Role of caveolin and caveolae in insulin signaling and diabetes

Alex W. Cohen,1 Terry P. Combs,2 Philipp E. Scherer,2 and Michael P. Lisanti1
Departments of 1Molecular Pharmacology and 2Cell Biology and the Albert Einstein Diabetes Research and Training Center, Albert Einstein College of Medicine, Bronx, New York 10461

Cohen, Alex W., Terry P. Combs, Philipp E. Scherer, and Michael P. Lisanti. Role of caveolin and caveolae in insulin signaling and diabetes. Am J Physiol Endocrinol Metab 285: E1151–E1160, 2003; 10.1152/ajpendo.00324.2003.—Caveolae are specialized membrane microdomains present within the plasma membrane of the vast majority of cell types. They have a unique composition in that they are highly enriched in cholesterol, sphingolipids, and their coat proteins the caveolines (-1, -2, and -3). In recent years it has been recognized that caveolae act as signaling platforms, serving as a concentrating point for numerous signaling molecules, as well as regulating flux through many distinct signaling cascades. Although caveolae are found in a variety of cell types, they are most abundant in adipose tissue. This fact has led to the intense study of the function of these organelles in adipocytes. It has now become apparent that effective insulin signaling in the adipocyte may be strictly dependent on localization of at least two insulin-responsive elements to caveolae (insulin receptor and GLUT4), as well as on a direct functional interaction between caveolin-1 and the insulin receptor. We present a critical discussion of these recent findings.

caveolin-1; insulin receptor; glucose transporter 4

TYPE II DIABETES is a genetically heterogeneous disease affecting more than 5% of the population of the Western world (5). Frequently, the first recognizable abnormality detected in individuals destined to develop type II diabetes is insulin resistance, characterized by hyperinsulinemia and often hyperglycemia (20). Whereas the exact pathophysiological mechanisms responsible for the development of insulin resistance remain unknown, it is believed that combined defects in β-cell function and peripheral insulin action are both important components (5, 20, 33).

Upon binding to its receptor, insulin causes rapid autophosphorylation and activation of the receptor’s intrinsic tyrosine kinase activity. This leads to the phosphorylation of numerous downstream signaling molecules, including the insulin receptor substrate (IRS) family (20, 84). The IRSs are then recognized by proteins containing Src homology 2 (SH2) domains, such as type 1A phosphatidylinositol 3-kinase (PI 3-kinase), leading to the subsequent activation of numerous signaling cascades that, taken together, result in maintenance of glucose levels and energy storage (1, 20, 41, 84). It is thought that dysfunction of many of these insulin-responsive signaling molecules can lead to impaired insulin responsiveness in peripheral tissues and, thus, the development of type II diabetes.

Recently, caveolin-1 and caveolae microdomains have emerged as potentially important regulatory elements in the control of insulin signaling (14, 53, 67, 87). Caveolin-1 and caveolae may play a dual role in the regulation of glucose homeostasis, both through a direct interaction between caveolin-1 and the insulin receptor and as an agent for GLUT4-mediated glucose uptake. In this brief review, the role of caveolin-1 and caveolae in the pathogenesis of type II diabetes, with a particular focus on the above two molecules, will be discussed.

CAVEOLAE AND CAVEOLINS

Nearly fifty years ago, caveolae were first identified as abundant plasma membrane invaginations in endothelial and epithelial cells (55, 86). At the ultrastructural level, these organelles most often take on the appearance of 50- to 100-nm flask-shaped invaginations. In addition, they can also fuse to form rosette-like structures, as well as long tubular structures (75). Caveolae are most numerous in well-differentiated cell types such as adipocytes, endothelial cells, myocytes.
and fibroblasts (69–71, 81). In a more diminished capacity, they have also been found in a wide variety of cells, including those of the immune and nervous systems (7, 24, 25).

Before the advent of molecular biological techniques to study their function, caveolae were thought to be chiefly involved in vesicular transport. Indeed, it is now known that caveolae do participate in cellular trafficking and that they contain many molecules known to be involved in this process, including dynamin and several SNARE proteins (i.e., syntaxin 1A, SNAP-25, and VAMP2) (8, 9, 57). Caveolae are distinguished from the majority of the plasma membrane in that they constitute a morphologically identifiable subset of the liquid-ordered domains referred to as lipid rafts. These membrane microdomains are highly enriched in cholesterol and sphingolipids, which impart a relative insolubility in detergents such as Triton X-100 at 4°C (22, 23, 51, 60, 64, 73). This characteristic allows for the biochemical isolation of caveolae and caveola-associated proteins from the bulk of the plasma membrane.

The rather recent discovery of the structural components of caveolae, the caveolin gene family, has allowed for a more thorough investigation into the function of these organelles. Caveolin (now called caveolin-1) was initially identified as a major tyrosine phosphoprotein in v-Src-transformed chicken embryonic fibroblasts (26). Soon thereafter, two other caveolin isoforms, caveolin-2 and -3, were identified (69, 71, 81). The caveolins are cholesterol-binding integral membrane proteins with a unique hairpin topology that allows both the NH₂ and COOH termini to face the cytoplasm. Mapping experiments and mutational analysis have identified several distinct domains within the caveolin proteins, including the membrane-spanning domain, the oligomerization domain, and the scaffolding domain (72, 74, 85). Each caveolin has a unique pattern of distribution, with caveolin-3 being expressed in skeletal, cardiac, and smooth muscle cells (81), whereas caveolin-1 is expressed in the majority of other cell types, with particularly high expression in adipocytes, fibroblasts, and epithelial and endothelial cells (70).

Caveolin-2 expression most closely follows that of caveolin-1. Generally, it is thought that caveolin-2 is not expressed in cells of the myocyte lineage; however, this view has recently been challenged by a group demonstrating coexpression of caveolin-2 and -3 in cultured cardiac myocytes (65). Caveolin-1 and -2 form hetero-oligomeric high-molecular-mass complexes with each other and have been shown to interact by numerous techniques, including coimmunoprecipitation and cosedimentation (69). Importantly, caveolin-2 expression is dependent on the presence of caveolin-1. Without caveolin-1, caveolin-2 remains trapped in the Golgi apparatus, where it is rapidly degraded by proteasomal mechanisms because treatment of caveolin-1-deficient cells with the proteasomal inhibitor MG-132 rescues caveolin-2 levels but not its membrane localization (58).

Caveolins and caveolae are now known to be involved in numerous aspects of cellular biology, including vesicular transport, cholesterol homeostasis, and, perhaps most importantly, signal transduction (54). By both morphological and biochemical techniques, numerous signaling molecules have been shown to localize to, or reside within, caveolae. These molecules include Src family tyrosine kinases, G protein-coupled receptors, members of the Ras MAPK cascade, nitric oxide synthase, and receptor tyrosine kinases (30, 45, 53, 61). Localization of these proteins to caveolae has been shown to involve a direct interaction with the scaffolding domain of caveolin-1 or -3, depending on the cell type. More than just acting as an anchor, however, this interaction is most often one of inhibition, holding a given signaling molecule in check until released by the appropriate stimulus (21, 61). Protein interactions with caveolin can also serve to augment or enhance signal transduction through a specific pathway (i.e., the insulin receptor) (14, 53, 56). It is through this interaction that caveolins and caveolae serve to compartmentalize and regulate numerous aspects of cellular metabolism.

Recognition of caveolae as specialized signaling compartments served to address an important concept, in that the seemingly nonspecific nature of intracellular events elicited by receptor binding of distinct substrates, such as growth factors, has long presented an interesting theoretical dilemma. How can different outcomes, such as the membrane translocation of GLUT4, be achieved by one growth factor (insulin) but not another (EGF), when they both seem to activate the same downstream molecules? In this regard, caveolae may serve to spatially organize receptors and downstream signaling molecules within specialized microdomains and thus limit the effect of a growth factor to molecules confined within that area.

**CAV EOLIN, CAVEOLAE, AND INSULIN SIGNALING**

Initial evidence suggesting that caveolae and caveolins may play a major role in cellular energy metabolism came from experiments in rat adipocytes demonstrating that gold-labeled insulin, but not α₂-macroglobulin-methyleamine (a nonhormonal ligand), is endocytosed by a mechanism involving uncoated invaginations (27, 28). These ultrastructural findings were later elaborated upon (30), demonstrating that the insulin receptor is highly enriched in caveolae by immunogold electron microscopy. In further studies, it was discovered that adipose tissue is the most abundant source of caveolae and that caveolin-1 mRNA and protein greatly increase (~25 fold) during the differentiation of 3T3-L1 cells from fibroblasts (pre adipocytes) to adipocytes. Furthermore, it was shown that treatment of 3T3-L1 adipocytes with insulin resulted in the association of the insulin-responsive glucose transporter GLUT4 within caveolin-rich membrane fractions (70). These results were the first indication that caveolae and caveolin-1 may play an important role in adipocyte energy metabolism through regulation of insulin signaling.
A role for caveolin-1 in insulin signaling was further established when insulin was found to cause the rapid tyrosine phosphorylation of caveolin-1 in 3T3-L1 cells (15). In a similar study (16), it was also shown that the phosphorylation of caveolin-1 was specific for insulin, as epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) did not elicit a similar response. These authors also found that the phosphorylation of caveolin-1 was dependent on cellular differentiation, since the insulin response could only be measured in fully differentiated 3T3-L1 adipocytes, but not in fibroblasts (preadipocytes) (16). In further exploration of this phenomenon, Lee et al. (44) found that insulin, but not EGF, PDGF, TNF-α, or IL-6, resulted in the phosphorylation of caveolin-1 on tyrosine 14. In their study, however, it was shown that insulin treatment of preadipocytes did result in caveolin-1 phosphorylation (44). This inconsistency could be due to differences in experimental protocols, such as the use of antiphosphotyrosine antibodies vs. more sensitive antiphospho-caveolin-1 (Y14) antibodies, in the later study (16, 44).

As stated previously, caveolae are highly enriched in cholesterol, a property that has become very useful in their study. Treatment of cells with cholesterol-chelating agents, such as β-methyl-cyclodextrin (βMCD), results in the reversible disruption and flattening of plasma membrane caveolae (30, 43, 56). Several researchers have employed this methodology in investigating the role of caveolae in insulin signaling. When Gustavsson et al. (30) treated isolated rat adipocytes with βMCD, they found that it resulted in a decrease in the amount of insulin-stimulated ATP citrate lyase phosphorylation, indicative of decreased insulin signaling. In addition, these investigators showed that insulin-mediated glucose uptake was also abrogated by βMCD treatment in both isolated adipocytes and 3T3-L1 cells. When cholesterol content was then restored in these cells, insulin-mediated protein phosphorylation returned to normal, and caveolae were again morphologically recognizable (30). These findings were confirmed by another group (56), who showed that βMCD attenuated insulin-mediated glucose uptake in 3T3-L1 adipocytes without affecting insulin receptor number or the ability of insulin to bind to its receptor. Furthermore, it was demonstrated that IRS-1 phosphorylation and activation of the downstream kinase PKB/Akt was inhibited by βMCD treatment. These results implicate structurally sound caveolae as being necessary for successful insulin signaling. Interestingly, these authors also found that βMCD treatment did not disrupt insulin-stimulated activation of extracellular signal-related kinases 1/2, indicating that caveolae are involved in processing insulin-mediated metabolic, but not mitogenic, signals in adipocytes.

Because of their high cholesterol and lipid content, caveolae/lipid rafts and their resident protein components can be isolated from the bulk of cellular constituents by use of two well-defined methodologies. As stated above, these membrane microdomains are relatively resistant to extraction by Triton X-100 at 4°C. In addition, the use of discontinuous sucrose density gradient centrifugation allows for the purification of caveolae/lipid rafts, because these microdomains have the propensity to “float.” Using a combination of these methodologies, Mastick et al. (15) demonstrated that a very small percentage of the phosphorylated insulin receptor is found in caveolin-containing Triton X-100-insoluble fractions of 3T3-L1 cells. In addition, these authors found that Triton X-100-insoluble complexes could be subfractionated into two distinct, buoyant density bands on a sucrose gradient, i.e., an upper and a lower band. These bands were then subjected to SDS-PAGE and Western blot analysis, which revealed that the majority of caveolin-1 localized to the lower band. Using the antiphosphotyrosine antibody 4G10 to identify a phosphorylated protein of ~92 kDa as the insulin receptor β-subunit, it was found that the majority of this protein was localized to the upper band. The authors conclude that, while ~2% of the total phosphorylated insulin receptor is detergent insoluble, only 0.3% of it localizes to the lower caveolin-containing insoluble band, and thus that caveolin-1 and the insulin receptor are found in different protein/lipid complexes. In support of these findings, a later study (52) also found very little insulin receptor β-subunit in caveolin-containing membrane fractions when prepared by two different methods. Here, Muller and colleagues (51, 52) used either detergent (Triton X-100) or carbonate extraction in conjunction with sucrose density centrifugation to prepare caveolin-containing detergent-insoluble rafts. Both methodologies revealed similarly low levels of the insulin receptor in these fractions, with the majority of the protein being found in noncaveolar fractions. In addition, Souto et al. (78) used a monoclonal antibody to affinity-purify vesicles containing caveolin-1. These affinity-purified vesicles were highly enriched in semicarbazide-sensitive amine oxidase and the scavenger lipoprotein receptor CD36, but they lacked the insulin receptor, flotillin, and GLUT4, all of which had previously been shown to biochemically copurify with caveolae. These authors report a relatively low recovery rate of these vesicles (on the order of 10%) and point out that they cannot rule out that they are studying only a subclass of caveolar vesicles.

In contrast to the findings detailed above, Gustavsson et al. (30) used a combination of detergent-free membrane preparation and discontinuous sucrose density centrifugation to prepare caveolin-enriched fractions that were found to contain the vast majority of plasma membrane insulin receptor (~36-fold enrichment). The validity of this methodology was confirmed by demonstrating a selective presence of caveolin-1 and an exclusion of noncaveolar resident proteins (i.e., clathrin and Na+/K+-ATPase α2-subunit) in the caveolae fractions in addition to a high concentration of cholesterol. Interestingly, these authors found that caveolae isolated by detergent resistance were devoid of the insulin receptor, suggesting that not all caveolin-associated proteins are detergent resistant. Taken together, these four studies present conflicting and in-
The insulin receptor is not drawn to scale. The insulin receptor precursor (1 amino acid), termed the caveolin-binding motif, which is found within most caveolin-interacting proteins. Note that –Fig. 1. Schematic representation of the proposed caveolin-1-insulin receptor interaction. 

The caveolin scaffolding domain (CSD) was eventually identified and mapped to residues 82–101 of caveolin-1 and residues 55–74 of caveolin-3 by a variety of approaches (17, 37, 77). Successive work focused on characterizing the molecular basis of scaffolding domain interactions with numerous signaling molecules, including the insulin receptor. Using bacteriophage display libraries and a peptide corresponding to the CSD, two similar caveolin-binding motifs, present within all known caveolin-associated proteins, were finally recognized (ΦXXXΦXXΦ and ΦXΦXXXΦ, where Φ represents an aromatic amino acid) (17).

A simple sequence screen of signaling molecules known to localize to caveolae revealed that the insulin receptor β-subunit contains the characteristic caveolin-binding motif (Fig. 1; 1193WSFGVVLW1200) (18). This finding led to the first functional study of the caveolin-insulin receptor interaction. In their study, Yamamoto et al. (87) showed that overexpression of caveolin-3 in HEK-293T cells resulted in an insulin-dependent increase in IRS-1 phosphorylation without affecting insulin receptor autophosphorylation. In addition, these authors showed, through a series of in vitro experiments, that the scaffolding domains of caveolin-1 and caveolin-3 could directly stimulate insulin receptor kinase activity. The cytoplasmic tyrosine kinase portion of the insulin receptor (BIRK), containing the caveolin-binding motif (Fig. 1), was purified from insect cells and incubated with purified IRS-1 and peptides corresponding to the scaffolding domains of caveolin-1 or -3. IRS-1 phosphorylation was then used as a measure of BIRK activity, and it was found that this phosphorylation was greatly increased by the addition of CSD peptides. Furthermore, by incubating immobilized CSD peptides with BIRK, it was demonstrated that the insulin receptor directly interacts with the scaffolding domain of caveolin-1 and -3 (87). Taken together, these findings indicate that the scaffolding domain of caveolin serves to augment the activity of the insulin receptor kinase as well as target the insulin receptor to caveolae.

In further exploration of this interaction, Nyström et al. (53) generated several insulin receptor constructs with mutations in the CSD (W1200T, F1195G, and a triple mutant W1193G/F1195G/W1200G). When these mutant constructs were transfected into Cos-7 cells, it was found that they were poorly expressed and failed to undergo autophosphorylation, unlike their wild-type counterpart.

Interestingly, similar findings were made with respect to insulin receptor mutants in the human population many years earlier, before the caveolin-binding motif was discovered and before the insulin receptor was known to interact with caveolin. Clinical studies of two distinct families with inherited severe insulin resistance and type II diabetes identified that these patients had either a W1193L or a W1200S mutation in the insulin receptor β-subunit (34–36, 49, 50, 68). Further analysis revealed that these mutations lead to rapid degradation of the insulin proreceptor, as well as markedly decreased kinase activity. In vitro studies of these mutants demonstrated that, when transfected into cells, they result in retention of the uncleaved insulin proreceptor in the endoplasmic reticulum and subsequent degradation. Interestingly, in the same report (68), these authors show that another insulin receptor mutant, Ser725, is transported normally to the plasma membrane. The Ser725 mutant is now known to

![Fig. 1. Schematic representation of the proposed caveolin-1-insulin receptor interaction. Residues 82–101 of caveolin-1 represent the caveolin scaffolding domain, which has been shown to mediate protein-protein interactions between caveolin-1 and numerous signaling molecules, including the insulin receptor. The insulin receptor contains a characteristic amino acid motif (1193WSFGVVLW1200; ΦXXXΦXXΦ, where Φ represents an aromatic amino acid), termed the caveolin-binding motif, which is found within most caveolin-interacting proteins. Note that the insulin receptor is not drawn to scale. The insulin receptor precursor (1–1382) undergoes posttranslational modification to remove the signal sequence (s.s.; 1–27) and is then cleaved into the α-subunit (28–758) and the β-subunit (763–1382). The position of the insulin receptor kinase domain is also shown (1023–1298).](http://ajpendo.physiology.org/)

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reside outside the caveolin-binding domain of the insulin receptor (residues 1193–1200). Although it was unknown at the time, these studies were the first to indicate that insulin signaling and stability of the insulin receptor are regulated by an interaction with the CSD.

Perhaps the most important advancements in understanding the relationship between caveolin-1, insulin signaling, and diabetes came from the development of caveolin (Cav)-1 null mice. This mouse model was independently generated and initially characterized by two different groups (19, 59), both showing that loss of caveolin-1 leads to hyperproliferation of lung endothelial cells with thickening of the alveolar septa. A more thorough analysis of Cav-1 null mice revealed that, whereas a caveolin-1 deficiency did not lead to an overt diabetic phenotype, it did result in abnormalities in lipid homeostasis, characterized by resistance to diet-induced obesity, ablation of the hypodermal fat layer, progressive white adipose tissue atrophy, and hypertrophy of brown adipose tissue (58). These changes occurred without any noticeable differences in insulin or glucose levels; however, both free fatty acid and triglyceride levels were found to be elevated in the serum, whereas leptin and Acrp30 levels were drastically decreased. The lack of weight gain could not be accounted for by reduced intestinal absorption (steatorrhea) or decreased food intake. In addition to these findings, Cav-1 null mice were also defective in triglyceride clearance when challenged with an oral triglyceride tolerance test, independent of lipoprotein lipase activity (58).

The molecular mechanism behind this adipocyte-based phenotype was recently elucidated in a follow-up study by our group (14). Because Cav-1 knockout mice did not develop an obvious diabetic phenotype on a normal chow diet, a cohort of these mice was placed on a high-fat diet (59% of calories from fat) at weaning. At 9 mo of age, serum insulin analysis revealed that Cav-1 null mice developed significant postprandial hyperinsulinemia without noticeable changes in glucose levels, compared with wild-type mice. On a normal chow diet, young Cav-1 null mice were shown to have a blunted response to an insulin tolerance test, indicative of insulin resistance. On further analysis, it was found that insulin signaling, as measured by insulin receptor phosphorylation and phosphorylation of several downstream targets, was selectively decreased in the adipocyte (a caveolin-1-expressing tissue) while signaling in both muscle and liver was normal. This defect in signaling was attributed to an ~90% decrease in insulin receptor protein content in the adipocyte, without changes in mRNA levels, indicating that caveolin-1 serves to stabilize the insulin receptor protein. In vitro experiments further proved that caveolin-1 stabilizes the insulin receptor and that the CSD mediates this stabilization. Briefly, cDNAs encoding full-length caveolin-1 or a CSD deletion mutant [caveolin-1 (Δ61–100)] were transfected into caveolin-1-deficient mouse embryonic fibroblasts (MEFs) and analyzed by Western blot and immunofluorescence microscopy for insulin receptor protein expression. It was found that only the full-length caveolin-1 cDNA was capable of restoring the insulin receptor protein to wild-type levels. In addition, it was shown that treatment of Cav-1 null MEFs with the proteosomal inhibitor MG-132 resulted in rescue of insulin receptor levels, indicating that caveolin-1 normally acts to stabilize the insulin receptor against degradation (14). In further support of the notion that the CSD acts to stabilize the insulin receptor against proteosomal degradation, we showed that incubation of Cav-1-deficient MEFs with a cell-permeable peptide corresponding to the CSD results in a rescue of insulin receptor protein levels (14) (Fig. 2). This finding indicates that the CSD alone is sufficient to functionally stabilize the insulin receptor.

Although it might be expected that an ~90% decrease in insulin receptor protein in the adipocyte would result in fasting hyperglycemia and hyperinsulinemia, several new findings suggest that our understanding of glucose homeostasis is less firm than previously thought. Recently, using the Cre-loxP system, Blüher et al. (4) developed an adipose tissue-selective insulin receptor knockout mouse (FIRKO). This mouse, unlike the Cav-1 null mouse that displays an adipose
tissue-specific reduction of the insulin receptor, completely lacks the insulin receptor in adipocytes. However, both a dramatic reduction and the complete absence of the insulin receptor lead to a similar phenotype. These authors (4) found that the FIRKO mouse, like the Cav-1 null mouse, was resistant to age-related obesity and showed markedly reduced overall body fat. In addition, the FIRKO mouse was found to have normal fasted and fed glucose and insulin levels on a chow diet, like Cav-1 null mice, despite significant tissue-specific insulin resistance, as evidenced by impaired insulin-dependent glucose uptake in isolated adipocytes.

Interestingly, unlike Cav-1 knockout mice, FIRKO mice were found to respond normally to an insulin tolerance test at the extreme age of 10 mo, when even wild-type mice show significant insulin resistance. Although the authors state that this is due to protection against obesity-related insulin resistance, because their mice are lean at this age, Cav-1 null mice were found to show a markedly blunted response to insulin at 5 mo of age, even though they are also resistant to age-related obesity (4, 14, 58). In any case, the FIRKO mouse serves to demonstrate that diminished adipocyte insulin receptor protein content does not lead to fasting hyperinsulinemia or an overtly diabetic state in a whole animal model.

The fact that functional insulin signaling is dependent on caveolin-1 helps to explain the lean-body phenotype of Cav-1 null mice just described. Insulin plays a dual role in governing adipose tissue homeostasis, in that it is responsible for de novo lipogenesis as well as inhibiting lipolysis (40, 66). When insulin binds to its cellular receptor, it triggers a host of signal transduction cascades, resulting in triglyceride and glycogen storage as well as inhibiting the lipolytic breakdown of stored fats. This inhibition is accomplished in part by the activation of phosphodiesterase-3B by the insulin receptor, which leads to a decrease in intracellular cAMP levels and a dampening of protein kinase A (PKA) activity (40). Although many ligands working through different receptors influence the rate of cellular lipolysis, all of their actions are directly dependent on cAMP levels and thus PKA activity (47). Therefore, PKA can be seen as the regulatory hub of lipolytic signals in the adipocyte.

Upon activation by increasing cAMP levels, PKA phosphorylates two lipolytic targets, hormone-sensitive lipase (HSL) and perilipin (29, 47, 79, 82). Phosphorylation of HSL results in a translocation of this molecule from the cytosol to the lipid droplet (11–13, 80). When perilipin is phosphorylated, it undergoes a conformational change and/or cytosolic translocation that allows HSL access to the lipid core (11–13). Caveolin-1 has been shown to regulate PKA activity via an inhibitory interaction with its catalytic subunit (61) and thus may serve to further govern the rate of adipocyte lipolysis, both through a stimulatory interaction with the insulin receptor and an inhibitory interaction with PKA. Therefore, it would be expected that loss of caveolin-1 would lead to diminished insulin signaling along with overactive lipolysis. This theory remains to be tested.

CAVEOLAE AND GLUT4

In addition to their role in directly governing insulin signaling, caveolae have also emerged as potentially important structures in downstream insulin-dependent cellular effects via a role in GLUT4-mediated glucose transport. It has been well established that effective insulin-stimulated glucose uptake in peripheral tissues such as muscle and fat requires the translocation of GLUT4 from intracellular stores to the plasma membrane, forming an aqueous pore that facilitates glucose entry into cells (see review in Ref. 6). GLUT4 translocation seems to be dependent on two divergent insulin-mediated signaling pathways.

The first pathway involves insulin receptor phosphorylation of insulin receptor substrates and the subsequent activation of PI 3-kinase (41). PI 3-kinase then recruits and activates the serine/threonine kinase PKB/Akt and atypical PKC isoforms (α/δ). Although the exact mechanisms by which these effector molecules lead to GLUT4 translocation are unclear, there is significant evidence to implicate them in this process. The other insulin-dependent signaling pathway required for GLUT4 translocation has been less well described but is important to note in detail here because of its relationship to caveolae/lipid rafts. This pathway is independent of PI 3-kinase activity and involves the insulin-dependent tyrosine phosphorylation of the proto-oncoprotein c-Cbl. At baseline, Cbl associates with the insulin receptor via an interaction with the adapter protein, Cbl-associated protein (CAP), through COOH-terminal SH3 domains in CAP (42). When Cbl is phosphorylated by the insulin receptor, which has been shown to be specific for differentiated 3T3-L1 cells, the Cbl-CAP complex is recruited to caveolin-enriched detergent-insoluble fractions (1, 2, 16, 62). Using the amino terminus of CAP as bait, Baumann and colleagues (1, 2) screened a yeast two-hybrid library derived from 3T3-L1 cells and identified the caveolar resident protein flotillin (see Ref. 3) as mediating the translocation of the Cbl-CAP complex into lipid rafts (2). In addition, these authors showed that overexpression of the amino-terminal domain of CAP in 3T3-L1 cells abrogates insulin-stimulated glucose uptake and glycogen and lipid synthesis without affecting PI 3-kinase-dependent signaling events. These results indicate that localization of Cbl to lipid rafts is necessary for proper insulin-dependent glucose uptake.

In a follow-up study (10), this group further explored this pathway, identifying the small GTP-binding protein TC10 as also necessary for insulin-mediated glucose uptake in 3T3-L1 adipocytes. When expressed in 3T3-L1 adipocytes, wild-type TC10 and a dominant-interfering TC10 mutant were found to significantly reduce insulin-mediated glucose uptake via inhibition of GLUT4 translocation. Although it is surprising that transfection of both the wild-type and mutant TC10
proteins inhibit glucose uptake, the authors suggest that overexpression of either form may cause altered GTPase stoichiometry and displacement of endogenous TC10 from effector molecules. In a search for the interrelationship between TC10 and the previously identified Cbl-CAP complex, Chiang et al. (10) found that the insulin-regulated guanyl nucleotide exchange factor (GEF) responsible for activating TC10, C3G, is recruited to lipid rafts by Cbl. Furthermore, by overexpressing C3G as well as the activated PI 3-kinase subunit p110 in 3T3-L1 adipocytes, the authors found that, together, these proteins mimic the effect of insulin on GLUT4 translocation, suggesting that the combination of these two pathways is necessary for insulin-stimulated GLUT4 translocation (10).

In another study, Watson et al. (83) used several methodologies to determine that TC10 localizes to caveolae/lipid rafts and that this localization is necessary for GLUT4 translocation. Here, the authors first transfected 3T3-L1 cells with TC10/H- or K-Ras chimeras that differentially target to either lipid rafts or nonraft plasma membranes, respectively. When these cells were then stimulated with insulin, it was found that only the raft-targeted TC10/H-Ras chimera-expressing cells behaved in a manner similar to cells transfected with wild-type TC10. In addition, differentiated 3T3-L1 adipocytes were coelectroporated with a dominant-interfering caveolin-3 mutant construct and a GLUT4-GFP or a TC10 construct. When plasma membrane preparations were analyzed by immunofluorescence microscopy, the authors (83) found that the dominant-interfering caveolin-3 mutant caused both a displacement of TC10 from caveolae and an inhibition of insulin-mediated GLUT4 translocation without affecting PI-3 kinase signaling. This study strongly suggests that proper localization of TC10 to caveolae is necessary for GLUT4 activation by insulin. Both the PI 3-kinase- and Cbl-CAP-signaling pathways seem to converge upon intracellular GLUT4 stores, resulting in translocation to the plasma membrane, most likely to caveolae. However, the exact role of caveolae and caveolin-1 in GLUT4-mediated glucose uptake remains controversial.

One of the first indications that caveolae could be important for proper GLUT4 function came from studies in 3T3-L1 adipocytes. Scherer et al. (70) used sucrose gradient centrifugation to demonstrate that insulin stimulation of 3T3-L1 adipocytes resulted in an increase in the amount of GLUT4-containing vesicles in caveolin-rich buoyant membrane fractions. Although this result suggests only that caveolin-1 and GLUT4 are in the same intracellular vesicles, these authors further explored this finding via immunopurification of GLUT4-containing vesicles and then used Western analysis to show that at least some of these vesicles contain caveolin-1. Other studies have lent further credibility to these initial findings, as Karlsson et al. (39) showed by both biochemical and morphological techniques that GLUT4 was indeed localized to caveolae. These authors exposed the inner surface of the plasma membrane of 3T3-L1 adipocytes by sonication and then double-immunolabeled these preparations with antibodies against GLUT4 and caveolin-1. This methodology showed that a large portion of plasma membrane GLUT4 colocalized with caveolin-1. In addition, immunogold electron microscopy of isolated plasma membranes again showed that the vast majority (85%) of GLUT4 is localized to caveolae. The morphological localization of GLUT4 to caveolae was then confirmed by detergent-free purification of caveolae from rat adipocytes, which showed ~3.5-fold enrichment of GLUT4 in the caveolar fraction. Furthermore, each of these methodologies revealed that GLUT4 was translocated into caveolae after insulin treatment. In another study (31), detergent solubilization and sucrose density centrifugation were used to show that insulin caused a biphasic translocation of GLUT4 from the cytosolic fraction to the plasma membrane in isolated rat epididymal adipocytes. Insulin induced the rapid appearance of GLUT4 in the bulk plasma membrane, which was followed by a slower translocation to caveolar membranes. It was found that the shift of GLUT4 into caveolae corresponded to an overall increase in glucose uptake (31).

The legitimacy of these findings has been questioned, however, as a study by Kandror et al. (38) found that GLUT4-containing vesicles are distinct from caveolae. These authors used anti-GLUT4 antibodies to immunoadsorb GLUT4-containing vesicles from rat adipocytes. Western analysis could not confirm that these vesicles were caveolae, as no caveolin-1 protein could be detected (38). Although this methodology is similar to that employed above by Scherer et al. (70) to show that immunopurified GLUT4 vesicles contain caveolin, the different results could be due to differences in experimental protocol. For instance, Kandror et al. isolated GLUT4-containing vesicles from rat adipocytes, whereas Scherer et al. used differentiated 3T3-L1 cells. 3T3-L1 cells are widely used as a cell culture model for adipocytes; however, these cells undoubtedly differ from purified adipocytes in many ways, including a multilocular rather than unilocular lipid droplet. In another study supporting the notion that GLUT4 does not localize to caveolae, Malide et al. (48) cryosectioned isolated rat adipocytes and then used immunoelectron microscopy to show that gold-labeled GLUT4 molecules do not localize to caveolae. Again, it is difficult to reconcile these ultrastructural findings with those mentioned above, except that very different experimental procedures, each with their own shortcomings, were employed in the preparation and identification of GLUT4 and caveolae. Whereas Malide et al. used intact adipose tissue for immunoelectron microscopy, Karlsson et al. (39) used fixed, purified plasma membranes. Thus it remains controversial whether GLUT4 truly localizes to caveolae.

A few reports have, however, shown that caveolae do play an interesting role in GLUT4 membrane trafficking. By use of cholesterol-chelating agents, it was shown (63) that disruption of plasma membrane caveolae did not affect insulin-stimulated glucose transport into 3T3-L1 adipocytes. However, this treatment re-
sulted in the kinetic buildup of GLUT4 at the plasma membrane, thus indicating that caveolae are necessary for GLUT4 endocytosis. In another study (76), it was shown that disruption of caveolae-mediated endocytosis with dominant-negative caveolin-1 mutants again resulted in increased plasma membrane-localized GLUT4. However, these authors showed that GLUT4 endocytosis was only partially dependent on caveolae, as only a 40% reduction in GLUT4 internalization was found in cells expressing the dominant-negative caveolin-1 mutant (76). In further demonstration that caveolae are important for GLUT4 homeostasis, our group (14) found that, in the Cav-1 null mouse, there is an overall upregulation of GLUT4 protein levels in the adipocyte by approximately three- to fourfold. Thus, although the exact role of caveolae and the caveolin proteins in GLUT4 function remains to be determined, it seems that there is indeed significant evidence to suggest an interrelation between these important molecules.

CONCLUSIONS

From initial evidence obtained over fifteen years ago to that gleaned rather recently from Cav-1 null mice, it has now become firmly established that caveolin-1 and caveolae play important roles in energy metabolism, through both augmenting insulin signaling and modulating GLUT4 function. Although it has now been shown that the scaffolding domain of caveolin-1 directly interacts with the insulin receptor and serves to stabilize the latter molecule against proteasomal degradation, many questions regarding the role of caveolin-1 in insulin signaling remain to be answered.

For instance, the function of insulin-mediated caveolin-1 phosphorylation remains unknown, as does the exact role of caveolin-1 in GLUT4 translocation and endocytosis. Furthermore, the clinical relevance of caveolin-1’s role in insulin signaling remains to be determined. Although patients have been identified with severe insulin resistance due to mutations in the caveolin-binding motif of the insulin receptor, these mutations are extremely rare. However, as mutations in caveolin-1 have been identified in the human population, albeit as somatic changes particularly in breast cancers (32), it is possible that germ-line caveolin-1 mutations also exist in a subset of patients with a thin, prediabetic phenotype similar to that observed in the Cav-1 null mouse.

DISCLOSURES

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