*Biochemical Society Transactions* (2020) **48** 165–177
https://doi.org/10.1042/BST20190388

PORTLAND PRESS

Review Article

# The caveolar-mitochondrial interface: regulation of cellular metabolism in physiology and pathophysiology

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The plasma membrane is an important cellular organelle that is often overlooked in terms of a primary factor in regulating physiology and pathophysiology. There is emerging evidence to suggest that the plasma membrane serves a greater purpose than a simple barrier or transporter of ions. New paradigms suggest that the membrane serves as a critical bridge to connect extracellular to intracellular communication particularly to regulate energy and metabolism by forming physical and biochemical associations with intracellular organelles. This review will focus on the relationship of a particular membrane microdomain — caveolae — with mitochondria and the particular implication of this to physiology and pathophysiology.

## Introduction

Mitochondria are classically known as the 'powerhouse' of the cell, sustaining high energy needs through oxidative phosphorylation. Additionally, with its own DNA, ribosomes, and replicative capacity, mitochondria are often regarded as independent and self-sustaining though their ultimate survival depends on a symbiosis with host cells. Broader roles for mitochondria include intracellular signaling, maintenance of reactive oxygen species, apoptosis, and adaptation to cellular stress [1]. Emerging evidence has shown that caveolae and caveolin proteins modulate mitochondrial function to protect from injury and stress in the heart [2–6]. Several studies highlight the importance of caveolae as critical regulators of intracellular mitochondrial trafficking, membrane structure, the antioxidant capacity of the respiratory chain, energy, and metabolism [4,7,8]. Nonetheless, the mechanisms by which caveolae and caveolin proteins directly modulate mitochondrial function and structure remain to be clarified. The goal of this review is to discuss how caveolin proteins and caveolae modulate mitochondrial biogenesis, structure, and function to help maintain balance and adaptation to cellular stress. We will also summarize microscopic, biochemical, proteomic, pharmacological, and genetic approaches for examining caveolin proteins and caveolae structure. Last, we will examine the protective role of caveolae against mitochondrial dysfunction in many disorders such as cardiovascular disease and diabetes.

## Caveolae and caveolin

In the early 1950s, Palade et al. first described caveolae, which are flask-like invaginations (<100 nm diameter) of the plasma membrane [9,10]. Caveolae are considered to be a subset of lipid rafts [11] and possess particular lipids (e.g. cholesterol, glycosphingolipids) and scaffolding proteins that interact with a wide variety of signaling molecules. Caveolae are found in many cell types such as adipocytes, endothelial cells, myocytes, and fibroblasts [12–14], yet the physiological roles are different depending

Received: 21 November 2019
Revised: 9 January 2020
Accepted: 10 January 2020

Version of Record published:
3 February 2020

Biochemical Society Transactions (2020) 48 165–177
https://doi.org/10.1042/BST20190388

on the cell type. However, some cell types such as neurons and leucocytes are essentially devoid of the physical caveolar structure though they contain caveolin protein suggesting the potential for the non-overlapping function of the structure and protein [15].

Caveolins, discovered in the early 1990s, are structural and scaffolding proteins in caveolae with a unique hairpin orientation that allows both the amino and carboxyl termini to face the cytoplasm. There are three caveolin isoforms, caveolin 1 (Cav-1), caveolin 2 (Cav-2), and caveolin 3 (Cav-3). Cav-1 and Cav-2 are expressed in multiple cell types, whereas Cav-3 is found primarily in striated (skeletal and cardiac) muscle and certain smooth muscle cells [16]. Caveolins have a 20 amino acid scaffolding domain (caveolin scaffolding domain, CSD) that anchors and recruits signaling molecules such as adenylyl cyclase (AC) [17], heterotrimeric Gα and Gβγ [18], Src [18,19], PI3 kinase (PI3K), endothelial nitric oxide synthase (eNOS, NOS 3) [20–22], protein kinase A (PKA) [23,24], protein kinase C (PKC) [25], and mitogen-activated protein kinase (MAPK) [26] to caveolae to provide direct temporal and spatial regulation of signal transduction. However, several studies (reviewed by Collins and Bryne) suggest that binding motifs and sites for the CSD within other proteins may exhibit structural constraints that preclude binding of the CSD region with these suggested signaling proteins [27–29]. Data from our laboratory also show that treatment of the CSD-3 in Cav-3 KO mice was not able to restore or compensate for hypertrophic loss and signaling pathways suggesting additional binding domains may be necessary for caveolin function [30].

Additional structural components of caveolae are the cavin family of proteins composed of cavin 1 [polymerase transcript release factor (PTRF)], cavin 2 [serum deprivation protein response (SDPR)], cavin 3 [srd-related gene product that binds to c-kinase (SRBC)], and cavin 4 [muscle-restricted coiled-coil protein (MURC)]. They form higher-order, hetero-oligomeric assemblies, which regulate distinct aspects of caveolar formation and function, such as recruitment of the cavin complex from the cytosol to the plasma membrane [31], the caveolar invaginations [32], and caveolar endocytosis [33]. Loss of the cavin proteins prevent caveolae formation and has been reviewed in many disease pathologies [34–36].

Genetic approaches have been used to provide convincing evidence in determining the phenotypes involved in the caveolar function and interface with other organelles. siRNAs targeted to caveolins are commonly used to decrease caveolin expression [37]. DNA constructs that increase caveolin expression wholly or partially are also widely used [38,39]. Genetic approaches using knockout (KO) caveolin-1, -2, -3 mice, and caveolin-1/-3 double KO mice have been generated [40]. Caveolin KO mice are viable and fertile but present a large number of phenotypes [41]. Many findings using these approaches suggest a link between caveolin and mitochondrial interactions [5,42,43] (Table 1). Both Cav-1 and -3 KO mouse models show metabolic dysregulation. Fridolfsson et Al. showed that Cav-3 KO mice have increased oxidative stress and Cav-1 KO studies using *Caenorhabditis elegans* also show similar mitochondrial dysregulation [5]. Additionally, work from our laboratory shows Cav-3 KO mice show more fragmented mitochondria with increased reactive oxygen species (ROS) production in these hearts compared with WT and mice overexpressing Cav-3 [5]. Taken together, these findings indicate that caveolae and caveolins may play a role in metabolic dysfunction.

## Caveolin in mitochondrial biogenesis and dynamics and mitophagy

Methods for ultrastructural and morphological characterization are available for assessment of the interface between caveolae and other cell components, including mitochondria [5]. Moreover, high-resolution 3D imaging such as confocal z-stacks, stimulated emission depletion, and electron microscopy tomography, allows for not only the spatial mapping of the positions but also interactions of macromolecules within their cellular components at an increasing resolution [55]. In particular, electron microscopy tomography revealed direct contacts of mitochondria and invaginations of the plasma membrane, which are difficult to identify without 3D tracing [56]. Furthermore, 4D live-cell imaging using viral vector transfection of GFP-tagged caveolin protein or fluorescence resonance energy transfer (FRET) are powerful tools for examination of temporally and spatially specific caveolae and the dynamic nanocontacts between caveolae and other organelles [57,58]. These tools have provided fundamental information on mitochondria dynamics identifying variations in morphology such as length, size, and number and how these changes compensate for cellular damage and to maintain function during stress.

Mitochondrial dynamics rely on co-ordinated control of mitochondrial biogenesis by balancing fusion, which is the mixing of healthy and damaged mitochondria to minimize damage, and fission, the generation of

**Table 1 Studies examining mitochondria–caveolin contacts and mitochondrial dysfunction**

| Mitochondrial structure/function | Cell type/ model | Method | Caveolin type and mechanism of action | Reference |
|---|---|---|---|---|
| Mitochondrial dynamics | | | | |
| Fusion and fission | Cardiac HL-1 Cell Line | Immunoblotting, Immunocytochemistry, Fluorescent Microscopy | Cav-3 increased Mfn1 protein levels | [44] |
| | He La Cells | Confocal Microscopy, EM, Cell Fractionation, Immunoblotting | Cav-1 increased Drp1 protein levels | [45] |
| Mitophagy | Lung Cancer cell line | Immunofluorescence, Immunoblotting | Cav-1 increased Parkin 1 | [46] |
| | Cardiac Myocyte | Coimmunoprecipitation, Fluorescent Microscopy, Immunoblotting | Cav-3, BECN1, Atg, p62, LC3II | [44] |
| Structure | | | | |
| SSM | Cardiac Myocyte | TEM, Cell Fractionation, Immunoblotting | Cav-3, SSM localization | [6,47,48] |
| Mitochondrial membrane | Cardiac Myocyte | EM, Immunohistochemistry | Cav-1/Cav-3 localization | [5,49] |
| Nanocontacts | | | | |
| ER-Mitochondria MAM's | Hepatocytes Microglia | Immunofluorescence, EM EM | Cav-1Cav-1 Cav-1 Cav-3 | [42] [50,51] |
| Adaptation to stress and injury | Cardiac myocytes *C. elegans* Colon Cancer Cell Line | EM, Cell Fractionation, Immunoblot | Cav-3 Cav-3 Cav-3 | [2,5,49] |
| ROS dysfunction | | Immunofluorescence, Immunoblot | NADPH Oxidase | [52,53] |
| Energy and metabolism | Endothelial | Immunocytochemistry | Cav-1 knockdown, decreased ATP and $Ca^{2+}$ flux | [54] |

new mitochondria to mitigate the consequence of damaged mitochondria (reviewed by van der Bliek and Youle) [59]. Regulation of both fusion and fission can vary by cell type, energy demand, and severity of damage [60–62]. Regulatory mitochondrial fusion proteins Mfn1 and 2 and dynamin-related GTPase, OPA1, are critical regulators for mitochondrial fusion [61,63] while dynamin-related protein 1 (Drp1) regulates mitochondrial fission [64]. The dynamic balance of fusion versus fission impacts individual mitochondrial networks, fragmentation, size, number, and overall efficiency of the organelle [61]. Several studies have identified that caveolin protein contacts with mitochondria provide protection against dysfunction, but few elucidate the role of caveolin on mitochondrial dynamics and biogenesis. In HL-1 cardiac myocytes, simulated ischemia–reperfusion (I/R) injury increased mitochondrial fusion protein Mfn1 in Cav-3 overexpressing cells but not in cells lacking Cav-3 [44]. Using a cancer cell line, Bravo-Sagua et al. [45] showed that Cav-1 enhanced mitochondrial fission protein Drp1. Such data suggest caveolin isoforms can regulate mitochondrial fusion dynamics in a variety of cell types.

If mitochondrial fission and fusion fail to restore damaged mitochondria, selective autophagic degradation known as mitophagy [65], can also restore the balance of the number of healthy mitochondria. While there are limited studies on the role of caveolin in mitochondrial autophagy, recent work by Liu et al. has demonstrated in a lung cancer cell model examining mechanisms of cisplatin chemotherapy resistance, that overexpression of Cav-1 increased mitophagy by inhibiting the Parkin1/Rho-associated coiled-coil kinase 1 pathway. They observed increased cisplatin resistance and cancer cell proliferation by restoring mitochondrial survival and

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https://doi.org/10.1042/BST20190388

function. However, loss of caveolin in these cells increased mitochondrial oxidative stress, decreased membrane potential, and increased the apoptotic marker caspase-9 [46]. While there are no specific studies on caveolin mediated mitophagy in the heart, our laboratory has shown that caveolin regulates autophagy in response to I/R injury. In cardiac myocytes, Cav-3 co-immunoprecipitated with autophagy markers BECN1, Atg, p62, and LC3 II, and siRNA knockdown of Cav-3 decreased this response [44]. However, differential effects of Cav-1 particularly in cancer cells have been shown to either stimulate or inhibit cell proliferation depending on the cancer type, subcellular location, and metabolic conditions. While the use of marker proteins, particularly with caveolin antibodies are widely used, coimmunoprecipitation of caveolins and components localized with caveolins have been useful to assess binding interactions that may have physiological consequences [38,66]. Given technical complications associated with such techniques (i.e. contamination, non-specificity of antibodies, secondary interactions, etc.), it is important to note that newer approaches such as proximity biotinylation (BioID) may overcome many of these limitations and lead to novel insights into caveolar/caveolin/cavin binding partners [67,68]. Future studies should include caveolin regulation of mitochondrial quality control of fission and fusion dynamics and association with Drp1, Mfn1 and 2 proteins, and mitophagy pathways during injury.

The distinct location and distribution of mitochondria throughout the cell can also modulate communication with subcellular compartments of the cell [69]. In cardiac myocytes, mitochondria localize in two distinct populations that differ in their spatial arrangement, structure, and function. Subsarcolemma mitochondria (SSM) are located directly beneath the plasma membrane and are more susceptible to modulation by signal transduction events, while interfibrillar mitochondria (IFM) are located in cardiac cells between myofibrils [70]. Transmission electron microscopy (TEM) revealed that SSM are elongated with more dense cristae to account for higher ATP production, whereas IFM are smaller and round in structure with fewer cristae [71]. Both SSM and IFM have been shown to respond differently to cellular injury and stress [71,72] in many cardiac pathologies such as I/R, diabetes, and aging [70,72]. However, recent studies have shown there is a differential association of caveolae with SSM versus IFM and that Cav-3 specifically protects SSM from post-ischemic injury [6,47,48]. Furthermore, following ischemic preconditioning, there was a higher association of Cav-3 with SSM and increased s-nitrosylated proteins and eNOS/NO signaling, which is cardioprotective [6,47]. However, the signaling mechanisms that allow caveolae to target only SSM versus IFM preferentially remain to be clarified [73]. The role of caveolin with these two populations may reflect that caveolae are located at the surface of the sarcolemma along transverse tubules near SSM. It is also possible that higher susceptibility of subsarcolemma to ischemic injury impairs membrane integrity, thus requiring a greater need for protection [74]. Lastly, the increased susceptibility of SSM to oxygen and whether this serves as a caveolae-mediated sensor to target SSM over IFM preferentially are areas for future study [69].

Numerous studies have shown that caveolin proteins bind cholesterol and regulate plasma membrane structure and fluidity in several intracellular organelles. Particularly in the mitochondria, caveolin transports cholesterol to the mitochondria promoting membrane fluidity for the diffusion of oxygen and stability for respiratory chain components [7,75,76]. Loss of caveolin, however, results in increased accumulation of cholesterol in mitochondrial membranes and disrupts mitochondrial function with reduced membrane fluidity, the efficiency of the respiratory chain, and diffusion of antioxidant enzymes across the membrane [77]. Oxygen availability to the mitochondria is a critical step for the efficiency of ATP synthesis and handling the generation of ROS, a process that may be modulated by caveolae [7,78] but needs further investigation.

## Caveolin and mitochondria contacts

The plasma membrane has been reported to be in close contact with mitochondria in several mammalian cell types [79–81]. It is, however, not clear whether and how mitochondria directly come in contact with caveolae at the plasma membrane. High-resolution morphological methods, such as electron microscopy and electron tomography, are available for anatomic assessment of contacts between mitochondria and caveolae at the plasma membrane [56]. Recent evidence from our laboratory using these methods show that caveolae form nanocontacts with mitochondria close to the plasma membrane which may serve to more efficiently manage metabolism and energy for stress adaptation [5,82]. Moreover, our group and others have shown in the heart and isolated cardiac myocytes from cardiac-specific Cav-3 overexpressing mice that upon stress these microdomains form a physical interaction that may be dependent on G-protein activation [2,5], and may specifically target mitochondrial function [6]. Proteomic methods revealed that a large number of proteins

localize to caveolae [83,84]. In-depth mass spectrometry of plasma membrane fractions by density gradient centrifugation [42,85] also suggests that caveolar proteins interact with elements of mitochondria [86]. Such approaches have the potential to reveal the full range of protein partners in caveolae, and such approaches may become invaluable in characterizing caveolae as they are dynamic entities forming and deforming in response to stimuli.

Several studies have shown caveolae are in direct juxtaposition to mitochondria and that caveolin proteins localize within mitochondrial membranes [5], and loss of these caveolin contacts negatively regulates mitochondrial function in many tissues. Work from our laboratory has shown that cardioprotective stimuli to reduce ischemic injury, increases caveolae formation, and caveolin contacts with the mitochondrial membrane [5,49]. Additional studies show that disruption of Cav-1 and mitochondrial contacts increased mitochondrial free radicals while overexpression of Cav-3 increased complex I and IV activity, decreased ROS production, and increased mitochondrial fragmentation. Gene targeted delivery of Cav-3 directly into the mitochondria further increased cardiac protection following I/R injury evidenced by increases in mitochondrial $Ca^{2+}$ flux, electron transport chain complex activity, and reduced infarct size [5]. Additionally, disruption of mitochondrial localized caveolin attenuated protection in these mice overexpressing Cav-3; these effects were not organismal, or cell-specific, as studies with caveolin mutant *C. elegans* also showed reduced mitochondrial function [5]. Even in proliferating colon cancer cells, caveolin reduced apoptotic cell death, increasing cell survival [5]. Altogether these studies show that the relationship between caveolae, caveolin, and the mitochondria appears to be critical for survival and adaptation to injury.

In addition to the plasma membrane and mitochondrial dynamics, caveolae and caveolin proteins mediate intra organelle contacts between the mitochondria and endoplasmic reticulum (ER) [8,42] in adipocytes [87], hepatocytes [42], and microglia [50,51]. Cav-1 is an integral component of mitochondria-associated domains (MAM's), directing ER communication with mitochondria and calcium signaling. For example, Cav-1 KO mice showed reduced ER-mitochondria contacts in the liver and increased lipid exchange [42]. Bravo-Sagua et al. [45] showed in HeLa cells that Cav-1 deficiency reduced the percent of ER-mitochondria contacts, and this was also associated with the increased mitochondrial length and free cholesterol accumulation at the ER membrane. Consequently, loss of caveolin increased apoptosis and calcium ion flux.

## Caveolin and mitochondrial energy, metabolism, and redox signaling

Mitochondrial bioenergetics generate the most substantial amount of ATP for the cell's metabolic needs. Structural mitochondrial dynamics mitigate metabolic efficiency, and several studies have examined the direct role that caveolin plays in this response. Cav-1 deficient mice display many phenotypes with an altered metabolic function such as lower body weight, insulin resistance, and reduced glucose metabolism with impaired pyruvate and fatty acid metabolism in the mitochondria [4,87,88]. Studies examining the impact of shear stress generated by hemodynamic blood flow showed increased ATP levels into caveolae of endothelial cells; however, disruption of caveolae by methyl-β-cyclodextrin (MCβD), a pharmacological agent commonly used to study caveolae function that binds to cholesterol destroying caveolae structure [66], reduced ATP levels [54]. Furthermore, knockdown of Cav-1 in endothelial cells using siRNA abrogated mitochondrial ATP generation and subsequent $Ca^{2+}$ release into the intracellular space [54]. The mechanism of how caveolin and caveolae trigger mitochondrial ATP production and translocation in this process remains unclear.

Caveolin rich mitochondrial membranes stabilize oxygen permeability and sequester superoxide within caveolar membrane structure and loss of caveolar structures as a result of aging, disease, or injury can reduce membrane oxygen permeability generating increased superoxide anions (Figure 1). Several studies have shown that caveolin proteins interact with many redox enzymes sequestered within caveolae and that these protein interactions modulate enzyme activity and ROS production [82]. For example, antioxidant flavonoids have been shown to reduce superoxide production by increasing Cav-1 levels. Additionally, ROS producing enzyme NADPH oxidase has been shown to localize to caveolae, and Cav-1 protein levels decreases its activity on superoxide production [52,53,89]. Thus, compartmentalization of ROS enzymes within caveolae can modulate their ability to modify and generate reactive species separately from the mitochondrial respiratory chain complexes. However, more recent studies have identified mitochondria–caveolin interactions in many cell types that directly result in abnormal increases of ROS due to respiratory chain damage, compromised ATP production, fatty acid, and glycolytic metabolic shifts [4]. For example, Volonte et al. showed that Cav-1 mediates

*Biochemical Society Transactions* (2020) **48** 165–177
https://doi.org/10.1042/BST20190388

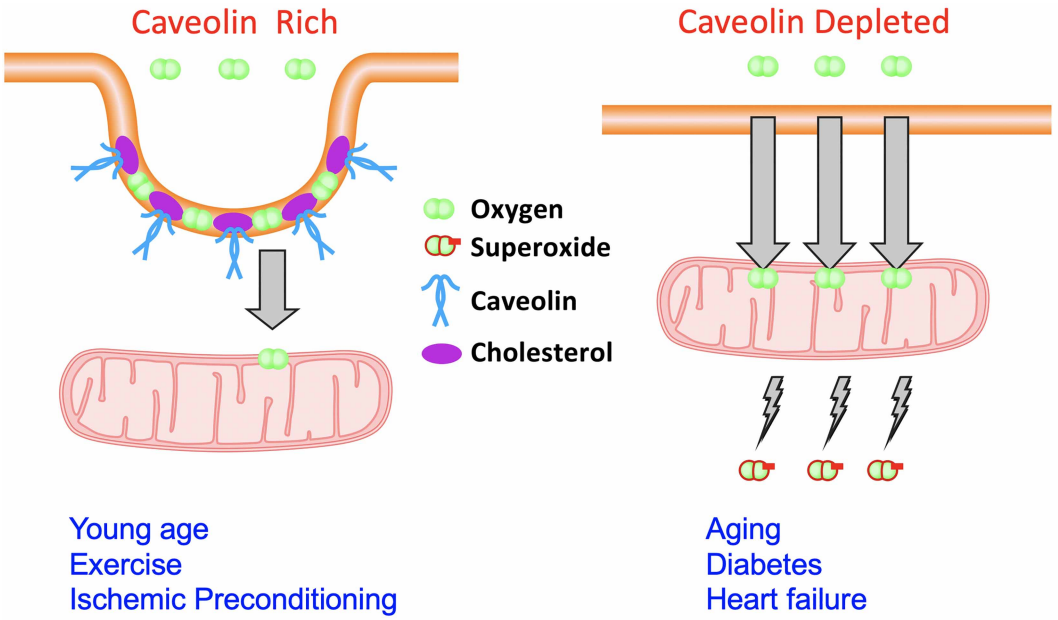


**Figure 1.** A cartoon depicting the association of caveolae with mitochondria leading to stress resilience and homeostasis, whereas loss of this relationship leads to pathophysiology.

translocation of the mitochondrial m-AAA protease AGL5445 to the mitochondria, and this prevents the degradation of complex I–IV in the presence of oxidative stress. This interaction was diminished in Cav-1 deficient cells [90]. Several studies also show caveolae and caveolin proteins localize with many mitochondrial specific oxidative/redox signaling components [4,5,91]. Cardiac specific overexpression of Cav-3 reduced superoxide production [5] and siRNA knockdown of Cav-1 increased $H_2O_2$ and ROS in endothelial cells [4]. These data indicate a dual role for caveolin via oxidative stress to modulate ROS production either by caveolae sequestering mitochondrial oxidant enzymes or direct caveolin-mitochondrial contacts to influence function.

# Caveolin, disease, and mitochondria

Caveolin knockout mice exhibit cardiovascular disease, diabetes, obesity, cancer, atherosclerosis, early aging, and pulmonary fibrosis [92,93], and mitochondrial dysfunction is a central element in each of these diseases. While this highlights a critical role for the caveolin proteins in disease, loss of caveolin does not result in a lethal phenotype suggesting loss of caveolin is essential for adaptation to injury or stress resulting in each of the above conditions. Additionally, several Cav-3 mutations have been described in human muscular dystrophies (MD) causing progressive weakness and degeneration of skeletal muscles [94–96]. Cav-1 KO mice also show increased death and premature aging often associated with redox stress [97]. On the other hand, Cav-1 may play a role in tumor growth. Some studies showed the expression of Cav-1 in human cell lines or tumor samples depends on tumor type and cell stage, thereby leading to oncogenic or tumor-suppressive functions [46,98,99].

## Cardiac protection

The heart is composed of several cell types (i.e. myocytes, endothelial cells, fibroblasts, and smooth muscle cells) that express different caveolin isoforms with cardiomyocytes expressing Cav-3, and the other cell types expressing Cav-1 and Cav-2 [14,16]. Myocardial infarction is a major cause of morbidity and mortality. It is known that I/R injury activates p42/44 and p38 MAPKs, redistributes Cav-3, and down-regulates the expression of Cav-1 suggesting the involvement of multiple cell types in the cardiac stress response. Ischemic preconditioning is one of the most potent interventions leading to protection of cardiomyocytes following I/R injury [100], and the role of caveolin in regulating IPC and the molecular signaling cascade

that has become known as the reperfusion injury salvage kinase (RISK) pathway is well described [101]. Studies in Cav-1 and Cav-3 KO mice showed loss of cardiac protection against ischemia stimuli [51,102–104] and restoration of the preconditioning phenotype by cardiac myocyte-specific overexpression of Cav-3 (Cav-3 OE) [49].

The distinct links between caveolar structure, caveolins, and cardiac protection regarding hypertrophy and heart failure also exist. A dilated cardiomyopathy characterizes impairment of heart function in Cav-1 KO mice with an enlarged left ventricular diameter, wall thinning, decreased systolic function, and decreased contractility [105,106]. Cav-3 KO mice result in hyperactivation of the Ras/extracellular signal-regulated kinases (ERK) 1/2 signaling pathway, cardiac hypertrophy, and reduced cardiac function [107]. Cav-1/Cav-3 double KO mice completely lack morphologically identifiable caveolae and develop a severe cardiomyopathic phenotype with left ventricular hypertrophy and dilation [108]. Overexpression of Cav-3 *in vitro* [109] and *in vivo* [38,110] results in blunted hypertrophy. There is evidence to suggest in Cav-3 KO, Cav-3 OE, and studies manipulating caveolin-protein interactions that the hypertrophic response may involve regulation of t-tubules and calcium channels [38,111–114]. These results suggest a potential role for caveolins in cardiac hypertrophy and heart failure though further investigation is necessary to determine the direct mechanistic role of Cav-3 in regulating cell hypertrophy.

## Diabetes

Several studies suggest that caveolae and caveolins may play a significant role in insulin signaling. Goldberg et al. [115,116] demonstrated that gold-labeled insulin, but not beta 2 macroglobulin-methylamine, is endocytosed by a mechanism involving uncoated invaginations in rat adipocytes, and the insulin receptor (IR) is highly enriched in caveolae [117]. A role for caveolae and caveolins in insulin signaling was further established when the IR beta-subunit was found to contain the characteristic caveolin-binding motif, and the scaffolding domain of caveolin was shown to augment the activity of the IR kinase as well as target the IR to caveolae [118,119], early discoveries important in linking caveolin to insulin. The exact role of caveolae and caveolin in GLUT4-mediated glucose uptake remains controversial. It is suggested that caveolae and caveolin also affect the downstream insulin-dependent cellular effects via a role in GLUT4-mediated glucose transport and are involved in 3T3-L1 adipocytes [120–122], but further investigation is necessary. More recent studies suggest there may be links to variations in Cav-1 gene and high serum triglyceride levels [123], potential of Cav-2 in regulating insulin sensitivity [124], impact of common Cav-3 mutations in muscle glucose disposal [125], and the impact of post-translational modification of caveolin in regulating pancreatic insulin signaling [126]. The plethora of research linking caveolin to diabetes has strong potential to propel future research in this area leading to clinical translation.

Cav-1 KO mice which lack of caveolin and caveolae in adipocytes, when exposed to high-fat diet, but not normal diet, showed postprandial hyperinsulinemia, in addition to elevated serum triglycerides and free fatty acids, suggesting the development of insulin resistance [127]. It was also demonstrated that a dramatic reduction in IR phosphorylation caused by the reduction in the IR protein in the presence of normal insulin expression was shown in fat pads of Cav-1 KO mice, indicating Cav-1 may be necessary for IR protein stability in adipose tissues [128]. Cav-3 KO mice showed blunted insulin-stimulated activation of the insulin signaling component in the skeletal muscles [129]. Taken together, these findings indicate that caveolae and caveolins may play a role disease such as diabetes that lead to metabolic dysfunction.

# Conclusion

Since their original discovery in the early 1950s, caveolae have emerged as dynamic entities that impact organ and cell physiology and pathophysiology. The discovery of caveolin proteins has led to further mechanistic insights into how these proteins and the microdomains they critically form regulate cellular processes. What has emerged is a sense that caveolins, though initially thought of as residing in and regulating cell membrane-associated functions, are more ubiquitously expressed in many cellular compartments. Their critical localization in multiple membranes is helping to drive and define the diverse role of these proteins in biology. Importantly, such discoveries are leading the way for novel insights into cell biology and metabolism that will hopefully someday lead to fundamental therapeutic approaches for cardiovascular disease, cancer, diabetes, neurodegeneration, diabetes, and many more ailments impacting human health.

*Biochemical Society Transactions* (2020) **48** 165–177
https://doi.org/10.1042/BST20190388

## Perspectives

- **Importance to the field:** Changes in mitochondrial function and structure correlate with the pathophysiology in many diseases. Caveolins may serve as a novel therapeutic target for disease where mitochondrial function and structure need to be modulated.

- **Current thinking:** Traditional thinking of caveolin and caveolae localized to the plasma membrane is evolving to consider these proteins and domains as regulators of other membranes found in a cell. This is leading to the idea that caveolin may cycle in a cell creating a homeostasis that is altered in pathophysiology.

- **Future directions:** With increasing evidence to suggest their role in diseases, effort needs to be places in developing therapies (i.e. gene therapy based, small peptides, etc.) that can modulate caveolin biology. Additional work needs to be done to determine how caveolae sense cellular and mechanical stress and transduce into survival signals to the mitochondria to modulate function and structure. Caveolae regulate many signal transduction molecules, thus, it will be critical to also delineate cross-talk pathways and how specific interactions are determined in a cell or domain of a cell. Insights into these open questions will further our understanding of caveolin in the regulation of biology.

### Abbreviations

Cav-1, caveolin 1; Cav-2, caveolin 2; Cav-3, caveolin 3; CSD, caveolin scaffolding domain; AC, adenylyl cyclase; PI3K, PI3 kinase; eNOS, endothelial nitric oxide synthase; PKA, protein kinase A; PKC, protein kinase C; MAPK, mitogen-activated protein kinase; PTRF, polymerase transcript release factor; SDPR, serum deprivation protein response; SRBC, srd-related gene product that binds to c-kinase; MURC, muscle-restricted coiled-coil protein; KO, knockout; FRET, fluorescence resonance energy transfer; Mfn1 and 2, mitochondrial fusion proteins; Drp1, dynamin-related protein 1; I/R, ischemia–reperfusion; SSM, subsarcolemma mitochondria; IFM, interfibrillar mitochondria; TEM, transmission electron microscopy; ROS, reactive oxygen species; MAM's, mitochondria-associated domains; ER, endoplasmic reticulum; MCβD, methyl-β-cyclodextrin; siRNA, small interfering RNA; NADPH, nicotinamide adenine dinucleotide phosphate; RISK, reperfusion injury salvage kinase; IR, insulin receptor.

### Author Contributions

C.R.F., S.S., Y.K., and H.H.P. drafted and revised the manuscript.

### Acknowledgements

This work was supported by grants from the American Heart Association (19AIREA34470006 to CRF), the National Institutes of Health (NIH) (HL091071, to H. H. Patel), and the Veterans Affairs (BX001963, to H. H. Patel).

### Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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