Caveolin-1-deficient Mice Are Lean, Resistant to Diet-induced Obesity, and Show Hypertriglyceridemia with Adipocyte Abnormalities*

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Babak Razani, Terry P. Combs, Xiao Bo Wang, Philippe G. Frank, Robert G. Russell, Maomi Li, Baiyu Tang, Linda A. Jelicks, Philipp E. Scherer, and Michael P. Lisanti

From the Departments of Molecular Pharmacology, Cell Biology, Pathology, and Physiology and Biophysics, The Albert Einstein Cancer Center, and The Institute for Animal Studies, Albert Einstein College of Medicine, Bronx, New York 10461

Caveolae organelles and caveolin-1 protein expression are most abundant in adipocytes and endothelial cells. Our initial report on mice lacking caveolin-1 (Cav-1) demonstrated a loss of caveolae and perturbations in endothelial cell function. More recently, however, observation of the Cav-1-deficient cohorts into old age revealed significantly lower body weights, as compared with wild-type controls. These results suggest that Cav-1 null mice may have problems with lipid metabolism and/or adipocyte functioning. To test this hypothesis directly, we placed a cohort of wild-type and Cav-1 null mice on a high fat diet. Interestingly, despite being hyperphagic, Cav-1 null mice show overt resistance to diet-induced obesity. As predicted, adipocytes from Cav-1 null null mice lack caveolae membranes. Early on, a lack of caveolin-1 selectively affects only the female mammary gland fat pad and results in a near complete ablation of the hypo-dermal fat layer. There are also indications of generalized adipose tissue pathology. With increasing age, a systemic decompensation in lipid accumulation occurs resulting in dramatically smaller fat pads, histologically reduced adipocyte cell diameter, and a poorly differentiated/hypercellular white adipose parenchyma. To gain mechanistic insights into this phenotype, we show that, although serum insulin, glucose, and cholesterol levels are entirely normal, Cav-1 null mice have severely elevated triglyceride and free fatty acid levels, especially in the post-prandial state. However, this build-up of triglyceride-rich chylomicrons/very low density lipoproteins is not due to perturbed lipoprotein lipase activity, a major culprit of isolated hypertriglyceridemia. The lean body phenotype and metabolic defects observed in Cav-1 null mice are consistent with the previously proposed functions of caveolin-1 and caveolae in adipocytes. Our results show for the first time a clear role for caveolins in systemic lipid homeostasis in vivo and place caveolin-1/caveolae as major factors in hyperlipidemias and obesity.

Uniform 50- to 100-nm invaginations of the plasma membrane called caveolae remain one of the most intriguing and enigmatic organelles in the cell. As early as the 1950s, at the inception of ultrastructural cell biology, caveolae were readily observable and morphologically distinct organelles described on the surface of epithelial/endothelial cells (1, 2). Although a profile of the tissue distribution of caveolae has never been reported, a compilation of various reports to date arrives at one main conclusion: two tissue types have an extremely high abundance of these structures in vivo, adipose tissue (due to the adipocytes) and lung tissue (due to endothelial cells and type I pneumocytes).

Based purely on ultrastructural comparisons, the adipocyte seems to have higher concentrations of caveolae than any other cell. Indeed, electron micrographs of adipocytes dating back to 1963 show that caveolae account for ~30% of the surface area of the adipocyte plasma membrane (3, 4). Furthermore, in 3T3-L1 cells, a widely used model system for studying adipogenesis, the number of caveolae increases ~10-fold during adipocyte differentiation as compared with the undifferentiated fibroblastic state (4).

An important advance in the study of caveolae was the discovery that caveolin-1 (Cav-1)1 is a marker protein for caveolae organelles and that Cav-1 plays an intrinsic role in caveolar functioning (5). Research in the past decade has shown that caveolae are specialized membrane microdomains formed as a result of the localized accumulation of cholesterol, glycosphingolipids, and caveolin-1 (6–8). Caveolin-1, an integral membrane protein that can directly bind cholesterol, most likely plays a major role in the invagination of caveolae from the plasma membrane proper, but the mechanisms underlying this process remain unknown. Although the function of caveolae and caveolins in vivo remains controversial, they have been

1 The abbreviations used are: Cav-1, caveolin-1; mAb, monoclonal antibody; MRI, magnetic resonance imaging; RIA, radioimmunoassay; WAT, white adipose tissue; M4/subQ, mammary gland 4/subcutaneous; VLDL, very low density lipoprotein; LDL, lipoprotein lipase; BAT, brown adipose tissue; PKA, protein kinase A; ER, endoplasmic reticulum.

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implicated in endocytosis/transcytosis, cholesterol transport/efflux, regulation of signal transduction, and tumorigenesis (reviewed in Ref. 9).

True to its description as a “marker of caveolae,” examination of caveolin-1 transcripts and protein levels in a panel of mouse tissues reveals that the two highest expressing tissues are adipose tissue and lung tissue (an endothelial-rich organ) (10). Also, concomitant with a ~10-fold increased number of caveolae in fully differentiated 3T3-L1 adipocytes, the levels of caveolin-1 mRNA and protein expression increase ~20-fold during differentiation from the fibroblastic to the adipocyte phenotype (10).

There are possible functional consequences for such high caveolin expression in adipocytes. Photoaffinity labeling has identified caveolin-1 as a major plasma membrane fatty-acid binding protein in adipocytes (11, 12). Furthermore, caveolin-1 moves from the plasma membrane to lipid droplets in response to free fatty acids (13, 14). As such, caveolin-1 is the first known integral membrane protein component of lipid droplets. Based on these studies, it has been proposed that caveolin-1 functions in the transport and/or storage of free fatty acids/triglycerides in lipid droplets. However, no functional data has been presented to support this hypothesis.

We and others have recently reported the generation and initial characterization of mice with a disrupted Cav-1 locus. Detailed analysis of this phenotype with the intriguing finding that a deficiency in caveolin-1 causes a gradual decompensation in several adipose tissues and imparts resistance to diet-induced obesity. Furthermore, we find that these mice have de-ranged metabolism of triglycerides and free fatty acids, thereby implicating caveolae and caveolins for the first time as important factors in lipid homeostasis and obesity.

**EXPERIMENTAL PROCEDURES**

**Materials—**Antibodies and their sources were as follows: anti-caveolin-1 mAb 2297 and anti-caveolin-3 mAb 26 (17-19) (gifts of Dr. Roberto Campos-Gonzalez, BD Transduction Laboratories, Inc.); anti-β-tubulin TUB-2.1 (Sigma Chemical Co.).

**Generation and Maintenance of Cav-1-deficient Mice—**The strategy used to target the caveolin-1 locus and generate Cav-1 null mice was as previously described (15). All animals used in these studies (mice homozygous null for the caveolin-1 gene and their wild-type littermates) were of a C57BL/6 × C3H/129 genetic background and were genotyped by PCR, as previously described (15). Housing and maintenance was provided by the Albert Einstein College of Medicine barrier facility; mice were kept on a 12-h light/dark cycle and, except where noted, had ad libitum access to Chow (Picolab 20, PMI Nutrition International) and water. All animal protocols used in this study were pre-approved by the Albert Einstein College of Medicine Institute for Animal Studies.

**Immunoblot Analysis—**Freshly dissected tissue samples were washed thoroughly with phosphate-buffered saline and either snap-frozen in liquid N2 or immediately homogenized with lysis buffer (10 mM Tris, pH 7.5, 50 mM NaCl; 1% Triton X-100; 60 mM octyl glucoside) containing protease inhibitors (Roche Molecular Biochemicals). Protein concentrations were quantified using the BCA reagent (Pierce), and the volume required to fill the room’s dye chamber (500 μl) was determined. Samples were separated by SDS-PAGE (12.5% acrylamide) and transferred to nitrocellulose. The nitrocellulose membranes were stained with Ponceau S (to visualize protein bands) followed by immunoblot analysis. All subsequent wash buffers contained 10 mM Tris, pH 8.0, 150 mM NaCl, 0.05% Tween 20, which was supplemented with 1% bovine serum albumin and 2% nonfat dry milk (Carnation) for the blocking solution and 1% bovine serum albumin for the antibody diluent. Primary antibodies were used at a 1:500 dilution. Horseradish peroxidase-conjugated secondary antibodies (1:5000 dilution, Pierce) were used to visualize bound primary antibodies with the Supersignal chemiluminescence substrate (Pierce).

**Transmission Electron Microscopy—**Adipose tissue samples (derived from subcutaneous, peri-epidymal, and perirenal fat depots) were minced with a razor blade into ~1 mm × 1 cm-long strips fixed with 2.5% glutaraldehyde/0.1 M cacodylate, post-fixed with OsO4, and stained with uranyl acetate and lead citrate. A cryotome was used to yield sections of 1-μm thickness, and the samples were examined under a LEICA transmission electron microscope and photographed at ×16,000 magnification (20). Caveolae were identified by their characteristic flask shape, size (50–100 nm), and location at or near the plasma membrane.

**Diet-induced Obesity Study—**A cohort of mice (composed of Cav-1(+/−) and Cav-1(−/−) mice) was generated by heterozygous interbreedings, some of which were placed on a high fat diet (59% of calories derived from fat, Research Diets, D12492), while others were placed on the equivalent chow diet (10% of calories derived from fat, Research Diets, D12450B) (see Fig. 2A). The diet study was begun upon weaning (3 weeks of age) and continued up to 45 weeks of age.

**Magnetic Resonance Imaging and Spectroscopy—**All images were obtained using a 4.4-Tesla magnet (GE Omega vertical wide bore system). Mice were first anesthetized by intraperitoneal injection with a ketamine/xylazine mixture (0.1 ml per 20 g of body weight). To quantitatively assess whole-body fat and water, each mouse was subjected to a 16-scan pulse-acquire sequence in a 40-mm 1H coil, and spectra, including the water and fat peaks, were obtained. For imaging, eight slices of 2-mm thickness spanning the whole body were obtained. Imaging were conducted using a 35-mm 1H coil and a routine spin-echo pulse sequence (18-ms echo time, 600-ms repetition time, and 4-signal averaging per scan).

**Fat Pad Measurements and Histology—**The indicated Cav-1(+/−) or (−/−) mice cohorts were sacrificed at an early age (3 months, when there were insignificant weight differences between wild-type and knockout mice) or at an older age (9 months, when diet-induced weight differences were statistically significant). Several tissues, including the significant white adipose depots (subcutaneous/mammary gland 4, perirenal/peri-epidymal, perirenal/retroperitoneal), the scapular brown adipose depots (inter-scapular and sub-scapular), and the liver were dissected and weighed. For routine histology, similar areas from all tissues were chosen, formalin-fixed, paraffin-embedded, sectioned, and stained with hematoxylin and eosin. All photographs were taken with a Zeiss digital imaging system.

**Metabolic Chamber Studies—**VO2, VCO2, body heat, and movements were measured from mice housed individually in Oxymax metabolic chambers (Columbus Instruments) with an air flow of 0.65 liter/min. Measurements were made every 12 min for a 24-h period. Total VO2 (ml/kg/h), VCO2 (ml/kg/h), and body heat (kcal/h) are the mean of all measurements made during the 24-h period. Total movements are a tabulation of the number of times the motion sensors detected movement during the 24-h period. Resting VO2, VCO2, and body heat are the mean of all measurements made during times when mouse movement was limited to ~20 per each 12-min measuring period. Respiratory quotient is the ratio of VCO2 to VO2.

**Analysis of Food Intake and Food Absorption—**Mice were placed in individual cages with ad libitum access to both food and water. Food weight was measured, and stool samples were collected daily for a period of 8 days. To collect fresh stool samples for analysis, food, cage beddings were changed daily. The content of the collected stools was determined as follows: samples (25 mg from each mouse) were ground to a powder form by mortar and pestle and extracted three times with absolute ethanol at 90 °C under reflux. The extracts were dried under a stream of N2 and resuspended in 50% ethanol, and triglyceride content was measured colorimetrically (Sigma).

**Analysis of Plasma Metabolites and Lipoproteins—**Mouse plasma was drawn from the tail vein and decanted directly into heparinized capillary tubes (Fisher Scientific). Where indicated, fasting blood samples were collected at 7:00 a.m., 12 h after removal of the food, and post-prandial blood samples were collected at 12:00 a.m, 3 h after the beginning of the room’s dye cycle (500 μl). Glucose, cholesterol, triglyceride, and free fatty acid levels were measured with standard enzymatic colorimetric assays (Sigma and Wako Biochemicals). Insulin and leptin levels were determined by radioimmunoassay (RIA) (Linco Research), whereas ACRP30 levels were determined by quantitative immunoblotting with an anti-ACRP30 pAb, as we described previously (21). For lipoprotein fractionation, plasma samples from 10 mice of the same
genotype (150 µl of total plasma) were separated by gel filtration chromatography using two Superose 6HR 10/30 columns in tandem (Amersham Biosciences). A series of 0.5-ml fractions corresponding to the migration of known lipoproteins were collected and subjected to the colorimetric cholesterol and triglyceride assays indicated above.

Oral Fat Tolerance Test—Weight-matched mice were fasted for 8 h before being gavaged with 0.5 ml of olive oil. Blood was collected via the tail vein at baseline and for the indicated times. Due to a rapid initial rise in plasma triglycerides and a slower clearance, blood collection frequency was begun at every 20 min and was gradually tapered over the 24-h time course. Triglycerides were measured with a colorimetric assay (Sigma).

Measurement of Hepatic and Lipoprotein Lipase Activity—Lipase activity was determined essentially as previously described, with minor modifications (22). Heparin (1.5 units/g, Sigma) was injected by tail vein, and blood was collected 5 min later from the retro-orbital plexus (control wild-type and knockout mice were injected with saline). A triglyceride emulsion composed of 3H-labeled triolein (PerkinElmer Life Sciences) and cold triolein (Sigma) was prepared, as previously described (23). Equal protein loading was assessed using the anti-β-tubulin mAb (clone 2297). Equal protein loading was assessed using the anti-β-tubulin mAb (clone 2297). Equal protein loading was assessed using the anti-β-tubulin mAb (clone 2297). Equal protein loading was assessed using the anti-β-tubulin mAb (clone 2297). Equal protein loading was assessed using the anti-β-tubulin mAb (clone 2297).

RESULTS
Caveolin-1 Null Mice Are Lean and Resistant to Diet-induced Obesity—Targeted disruption of exons 1 and 2 of the caveolin-1 locus produces a null mutation (15). Immunoblot analysis of peri-gonadal white adipose tissue (WAT), one of the sites of highest caveolin expression in the mouse, verified that caveolin-1 expression is ablated in the caveolin-1 knockout animals (Fig. 1A). It should also be noted that, in adipose tissue, the heterozygote mice have nearly the same levels of caveolin as wild-types, indicating that loss of one allele is compensated for by the other. To assess the state of adipocytes ultrastructurally, we also performed transmission electron microscopy on the peri-gonadal WAT. Fig. 1B shows the characteristically marginalized cytoplasm of the Cav-1 (+/+) adipocyte and the extremely high number of plasma membrane-associated and invaginated caveolae (see arrows). In contrast, Cav-1 null adipocytes display barren membrane architecture (loss of caveolae), but interestingly have no other overt structural abnormalities.

In our follow-up of the progressively older Cav-1 null cohorts, we noticed a tendency for the Cav-1 null mice to remain smaller than their wild-type littermates. In fact, at around 1 year of age, the difference is quantifiable with both the male and female knockout mice being approximately 5–7 g lighter than the wild-type controls (females: 39.8 ± 2.6 g for Cav-1+/+, versus 33.0 ± 2.1 g for Cav-1−/− mice (n = 10 each, p < 0.05) and males: 45.4 ± 2.1 g for Cav-1+/+ versus 40.1 ± 1.1 g for Cav-1−/− mice (n = 10 each, p < 0.05)). To assess whether the lack of weight gain was due to adiposity or other age-related changes, we placed a cohort of age-matched male and female mice on a high fat diet (59% of...
We conducted routine histopathology on the 12- and 36-week-old mice above with a focus on WAT from several regions. At 12 weeks, two striking differences could be observed. The Cav-1−/− females have significantly reduced lipid droplets thereby explaining the reduction in weight at such an early age (Fig. 3A). Additionally, the mammary ducts are noticeably perturbed: The number of ducts per field is increased, and the ductal epithelia exhibit hyperplasia. Although the size of the Cav-1−/− mammary glands is overtly reduced, the overall architecture is intact and the total number of ducts per gland does not seem to be altered (quantification of ductal density is also shown (Fig. 3B inset)). We therefore conclude that the reduction in mammary gland size in Cav-1−/− females is primarily due to reduced adipocyte diameter.

A second interesting finding at 12 weeks of age is the near complete ablation of adipocytes in the hypodermal fat layer of both Cav-1−/− males and females (Fig. 3A). Normally, the epidermis overlies a layer of adipocytes ~3-4 cell layers thick; this layer is notably absent in Cav-1-deficient mice. Obviously, the lack of the hypodermal fat layer does not contribute significantly to the reduced body weight of the knockout animals; nevertheless, it is intriguing that there is a selective early effect on the hypodermal and female mammary/subQ WAT.

Histological analysis of similar fat pads in the 36-week-old high fat diet mice indicates a systemic effect on adipose tissue. The most highly affected tissues are the mammary gland/subcutaneous and the peri-gonadal WAT in both Cav-1−/− males and females, although all fat pads are affected to some degree (Fig. 3C shows the female M4/subQ WAT and peri-gonadal tissue). There are several notable observations: The M4/subQ in the Cav-1 null females is so severely perturbed that it no longer resembles mammary tissue. The adipocytes are highly diminished in number and display heterogeneity in size, and there is marked interstitial fibrosis and hypercellularity (Fig. 3C, left panel). All other major fat pads examined (the male M4/subQ, the male/female peri-gonadal, and peri-renal/retroperitoneal) show similar histological abnormalities (Fig. 3C, right panel). Lipid droplet size in Cav-1 null adipocytes is generally 2- to 3-fold smaller than in wild-type adipocytes. Additionally, there is marked extracellular matrix deposition and hypercellularity (possibly of adipocyte precursors) surrounding the existing adipocytes.

As mentioned above, the initial characterization of Cav-1−/− mice revealed abnormalities in the lung and associated vasculature, conditions that result in exercise intolerance in these Cav-1−/− mice (15, 16). It could be imagined that these pulmonary/vascular defects could interfere with oxygen tension and delivery, thereby causing the mice to lose weight simply by increased energy expenditure. To rule out these confounding variables as possible causes of the observed loss of adiposity, metabolic chamber studies were conducted on 4-month-old wild-type (n = 4) and knockout (n = 4) mice (Table 1), an age at which dramatic lung/vascular abnormalities and exercise intolerance are fully present (15). In this way, if breathing difficul-
ties or fatigue cause Cav-1 null mice to use more energy (i.e., from adipose stores) we should observe differences in the metabolic parameters measured via the chamber (VO2, VCO2, respiratory quotient, heat release, or movement). However, there are no changes in these parameters, directly indicating that Cav-1 null mice do not have higher energy expenditures (Table 1); clearly, the leanness is based on factors independent of the lungs and related vasculature.

**Caveolin-1 Null Mice Have Metabolic Derangements in the Handling of Triglycerides and Free Fatty Acids**—Because obesity/leanness are primarily disorders of energy balance, three mechanisms could lead to a lean body phenotype: 1) diminished...

**FIG. 3.** Histological analysis of adipose tissues from 12-week and 36-week-old high fat diet Cav-1(+/+) and (−/−) mice. A, routine histology (hematoxylin/eosin) was performed on several different adipose tissue regions of 12-week-old wild-type and Cav-1 null mice. At this age, significant differences were only found in two tissues (the female subcutaneous/mammary gland 4 and both male/female hypodermal fat layers). All other regions assessed (i.e., male subcutaneous, male/female peri-gonadal, and male/female peri-renal/retroperitoneal) were unremarkable. B, the entire left-side mammary gland #4 of Cav-1(+/+) and (−/−) females was dissected and subjected to whole-mount preparation. Carminic dye staining of the mammary tissue allowed visualization of the ductal architecture and quantification of ductal density. C, similar histological analysis of 36-week high fat diet wild-type and Cav-1 null mice. All adipose tissues examined were dramatically altered with the most significant changes occurring in the male/female subcutaneous/mammary gland 4 and peri-gonadal WAT.
food intake; 2) reduced nutrient absorption/steatorrhea; and 3) altered metabolic storage, leading either to storage in non-adipose ectopic sites or to increased metabolism (reviewed in Ref. 24). We tested the first two possibilities on a cohort of 12-week-old wild-type (n = 6 male, n = 8 female) and knockout (n = 7 male, n = 8 female) mice by isolating each mouse in a separate cage and measuring daily food intake for an 8-day period as well as determining the triglyceride content of stools (Fig. A, A and B). Surprisingly, the daily food intake of the Cav-1(−/−) mice was on average even higher than the wild-type controls, reaching statistical significance for the females (females: p < 0.05; males: p < 0.1) (Fig. A4). Furthermore, stool fat content remained unchanged, indicating that absorption was not a factor in the weight phenotype (Fig. A4B). Similar results were obtained in an older cohort of mice (~8 months of age) (data not shown) indicating the leaner body weights observed in Cav-1 null mice are independent of food intake or intestinal absorption.

We thus considered a metabolic explanation for the reduction in adipose tissue, in light of the following: 1) alterations in several serum metabolites, including insulin and lipids are hallmarks of diabetic and obesity disorders, and 2) caveolin-1 has previously been implicated in cholesterol metabolism and insulin signaling (25, 26). We measured the plasma values of the several common metabolites in 12-week-old mice (both fasted and post-prandial states; Fig. 5, A and B). As expected, both insulin and glucose levels rose in the post-prandial state, albeit to similar levels in the wild-type and Cav-1 knockout mice (Fig. 5A). Furthermore, cholesterol levels were not altered in both fed and fasted states, indicating that a lack of caveolae does not necessarily disrupt cholesterol homeostasis as previously proposed (25). We instead discovered dramatic alterations in triglycerides and free fatty acid levels (Fig. 5B).

Fasted triglyceride levels were on average 2-fold higher in the Cav-1 knockout mice (baseline hypertriglyceridemia), whereas free fatty acid levels remained unaffected. Post-prandially, the hypertriglyceridemia was exacerbated, reaching as high as 500 mg/dl; also, the normally observed drop in free fatty acid levels (due to insulin-mediated suppression of lipolysis) was compromised in the Cav-1 knockout mice (Fig. 5B).

Adipocytes are not mere passive storage cells, but are rather highly active endocrine compartments with the ability to regulate whole-body metabolism through the secretion of numerous factors (reviewed in Ref. 27). We also set out to determine the plasma levels of certain adipocyte-specific secretory proteins, namely leptin and Acrp30, for the following reasons: Microarray analysis indicates that caveolin-1 is a major gene up-regulated in leptin-deficient Ob/Ob adipose tissue (28), and Acrp30 is a major adipocyte-secreted factor, induced by ~100-fold in fully differentiated 3T3-L1 adipocytes (29). Interestingly, plasma leptin levels were reduced by over 2-fold in Cav-1-deficient mice (Fig. 5C). Because leptin primarily acts to suppress appetite (30), its reduced levels could in large part explain the hyperphagia in Cav-1 null mice (see Fig. 4A). Furthermore, plasma ACRP30 levels were significantly reduced, ~8- to 10-fold, irrespective of the normally observed sexual dimorphism seen between male and female rodents.2 These two results along with the hypertriglyceridemia/elevation of free fatty acids indicate that the absence of caveolin-1 can lead to pleiotropic metabolic perturbations in lipid metabolism.

Given that plasma levels of triglycerides and not cholesterol were perturbed in Cav-1 null mice, we next assessed possible lipoprotein profile differences between wild-type and Cav-1 knockout mice. We utilized size-exclusion chromatography (fast-performance liquid chromatography) to fractionate plasma lipoproteins in both Cav-1(+/+) and (−/−) mice during fed and fasted states (Fig. 6A). As expected, the majority of cholesterol in plasma was present in high density lipoprotein particles and remained in large part unaffected in Cav-1 null mice. However, there is a second significant post-prandial cholesterol peak in Cav-1(−/−) mice, which is due to an increased chylomicron/VLDL fraction. Triglyceride analysis of the same fractions more clearly demonstrates this finding. The chylomicron/VLDL fraction is increased at baseline (i.e. during fasting) in the Cav-1 knockout mice and increases dramatically above wild-type mice in the post-prandial state (Fig. 6A). From the above data, we can conclude that a deficiency in Cav-1 leads to a build-up of triglyceride-rich chylomicrons and VLDLs in the blood.

Caveolin-1 Deficiency Leads to Kinetically Delayed Triglyceride Clearance, Independently of Lipoprotein Lipase Activity—A possible confounding variable in the above analysis is the propensity of Cav-1 null mice to be hyperphagic. To control for this and variations in the feeding behavior of individual mice and to obtain a more thorough kinetic analysis of triglyceride metabolism, we also tested the ability of mice to clear a bolus of fat administered via gastric gavage (i.e. an oral fat tolerance test) (Fig. 6B). Both wild-type and Cav-1 knockout mice begin to absorb released fatty acids and produce chylomicrons within a few minutes of gavage. As can be seen, the steady-state rate of absorption and clearance occurs rather quickly in wild-type mice (peak at 2 h and gradual clearance thereafter). In contrast, Cav-1 null mice have a severely compromised clearance mechanism and continue to build up plasma triglycerides (peak at 2 h and diminished clearance thereafter).

Lipoprotein lipase (LPL), an adipocyte and muscle-secreted enzyme, is the primary mechanism by which triglycerides are hydrolyzed for storage or metabolism in peripheral tissues (31). A deficiency or an inactivity of this enzyme plays a central role in many described hypertriglyceridemias (32). We took advantage of a well characterized assay in which the readily available pools of LPL and hepatic lipase are released into the blood upon intravenous injection of heparin in mice (22). Biochemically, one can differentiate between the two enzymes, because LPL activity is abrogated at high salt concentrations, whereas hepatic lipase activity is not. As shown in Fig. 6C, saline injection of both wild-type and Cav-1 knockout mice only measures baseline plasma lipase activity, of which nearly 100% is from de novo circulating hepatic lipases (33). Upon heparin injection, total lipase activity increases significantly (Fig. 6C), of which nearly two-thirds is NaCl-inhibitable (i.e. a reflection of LPL activity). In all cases, Cav-1 null mice have lipase activities that are indistinguishable from the wild-type mice, indicating that the disrupted lipid metabolism in Cav-1 knockout is independent of LPL function.

Brown Adipose Tissue in Caveolin-1 Null Mice Is Hyperplastic, Possibly as a Compensatory Reaction to Hypertriglyceridemia—A presumed lack of lipid uptake and storage in

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2 T. P. Combs and P. E. Scherer, manuscript in preparation

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**Table I**

| Parameter tested | Wild-type | Cav-1 KO |
|------------------|-----------|----------|
| Total VO2 (ml/kg) | 2290 ± 13 | 3047 ± 14 |
| Total VCO2 (ml/kg) | 1905 ± 11 | 2819 ± 13 |
| Total RQ          | 0.70 ± 0.01 | 0.74 ± 0.01 |
| Total Heat (kcal/l) | 0.37 ± 0.02 | 0.39 ± 0.03 |
| Total movements   | 22904 ± 1921 | 26092 ± 3957 |

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adipocytes would lead to shunting of triglycerides to other organ-systems. An attractive candidate for such ectopic storage would be hepatocytes for two reasons: 1) Many diet-induced obesities lead to partially fatty livers as a compensatory mechanism and 2) caveolin-1 expression is nearly absent in liver parenchyma, thereby making hepatocytes the least likely to be affected in Cav-1 null mice. However, examination of livers in both young and older high fat diet Cav-1 null mice revealed no increase in weight (Fig. 7A) or histologically obvious steatosis above that observed in wild-type mice (data not shown).

Because we found that brown adipose tissue (BAT) is a major triglyceride-storing/metabolizing tissue with much less caveolin-1 expression than WAT (Fig. 7C), we assessed the characteristics of the major site of brown fat accumulation in rodents, the sub/intra-scapular region. In contrast to white fat, the Cav-1(−/−) BAT was 3- to 4-fold larger in the 36-week-old high fat diet cohorts (Fig. 7A shows quantification of the BAT weights). Histologically, lipid droplets and cellular morphology was not different than that of wild-type mice (data not shown), indicating that the larger BAT mass is due mainly to hyperproliferation, possibly signaling compensatory responses to the hypertriglyceridemia. A photograph of this hyperplasia is shown grossly in Fig. 7B. The inter-scapular BAT is displayed on the right side of each mouse and the sub-scapular brown fat remains undissected. Normally unnoticeable in wild-type mice, the sub-scapular brown fat is so enlarged that it protrudes out of the overlying scapula in the Cav-1 null mice.

**DISCUSSION**

In this study, we have elucidated the reason why progressively older Cav-1 null mice have smaller body sizes than their wild-type littermates. We show that Cav-1 null mice are overtly resistant to obesity when challenged with a high fat diet for 36 weeks. The body weight differences are primarily due to reduced adiposity as determined by MRI and quantitation of the major fat pad weights. Using metabolic chamber analysis, we ruled out the pulmonary/vascular defects initially reported for Cav-1 null mice as an indirect cause of this reduced adiposity. Cav-1 null mice are also not lean due to diminished feeding behavior or fat absorption; in contrast, these mice are even slightly hyperphagic compared with wild-type controls. An analysis of plasma metabolites reveals that neither glucose, insulin, nor cholesterol, a lipid previously implicated in caveolar function, are altered. Rather surprisingly, however, in Cav-1 null mice, the triglyceride levels are elevated in the fasted state and increase even more dramatically post-prandially, whereas free fatty acids levels fail to undergo the expected post-prandial reduction observed in wild-type mice. Additionally, the plasma values of two major adipocyte-secreted factors (i.e. leptin and Acrp30) are significantly reduced. Our analysis of the hypertriglyceridemia phenotype indicates that Cav-1 null mice have a predicted build-up of the triglyceride-rich chylomicrons/VLDLs post-prandially and show an inability to rapidly clear an orally administered fat load. This lipid accumulation does not appear to be due to alterations in lipoprotein metabolism.
Fig. 5. Analysis of plasma parameters in Cav-1 null mice reveals significantly altered levels of triglycerides, post-prandial free fatty acids, and two major adipocyte-secreted factors (leptin and Acrp30). A and B, fasting blood samples were collected at 7:00 a.m. (12 h after removal of food), and post-prandial blood was collected at 12:00 a.m. (after 3 h of feeding in the dark) from 12-week-old wild-type and Cav-1 knockout mice. Plasma insulin was measured by radioimmunoassay (RIA) while plasma glucose, cholesterol, triglycerides, and free fatty acids were measured colorimetrically. C, plasma leptin levels were measured by RIA, while plasma Acrp30 levels were measured by quantitative immunoblotting, as we described previously (21).
FIG. 6. Lipoprotein fractionation of plasma from Cav-1 null mice reveals an increase in triglyceride-rich VLDLs/chylomicrons: Cav-1 null mice display marked intolerance in the clearance of an oral fat load independent of lipoprotein and hepatic lipase activity

A. Pooled plasma samples from 12-week-old mice (Cav-1+/+, n = 10 and Cav-1−/−, n = 13) in fasted and post-prandial states were subjected to size-exclusion chromatography (fast-performance liquid chromatography). Colorimetric analysis of triglycerides and cholesterol was performed on a series of 0.5-ml fractions that corresponded to the pre-determined migration of pre-purified lipoproteins (chylomicrons/VLDL, IDL/LDL, and high density lipoprotein).

B. An oral fat load (0.5 ml of olive oil) was administered to a cohort of 12-week-old wild-type and Cav-1 null mice by gastric gavage. Blood was obtained by tail vein at baseline and for the following times: 20 min, 40 min, 1 h, 80 min, 100 min, 2 h, 2.5 h, 3 h, 4 h, 6 h, 8 h, 12 h, 14 h, and 24 h. Triglycerides were measured for all mice and time points colorimetrically. An asterisk indicates the time points at which statistically significant differences were observed.

C. A cohort of 12-week-old wild-type and Cav-1 null mice were injected with heparin (to release proteoglycan-bound lipases) and saline as control; blood was collected 5 min later retro-orbitally. In each case, lipase activity was determined by incubating plasma with a lipid emulsion consisting of [3H]triolein and measuring released [3H]oleic acid via scintillation counts. To differentiate between lipoprotein lipase (LPL) activity and hepatic lipase activity, all incubations were performed with or without 1 M NaCl (a salt concentration that inhibits LPL, but not hepatic lipase activity).
lipase (LPL) function, because Cav-1 null mice have normal LPL activities similar to wild-type mice. The observed hyperlipidemia could partially be compensated for by shunting through brown fat stores, as there is marked hyperplasia in the scapular brown fat of older Cav-1 null mice.

Taken together, our results clearly demonstrate that obesity resistance in Cav-1 null mice lies in an inability to convert triglycerides in lipoprotein form to triglycerides in lipid droplet storage form. We have attempted to rule out the central rate-limiting enzyme in this process, LPL; however, many possibilities still exist. Because LPL is secreted by both muscle and adipose tissue (31), we do not as yet know if Cav-1 null mice have a selective disruption of LPL synthesis, secretion, or transport in adipose tissue. This possibility is unlikely given that mice lacking LPL expression in adipose tissue, but not in skeletal or heart muscle, have normal plasma triglycerides and fat mass (34, 35).

Histologically, younger Cav-1 null mice have relatively in-
Caveolin-1, Obesity, and Adipocyte Dysfunction

The uptake and storage of fatty acids as triglycerides is a major function of adipocytes. Because the flux of fatty acids into primary adipocytes and 3T3-L1 cells follows saturable kinetics, facilitated membrane transport has been proposed as the uptake mechanism (39, 40). Several proteins have been proposed to mediate this function, including caveolin-1 (for review see Ref. 41). Labeling of membrane proteins with photoreactive long chain fatty acids identifies caveolin-1 as a major fatty acid binding protein in adipocytes (11, 12). In addition to acting as storage compartments for triglycerides, adipocytes also take up significant amounts of non-esterified cholesterol (42). A high localized concentration of cholesterol is one of the hallmarks of caveolar/lipid raft biogenesis (43), and caveolin-1 has been shown to directly bind cholesterol both in vitro and in vivo (8, 44). A defect in the transport/storage of fatty acids or cholesterol in adipocytes would certainly lead to defects in lipid homeostasis and also result in a lean body phenotype.

By morphological and biochemical means, adipocyte caveolae have been shown to be enriched in several components of the PKA (45) and insulin signaling apparatus, including the insulin receptor and Glut4 (10, 46, 47). Interestingly, caveolin-1 has been shown to interact with the insulin receptor and enhance insulin-dependent activation of downstream targets (26, 48, 49), whereas its interaction with PKA is inhibitory (50). Localized insulin resistance, kinetically slower insulin signaling, or constitutive activation of PKA at the level of the adipocyte could explain some of the phenotypes observed. A major effect of insulin on the adipocyte is the favoring of lipid accumulation processes over the PKA-mediated lipolytic pathways (reviewed in Ref. 51). Perturbations/inefficiencies in this highly regulated process could explain the hypertriglyceridemia, the histologically smaller lipid droplets in older mice, and the lack of a post-prandial drop in free fatty acids (i.e. unregulated lipolysis). A relationship between caveolin-1 and insulin signaling is also interesting in light of the fact that plasma Acrp30 levels were drastically reduced in Cav-1 null mice. Recently, Acrp30, a major secreted factor from adipocytes, has been shown to sensitize cells to sub-physiological levels of insulin, thereby enhancing insulin-mediated responses in vivo (21). To address some of these observations, future studies will be needed to dissect the in vivo relationship between Cav-1 and insulin/PKA signaling in detail.

The literature is replete with reports of successes or failures in eliciting leanness/obesity resistance in mice with targeted disruption of distinct genetic loci. A survey of these genes indicates a common theme: Many have previously been implicated in pathways of metabolic control. For example, genetic ablation of the PKA regulatory subunit (Type II), ap2, perilipin, or acyl-CoA:diacylglycerol transferase all result in a lean body phenotype. Our finding that a deficiency in caveolin-1 imparts resistance to diet-induced obesity and causes hypertriglyceridemia suggests for the first time a clear functional role for caveolin-1 and caveolae in disorders of obesity and lipid homeostasis.

Interestingly, the adipose tissue phenotype of Cav-1 null mice is specific for caveolin-1, because Cav-2 null mice do not show any problems with weight gain, adipocyte abnormalities, or hypertriglyceridemia (52). However, Cav-2 null mice do show the same lung phenotype as Cav-1 null mice, including exercise intolerance (52). Thus, the lung phenotype is clearly independent of the adipose tissue phenotype that we describe here. This idea is consistent with our metabolic chamber studies described above (Table I).

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Babak Razani, Terry P. Combs, Xiao Bo Wang, Philippe G. Frank, David S. Park, Robert G. Russell, Maomi Li, Baiyu Tang, Linda A. Jelicks, Philipp E. Scherer and Michael P. Lisanti

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