caveolae. Their mis-localization in Cav-1(-/-) hepatocytes and other cell types may affect lipoprotein uptake and, therefore, affect lipoprotein and TG metabolisms. LRP is an important receptor for LDL and remnant lipoproteins, and its increased expression levels may be associated with increased hepatic uptake of these lipoproteins. Moreover, as caveolin-1 and CD36 have been suggested to play a role in the intracellular transport of fatty acids, caveolin-1 deficiency appears to be associated with altered metabolism of cellular fatty acids that may indirectly affect plasma lipoprotein levels. In fact, plasma levels of NEFA were found to be increased in the postprandial state in Cav-1(-/-) mice (33).

Caveolin-1 deficient mice show defects in the aortic uptake of LDL particles, both in vitro and in vivo.

Many electron microscopy studies have now established that endothelial caveolae (a.k.a., plasmalemmal vesicles) are involved in the transcytosis of macromolecules, such as albumin, LDL, and oxidized LDL, from the blood vessel lumen to the sub-endothelial space (18, 20, 41).
In support of this notion, we recently demonstrated that caveolin-1 deficient endothelial cells are indeed defective in the uptake and transport of serum albumin (37). However, it remains unknown whether the uptake and transport of LDL is affected in Cav-1(-/-) null mice. To test this hypothesis directly, we examined the ability of isolated aortic segments to take up $^{125}$I-LDL at 37°C in vitro (Figure 5). Our results indicate that loss of caveolin-1 reduces $^{125}$I-LDL uptake by ~ 45-50 %. In contrast, in vitro binding of $^{125}$I-LDL at 4°C was not affected.

To establish the in vivo relevance of these findings, we next evaluated the in vivo clearance of $^{125}$I-LDL in caveolin-1 deficient and wild-type control female mice, using tail vein injections (Figure 6A). Interestingly, Cav-1(-/-) mice exhibited a reduced initial rate of clearance, as compared with wild-type control animals (See the 45 min time point; % initial plasma value; 9.18 % ± 0.98 vs. 18.53 % ± 5.81). However, at 2 hours post-injection, no significant differences in $^{125}$I-LDL plasma levels were noted.

Twenty-four hours post-injection, Cav-1(-/-) null and wild-type mice were sacrificed and the tissue distribution of $^{125}$I-LDL was determined. Figure 6B shows the distribution of $^{125}$I-LDL in the various tissues examined. Note that in the aortas of Cav-1(-/-) null mice, the uptake of $^{125}$I-LDL was reduced by > 50 % (cpm/g; 331,351.9 ± 128,763.6 vs. 155,525.1 ± 31,089.4). In contrast, the livers of Cav-1(-/-) null mice showed a > 30 % increase in the uptake of $^{125}$I-LDL (cpm/g; 1,481,041.2 ± 440,051.3 vs. 2,075,403.8 ± 272,991.9). However, no significant differences were noted in the other tissues examined.
Caveolin-1 deficiency is associated with altered lipoprotein composition.

We also present data indicating that plasma obtained from Cav-1(-/-) mice shows increased HDL-cholesterol levels as observed after separation of lipoproteins by gradient density ultracentrifugation (Figure 7A). In addition, we demonstrate that these HDL particles are enriched in esterified cholesterol (Figure 7B) and to a lesser extent in free cholesterol (Figure 7C). However, the ratio phospholipids/total cholesterol (Figure 7D) is not affected in caveolin-1 deficient mice. These data suggest a defect in the metabolism of plasma HDL in Cav-1(-/-) mice and that this defect does not appear to be due to reduced cholesterol efflux from peripheral cells, as we have shown previously. Rather, this effect may be due to a defect in HDL catabolism, presumably in the liver.
Discussion

The present study was performed to determine the role of caveolin-1 in the regulation of lipoprotein metabolism. Our data show that caveolin-1 controls both production and degradation of plasma triglyceride. As a consequence, caveolin-1 directly regulates hepatic lipid metabolism. Additionally, we show that caveolin-1 is involved in the regulation of plasma cholesterol metabolism. In agreement with its role in the regulation of SR-BI function, we now show that a caveolin-1 deficiency is associated with altered cholesteryl ester metabolism.

Role of caveolin-1 in the regulation of plasma triglyceride metabolism.

Our data demonstrate that the increase in plasma TG levels observed in Cav-1(-/-) mice is not due to an increase in VLDL production, but rather due to reduced degradation of plasma TG. Since post-heparin lipoprotein lipase and hepatic lipase activity are not affected in Cav-1(-/-) mice (33), it follows that the tissue uptake and catabolism of non-esterified fatty acids (NEFA) is reduced in these animals. This finding is in agreement with the increased plasma NEFA levels observed in the postprandial state of Cav-1(-/-) mice (33). Concerning the catabolism of VLDL, our results suggest a reduction in VLDL degradation in caveolin-1 deficient mice. One possible mechanism by which caveolin-1 may regulate VLDL degradation might be via its ability to regulate insulin signaling in adipose tissue. Early studies have shown that caveolin-1 can act as an activator of insulin receptor signaling (44). Alterations in the regulation of the insulin signaling pathway in adipose tissue have indeed been demonstrated in caveolin-1 deficient mice. In fact, caveolin-1 deficient mice have recently been shown to be unresponsive to insulin when compared to wild-type mice (7). For the present work, this observation is important since it is well documented that insulin can regulate LPL activity and its release from adipocytes (32, 40). However, in our previous studies, we did not observe a reduced lipoprotein lipase (LPL) activity
in caveolin-1 deficient mice (33). In addition, increased levels of non-esterified fatty acids were also observed in the postprandial state in caveolin-1 deficient mice (33). Taken together, these results suggest that caveolin-1 deficiency may be associated with a reduced fatty acid uptake by adipose tissue. In support of this hypothesis, Ring et al have shown that caveolin-1 regulates the function of CD36, a fatty acid translocase (35). Overall, these findings suggest that caveolin-1 must play a key role in the regulation of fatty acid metabolism.

Caveolin and liver lipid metabolism

We show that the rate of VLDL production is reduced in Cav-1(-/-) mice (compare slopes for the increase in plasma TG in Figure 2). We also show that liver TG levels are decreased in Cav-1(-/-) mice. Accordingly, this decrease is associated with reduced ADRP protein levels. Since VLDL secretion is dependent on the availability of hepatocyte TG (38), we can hypothesize that the decrease in cellular TG levels in Cav-1(-/-) hepatocytes leads to reduced VLDL production. This finding is reminiscent of the one that was made for adipose tissue in Cav-1(-/-) mice (33) and suggest that these mice have a defective ability to store TG in the liver and adipose tissue.

Our results also suggest that liver TG (decreased by 35%) and cholesterol (increased by 30%) metabolisms are differently regulated in Cav-1(-/-) mice. These findings are consistent with a role for caveolin-1 in the regulation of cellular TG and cholesterol metabolism, as previously suggested (12, 33). We have already shown that caveolin-1 deficiency is associated with reduced accumulation of cholesteryl ester in mouse embryonic fibroblasts and in macrophages (12). In this previous paper, we found that caveolin-1 is an important regulator of intracellular cholesterol metabolism, and the present study confirms this finding. This result may have important consequences as cellular cholesterol regulates important cellular signaling pathways and could have important impact on the development of various diseases. In addition, increase
hepatic cholesterol levels may also be associated with the increased ABCA1 that we observed in the liver. As a consequence, a small increase in plasma HDL levels was observed in caveolin-1 deficient mice.

Liver regeneration studies have suggested an important role for caveolin-1 in the process (10, 15, 26). It was shown that caveolin-1 expression is upregulated after partial hepatectomy, and it participates in the regulation of fatty acid metabolism in hepatocytes, where it associates with lipid droplets. Therefore, the decreased triglyceride levels observed in caveolin-1 deficient liver is consistent with these findings. The increased expression of CD36 that we observed in Cav-1(-/-) liver may be a compensatory mechanism to improve defective fatty acid uptake by this organ (9). In this model, CD36 may not be functional, as previous studies have shown that caveolin-1 is required for CD36 function using mouse embryonic fibroblasts (35). In agreement with this interpretation, CD36(-/-) and Cav-1(-/-) mice present very similar lipoprotein profiles and metabolisms (9). It is also important to note that CD36 deficiency has been associated with insulin resistance, defective fatty acid metabolism, and hypertriglyceridemia (1).

**Caveolin-1 and Atherosclerosis**

How does loss of caveolae and caveolin-1 protect against the development of atherosclerosis? One possibility is that loss of endothelial cell caveolae prevents the transcytotic movement of LDL particles from the blood to the sub-endothelial space, where they become trapped and accumulate. Here, we show that caveolin-1 deficiency is associated with a change in the clearance and the metabolism of LDL lipoproteins. This finding is important, as the transcytosis of LDL plays a critical role in the development of atherosclerosis. In fact, the transcytosis of LDL may lead to the accumulation of lipids in blood vessels and may accelerate the inflammation process that is a key step in the development of atherosclerosis. We have
previously shown that, in the apolipoprotein E deficient (apoE(-/-)) background, caveolin-1 deficient mice presented remarkable increases in the level of plasma apoB-containing lipoproteins. Our current data suggest that the clearance of LDL is reduced in Cav-1(-/-) mice, and, as a consequence, it may prevent the development of atherosclerosis in apoE(-/-) mice.

Taken together, our data indicate that caveolin-1 deficiency leads to reduced aortic uptake or accumulation of pro-atherogenic lipoproteins, such as LDL. This is consistent with the notion that endothelial caveolae normally transport LDL particles from the blood vessel lumen to the sub-endothelial space.

**Role of Caveolin-1 in the regulation of HDL metabolism**

Several studies have now suggested that caveolin-1 may regulate plasma HDL-cholesterol metabolism (17, 28). In fact, caveolin-1 may be involved in the regulation of key proteins implicated in lipoprotein metabolism. Some of the best targets include SR-BI. We and others have shown that caveolin-1 may negatively regulate selective HDL-CE uptake mediated by SR-BI (16, 24). While some authors have suggested that caveolin-1 may facilitate this process (25), others have proposed that it had no effect (42). We have shown that Cav-1(-/-) mice present increased plasma levels of HDL-CE and therefore, caveolin-1 may facilitate, on the whole, the uptake of HDL-CE. We cannot rule out that caveolin-1 may have different effect depending on the cellular context. However, the observed increase in plasma HDL-chol levels may also be related to the increased hepatic ABCA1 expression that is observed in Cav-1(-/-) mice (Figure 4).

Taken together, our data suggest that caveolin-1 plays an important role in the regulation of triglyceride and cholesterol homeostasis, as well as in the regulation of lipoprotein metabolism.
(Figure 8). In adipose tissue, it promotes triglyceride storage but also its mobilization. In blood vessels and possibly in atherosclerotic lesions, it may promote cholesterol accumulation via LDL transcytosis across endothelial cells. The latter property may account for the pro-atherogenic properties of caveolin-1. Concerning the metabolism of lipoprotein, caveolin-1 has a direct impact on the regulation of VLDL production and is also involved in the regulation of plasma HDL levels.


Table I. Fasting plasma cholesterol and triglyceride levels observed in Cav-1(-/-) and Cav-1(+/+) animals fed a chow diet.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Plasma Cholesterol (mg/dL)</th>
<th>Plasma TGs (mg/dL)</th>
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<tbody>
<tr>
<td>Cav-1(+/-)</td>
<td>106.0 ± 8.2</td>
<td>175.0 ± 44.6</td>
</tr>
<tr>
<td>Cav-1(-/-)</td>
<td>139.7 ± 20.9*</td>
<td>282.8 ± 90.1*</td>
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Values are the average ± the standard deviation. An asterisk (*) indicates a significant difference, as compared with control animals (P<0.05). n=5 animals for each experimental group.
Table II: Fasting plasma cholesterol and triglyceride levels observed in 1 year-old Cav-1(-/-) and Cav-1(+/+) animals.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Plasma Cholesterol (mg/dL)</th>
<th>Plasma TGs (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cav-1(+/+)</td>
<td>182 ± 23</td>
<td>117 ± 11</td>
</tr>
<tr>
<td>Cav-1(-/-)</td>
<td>181 ± 9</td>
<td>139 ± 19</td>
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</table>

Values are the average ± the standard deviation. n=3 animals for each experimental group.
Figures

Figure 1: **Lipoprotein profile observed in caveolin-1 deficient mice.** Mice fed a chow or Paigen diet. Fasting plasma samples isolated from 3 mice from each group (Wt, ■, and Cav-1(-/-), □) were pooled and loaded atop one Superose 6 column. Fractions were then collected and analyzed for cholesterol content. Profiles shown were obtained 3-month old (A) or 1-year old (B) animals.

Figure 2: **Effect of caveolin-1 deficiency on hepatic TGs production.** The measurement of hepatic VLDL production was carried out after blocking VLDL catabolism with Triton WR1339, an inhibitor of plasma TG lipolysis (30). Baseline murine plasma TG were first determined. Mice were then injected with 15% Triton WR-1339 (Sigma-Aldrich, St. Louis, MO) in 0.9% NaCl solution (0.5g/kg body weight). Blood samples were collected after injection and plasma TG were assayed. * Linear regression was performed and an F-test was used to compare slope values, which were found to be significantly different (p<0.001).

Figure 3: **Liver lipid content observed in 3-month old male wild-type and Cav-1(-/-) mice.** Livers were collected after a 4-hour fasting period and snap-frozen in liquid N2. After solubilization of the tissue, tissue lipids were extracted by the method of Bligh and Dyer (2). TG and cholesterol concentrations were determined using colorimetric cholesterol and TG assays. An asterisk (*) denotes statistical significance (P < 0.05) and n=5 for each group of animals.

Figure 4: **Expression of key hepatic proteins involved in lipoprotein metabolism in Cav-1(-/-) and WT mice.** Livers were harvested from 3-month old male wild-type and Cav-1(-/-) mice and solubilized. Equivalent amounts of total protein were then separated by SDS-PAGE and transferred to nitrocellulose. The expression levels of caveolin-1, ABCA1, LRP, CD36, and ADRP were assessed using specific antibodies. Results with two representative animals are shown for each genotype (3-month old male Cav-1(-/-) and WT mice).

Figure 5: **Cav-1(-/-) deficient mice show defects in the aortic uptake of LDL particles in vitro.** Aortic ring segments were collected from Cav-1(-/-) and WT control mice and incubated with [125I]-LDL for a period of 15 minutes at either 37ºC (Left, for internalization) or 4ºC (Right, for binding only). Note that despite normal binding activity, caveolin-1 deficient aortic segments cannot internalize [125I]-LDL as efficiently as aortic rings obtained from WT control animals. Thus, our results indicate that loss of caveolin-1 reduces [125I]-LDL uptake by ~45-50 %. An asterisk (*) denotes statistical significance (P < 0.05).

Figure 6: **Cav-1(-/-) deficient mice show defects in the aortic uptake of LDL particles in vivo.** A. LDL clearance in vivo. [125I]-LDL was injected into the tail veins of 3-month old female mice (WT vs. Cav-1(-/-) animals). Note that Cav-1(-/-) mice exhibited a reduced initial rate of clearance, as compared with WT control animals (See asterisk at 45 min time point; % initial plasma value; 9.18 % ± 0.98 vs. 18.53 % ± 5.81). However, at 2 hours post-injection, no significant difference in [125I]-LDL plasma levels was noted. B. LDL uptake in vivo. Twenty-four hours post-injection, WT and Cav-1(-/-) mice were sacrificed, and the tissue distribution of [125I]-LDL was determined. Note that in the aortas of Cav-1(-/-) the uptake of [125I]-LDL was reduced by > 50 % (cpm/g; 331,351.9 ± 128,763.6 vs. 155,525.1 ± 31,089.4; See also the inset at right). These data indicate that a caveolin-1 deficiency leads to reduced aortic uptake or
accumulation of pro-atherogenic lipoproteins, such as LDL. In contrast, the livers of Cav-1(-/-) mice showed an increase in the uptake of [125I]-LDL (cpm/g; 1,481,041.2 ± 440,051.3 vs. 2,075,403.8 ± 272,991.9). No significant difference was noted in the other tissues examined.

Figure 7: Lipid content of plasma lipoproteins isolated by gradient density ultracentrifugation. Fasting plasma samples were isolated from five wild-type and Cav-1(-/-) mice (2-month old male) and applied to discontinuous gradient density ultracentrifugation as described by McManus et al. (27). After ultracentrifugation, fractions (1 ml) were collected from the top to the bottom of the gradient, yielding a total of 12 fractions. The lipid content of each fraction was determined and plotted as a function of the fraction number. HDL-containing fractions were identified as fractions 5 and 6 (characterized by their density and apolipoprotein A-I content; asterisk [*]). Note that for plasma obtained from Cav-1(-/-) mice, the HDL fraction was especially enriched in esterified cholesterol (see panel B).

Figure 8: Role of caveolin-1 in the regulation of lipid and lipoprotein metabolism. Our results and those of others suggest that caveolin-1 plays various functions in different organs. In adipose tissue, it promotes triglyceride (TG) storage but also its mobilization. In blood vessels and possibly in atherosclerotic lesions, it may promote cholesterol accumulation via LDL transcytosis across endothelial cells. The latter property may account for the pro-atherogenic properties of caveolin-1. Concerning the metabolism of lipoprotein, caveolin-1 has a direct impact on the regulation of VLDL production and is also involved in the regulation of plasma HDL levels.
Lipoprotein Profile of 3 month-old

![Graph showing lipoprotein profile with elution volume (ml) on the x-axis and cholesterol/fraction (µg/fraction) on the y-axis. The graph compares WT and Cav-1(-/-) groups. VLDL, IDL/LDL, and HDL are indicated.](image)
Lipoprotein Profile of 13 month Mice

Elution Volume (ml)

Cholesterol/Fraction (µg/fraction)

WT
Cav-1(-/-)

Lipoprotein Profile:
- VLDL
- IDL/LDL
- HDL
Figure 2, Frank et al.

Plasma TG after Triton WR-1339 injection

WT

Cav-1(-/-) *

Plasma TG (mg/ml)

Time (min)
Figure 3, Frank et al.

Liver Lipid Content

- WT
- Cav-1(-/-)

Liver Triglyceride Content
Liver Total Cholesterol Content

Lipid (µg/mg of protein)
Western Blot Analysis of the Liver

Cav-1  

+/-  -/-

ABCA1
LRP
CD36
ADRP
Caveolin-1
Figure 5, Frank et al.

**LDL Uptake--Aortic Segments *In Vitro***

![Graph showing LDL uptake at 37°C and 4°C for Cav-1 genotypes (+/+ and -/-). The graph indicates a significant difference at 37°C for Cav-1 -/- genotype compared to +/+ genotype.](image)
Figure 6, Frank et al.

B

**LDL Tissue Uptake--*In Vivo***

\[ \text{125I-LDL uptake (cpm/g of tissue)} \]

- **WT**
- **Cav-1(-/-)**

- Lung
- Heart
- Muscle
- Kidney
- Liver
- Adipose Tissue
- Aorta

* indicates statistically significant difference.
A. Cholesterol content of lipoprotein fractions after ultracentrifugation

B. Esterified Cholesterol content of lipoprotein fractions after ultracentrifugation

C. Free Cholesterol content of lipoprotein fractions after ultracentrifugation

D. Total Cholesterol/phospholipid ratio of lipoprotein fractions after ultracentrifugation

Figure 7, Frank et al.
Figure 8, Frank et al.

Caveolin-1

\[ \downarrow \]

Lipid homeostasis

\[ \downarrow \]

Adipose Tissue

\[ \downarrow \]

TG storage

\[ \downarrow \]

TG mobilization

\[ \downarrow \]

Blood Vessels

\[ \downarrow \]

Cholesterol Accumulation

\[ \downarrow \]

Liver

\[ \downarrow \]

VLDL production

\[ \downarrow \]

HDL levels