BJP British Journal of Pharmacology

DOI:10.1111/bph.13009
www.brjpharmacol.org

Themed Section: Conditioning the Heart – Pathways to Translation

REVIEW

Caveolins in cardioprotection – translatability and mechanisms

Jan M Schilling, David M Roth and Hemal H Patel

VA San Diego Healthcare System, San Diego, CA, USA, and Department of Anesthesiology, University of California, San Diego, La Jolla, CA, USA

Correspondence
Hemal H Patel, University of California, San Diego, Department of Anesthesiology, VASDHS (9125), 3350 La Jolla Village Drive, San Diego, CA 92161, USA. E-mail: hepatel@ucsd.edu

Received
29 September 2014
Revised
27 October 2014
Accepted
3 November 2014

Translation of preclinical treatments for ischaemia-reperfusion injury into clinical therapies has been limited by a number of factors. This review will focus on a single mode of cardiac protection related to a membrane scaffolding protein, caveolin, which regulates protective signalling as well as myocyte ultrastructure in the setting of ischaemic stress. Factors that have limited the clinical translation of protection will be considered specifically in terms of signalling and structural defects. The potential of caveolin to overcome barriers to protection with the ultimate hope of clinical translation will be discussed.

LINKED ARTICLES

This article is part of a themed section on Conditioning the Heart – Pathways to Translation. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2015.172.issue-8

Abbreviations

ANT, adenine nucleotide transporter; Cav, caveolin; CR, caloric restriction; CSD, caveolin-scaffolding domain; eNOS, endothelial NOS; GLUT4, glucose transporter 4; IPC, ischaemic preconditioning; KO, knockout; mPTP, mitochondrial permeability transition pore; ROS, reactive oxygen species

Tables of Links

TARGETS	
Enzymes[a]	**Transporters**[b]
Akt	ANT, adenine nucleotide transporter
eNOS	GLUT4, glucose transporter 4
ERK	**Catalytic receptor**[c]
F_0F_1 ATP synthase	Insulin receptor
Glycogen synthase kinase-3β	**Ion channels**[d]
PI3K	K_{ATP} channels, $K_{ir}6.2$
PKC	**GPCR**[e]
Src kinase	Adenosine A_1 receptor

LIGANDS
Resveratrol
TNFα

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b,c,d,c]Alexander *et al.*, 2013a,b,c,d,e).

Introduction

Myocardial infarction is a major cause of death in the United States. One of the most beneficial experimental interventions to produce cardiac protection is termed ischaemic preconditioning (IPC) where sublethal ischaemia protects from subsequent lethal injury (Murry *et al.*, 1986). Defining molecular events regulating IPC has potential implications for therapeutic strategies for ischaemic heart disease and broader implications for cardiac hypertrophy, heart failure, diseases that secondarily lead to cardiac dysfunction (e.g. diabetes, hypertension) and other conditions where the balance between cell death and survival is critical. Despite a considerable amount of data describing preconditioning signalling, a precise pharmacological target and therefore a specific therapeutic agent remains elusive. This review will focus on limitations of protective interventions in the heart and a novel observation that caveolae, lipid-rich membrane microdomains enriched in caveolin-scaffolding proteins, may be a means to break down these barriers to the translation of experimental cardiac protection to clinical practice.

What is the state of understanding of mechanisms leading to IPC?

Following the seminal discovery of IPC by Murry, Jennings and Reimer in 1986 (Murry *et al.*, 1986), two parallel ideas have developed over the last ~25 years to account for IPC and provide a path for therapeutic development. One is the *signalling* hypothesis which proposes molecules converge to change the biochemistry and metabolism of the cell to affect protection and the other is the *structural* hypothesis in which IPC provides a physical, structural resiliency to the cardiac myocyte. The ratio of published papers is skewed 100-fold in favour of investigations focused on the signalling hypothesis.

Many studies have described IPC as a promiscuous stimulus that involves the initiation of many shared and interconnected signalling pathways that ultimately converge upon the mitochondria to cause cell protection and survival (Hausenloy *et al.*, 2005; Hausenloy and Yellon, 2006). The signal transduction pathways involve sequential triggers, mediators and end effects that culminate in modulation of mitochondrial function (Juhaszova *et al.*, 2004; Hausenloy and Yellon, 2006). Three main mitochondrial phenomenon – (i) opening of mitochondrial ATP-sensitive potassium (K_{ATP}) channels; (ii) generation of a small burst of reactive oxygen species (ROS) and (iii) maintenance of the mitochondrial permeability transition pore (mPTP) – have all been linked to the cardiac protective effects of IPC (Auchampach *et al.*, 1992; Gross and Auchampach, 1992; VandenHoek *et al.*, 1998; 2000; Becker *et al.*, 1999; Yao *et al.*, 1999; Pain *et al.*, 2000).

Following their initial discovery of IPC, Murry *et al.* showed in 1990 that IPC delays ultrastructural myocardial damage (i.e. to membrane and mitochondria) during subsequent lethal ischaemia (Murry *et al.*, 1990). This led to exploration of the 'structural protective' hypothesis where IPC was shown to preserve membrane integrity and mitochondrial structure (Moolman *et al.*, 1995; Armstrong *et al.*, 2001). Although limited in number, some proposed effectors of this structural preservation have been postulated: the mPTP (Fang *et al.*, 2008), dystrophin and spectrin (Armstrong *et al.*, 2001; Kido *et al.*, 2004), ROS (Miyamae *et al.*, 2002) and K_{ATP} channels (Geshi *et al.*, 1998). This concept was expanded to include preservation of mitochondrial function and structure by IPC and postconditioning in heart and liver (Zhong *et al.*, 2000; Giovanardi *et al.*, 2009; Penna *et al.*, 2009; Quarrie *et al.*, 2012). What remained unknown was whether IPC prevented injury by directly modulating structure or if signalling events initiated by IPC activated membrane repair processes that helped to maintain membrane integrity during periods of stress. The latter possibility would suggest that the molecular signalling and structural protection afforded by IPC could be linked through some common factor. Preservation of cellular ultrastructure is a tightly regulated process that has at its core molecular signalling leading to membrane repair and dynamics in the sarcolemma and mitochondria (Donaldson *et al.*, 2009; Hausenloy and Yellon, 2010; Cao *et al.*, 2011; Gottlieb and Gustafsson, 2011; Mousley *et al.*, 2012). Finding a molecule that bridges signalling to preservation of cellular ultrastructure may provide a novel therapeutic target to protect against ischaemia-reperfusion injury.

Caveolae and caveolins: a bridge that provides a unifying feature to cardiac protection

Caveolae, or 'little caves', are cholesterol and sphingolipid-enriched invaginations of the plasma membrane (Palade, 1953) and are considered to be a subset of lipid rafts (Pike, 2003). Caveolins, the structural proteins essential for caveolae formation, are present in three isoforms (Chun *et al.*, 1994; Parton *et al.*, 1997). Caveolins have a 20 amino acid scaffolding domain (caveolin-scaffolding domain, CSD) that anchors and regulates proteins (Sargiacomo *et al.*, 1995; Feron and Balligand, 2006). Canonically, caveolin (Cav)-1 and Cav-2 were thought to be expressed in many cell types, while Cav-3 was found primarily in striated (skeletal and cardiac) muscle and certain smooth muscle cells (Song *et al.*, 1996). Such concepts are currently being challenged with the identification and description of the structural importance for Cav-3 in non-muscle cells (Niesman *et al.*, 2013) and the identification of a functional consequence of caveolin localized to a variety of cellular compartments (Head and Insel, 2007; Fridolfsson *et al.*, 2014).

The expression of caveolin isoforms in the heart has been hotly debated. It is accepted that cardiac myocytes express Cav-3, the muscle-specific isoform (Song *et al.*, 1996; Tang *et al.*, 1996), and that other cell types in the heart express Cav-1 and Cav-2. Recent studies have provided evidence for the existence of and a signalling role for Cav-1 in cardiac myocytes with respect to ischaemia-reperfusion injury and maintenance of cardiac gap junctions (Patel *et al.*, 2007; Yang *et al.*, 2014). It was previously thought that Cav-1 and Cav-2 form hetero-oligomers and Cav-3 forms homo-oligomers (Tang *et al.*, 1996; Scherer *et al.*, 1997) but more recent data indicate co-expression and interaction of Cav-2 and Cav-3 in neonatal cardiac myocytes (Rybin *et al.*, 2003) and interaction of Cav-1, Cav-2 and Cav-3 in adult cardiac myocytes

(Hagiwara *et al.*, 2002; Head *et al.*, 2006). Other findings show that cell type-specific environments may regulate the interaction of caveolins. Thus, in fibroblasts, Cav-1 and Cav-2, but not Cav-3, interact, whereas in myoblasts, all three caveolin isoforms co-immunoprecipitate (Capozza *et al.*, 2005) and in microglia Cav-1 and Cav-3 have very distinct roles depending on the metabolic and structural state of the cell (Niesman *et al.*, 2013). Studies of mice with knockout (KO) or transgenic overexpression of caveolins demonstrate that the expression of caveolin is both necessary and sufficient for the formation of caveolae. Recent studies have identified another protein, cavin, as a key component of caveolae although studies on the physiological and pathophysiological role of cavin in the heart are limited (Vinten *et al.*, 2005; Hill *et al.*, 2008; Liu and Pilch, 2008a).

Regulation of caveolae and caveolins is complex and may help explain the general role of this structural protein in regulation of a wide range of physiological cellular functions. Protein–protein interactions as a function of charge, size and/or steric factors may contribute to the localization of proteins within the caveolae (Yamabhai and Anderson, 2002; Nichols, 2003; Pike, 2005). In addition, being lipid-rich microdomains distinct from surrounding membranes, caveolae may facilitate lipid–protein interaction (Rothberg *et al.*, 1992; Park *et al.*, 2004). Lipid modification of proteins, in particular palmitoylation and myristoylation, contribute, for example, to the localization of G-protein signalling components in raft/caveolae domains (Ratajczak *et al.*, 2003; Razzaq *et al.*, 2004; Rodgers *et al.*, 2005; Kim *et al.*, 2006). As noted earlier, the CSD, a hydrophobic region in the cytoplasmic amino terminal tail that interacts with protein 'partners' through hydrophobic interactions, has been proposed as a critical region by which signalling proteins interact with caveolins (Chini and Parenti, 2004; Becher and McIlhinney, 2005), although this notion has been recently challenged (Collins *et al.*, 2012). Finally, caveolins can undergo post-translational modification that may be critical to regulating not only signalling proteins, but also the response to pathophysiology. Caveolins contain three C-terminal cysteine residues that contain putative palmitoylation sites (Cys^{133}, Cys^{144} and Cys^{156}) (Dietzen *et al.*, 1995). Cav-1 undergoes phosphorylation (at Tyr^{14}) (Rothberg *et al.*, 1992; Li *et al.*, 1996) and Src-mediated phosphorylation alters the properties of Cav-1, including its interaction with extracellular matrix proteins (Grande-Garcia *et al.*, 2007; Grande-Garcia and Del Pozo, 2008) and has other potential physiological functions (Patel *et al.*, 2007; Yang *et al.*, 2014). Although Cav-3 has a putative phosphorylation site, no studies have been published to identify this site or its functional significance. However, Cav-3 has been reported to be sumoylated which affects receptor desensitization (Fuhs and Insel, 2011). Thus, caveolae and caveolins appear to have a variety of ways to regulate cell function.

Cav-3 has been identified in the sarcolemmal membrane, transverse tubules (T-tubules), the I-band/A-band interface and localized with ryanodine receptors in myocytes (Ralston and Ploug, 1999; Scriven *et al.*, 2005). Caveolins are involved in many cellular processes including vesicular transport, cholesterol and calcium homeostasis (Fujimoto *et al.*, 1992; Fujimoto, 1993; Scriven *et al.*, 2002; Jones *et al.*, 2004; Peng *et al.*, 2004), signal transduction (Lisanti *et al.*, 1994; Steinberg and Brunton, 2001; Cohen *et al.*, 2004; Williams and Lisanti, 2004) and have been recently detected in the mitochondria (Li *et al.*, 2001; Fridolfsson *et al.*, 2012). Caveolins function as chaperones and scaffolds, recruiting signalling molecules to caveolae to provide direct temporal and spatial regulation of signal transduction (Shaul and Anderson, 1998; Williams and Lisanti, 2004). Caveolins can inhibit activity of signalling proteins by interaction of the CSD with a caveolin binding motif present in many proteins found in caveolae including endothelial NOS (eNOS) and ERK1/2 (Engelman *et al.*, 1998; Feron *et al.*, 1998; Kamoun *et al.*, 2006). Alternatively, caveolins can promote signalling via enhanced receptor-effector coupling or enhanced receptor affinity when caveolins are up-regulated or overexpressed (Feron and Balligand, 2006; Raikar *et al.*, 2006). This has led to the concept of a 'caveolar paradox' in which caveolins may produce direct allosteric inhibition of molecules such as eNOS under basal conditions but facilitate increased signalling upon agonist stimulation through compartmentation (Feron and Kelly, 2001; Feron and Balligand, 2006).

An emerging concept suggests that signalling molecules exist as multiprotein complexes, 'signalosomes', continuously forming and dissociating under basal or stimulated conditions (Feron and Balligand, 2006). Caveolins are thought to play an integral role in the dynamics of these multiprotein complexes. Specifically, in regard to signalling molecules involved in cardiac protection, many GPCRs including opioid (Head *et al.*, 2005) and adenosine receptors (Lasley *et al.*, 2000) localize to caveolae and co-immunoprecipitate with caveolins. Additionally, many of the signalling molecules involved in cardiac protection, including the Gα subunit of heterotrimeric G-proteins, Src kinases, PI3K, eNOS, PKC isoforms and ERK are known to bind with the scaffolding domain of caveolin and be regulated by caveolin (Krajewska and Maslowska, 2004; Ballard-Croft *et al.*, 2006). Caveolin is known to be a key component and activator of PI3K/Akt signalling (Fecchi *et al.*, 2006), a pro-cell survival pathway that plays a significant role in preconditioning in the heart.

Initial evidence implicating a role for caveolin in cardiac protection was confirmed by infusion of the CSD peptide of Cav-1 into ischaemic/reperfused hearts which resulted in recovery of cardiac function (Young *et al.*, 2001). It was later shown that ischaemia/reperfusion injury activates p42/44 and p38 MAPK, redistributes Cav-3 and down-regulates expression of Cav-1 (Ballard-Croft *et al.*, 2006). The critical links between caveolar structure, caveolin protein and cardiac protection have emerged from a series of studies conducted by our group showing that physical disruption of caveolae negated protection in adult cardiac myocytes (Patel *et al.*, 2006), loss of protection in Cav-1 and Cav-3 KO mice (Patel *et al.*, 2007; Horikawa *et al.*, 2008; Tsutsumi *et al.*, 2010a,b) and restoration of the preconditioning phenotype by cardiac-specific overexpression of Cav-3 (Tsutsumi *et al.*, 2008).

Why is clinical translation so difficult and is caveolin a potential solution?

Since the original discovery of IPC nearly three decades ago, numerous molecular mechanisms and a host of therapeutic

targets have been identified but not clinically translated. The root cause of this problem in clinical translation is likely to be complex. Simply put, the problem derives from the many complicating factors leading to a disconnection between the robustness of preclinical models, which are almost always performed in healthy young animals with no ongoing pharmacological treatments, or the various modifiers of protection, including age, sex, existing disease and drug treatment, that are present in patients. Could these complicating factors be somehow limited? The remainder of this review will explore the role of caveolin as a key feature to rescue protective pathways in pathophysiological settings.

Ageing

Why does the aged heart have decreased ischaemic tolerance?

As the population ages, there is an increasing challenge to preserve organ function in the face of disease and the well-known age-related changes in organ function and functional reserve. Age is the most important predictor of mortality in patients with ischaemic heart disease (Boersma *et al.*, 2000). Consistent with this clinical result, aged human atrial myocytes are not protected from ischaemic insults (Mio *et al.*, 2008). Studies with preclinical models also reveal an increased sensitivity and decreased tolerance to ischaemia-reperfusion injury in the aged heart (Headrick *et al.*, 2003; Willems *et al.*, 2005). Mechanisms that underlie this age-related deficit are not clear but are postulated to involve abnormalities in cellular signalling and mitochondrial function (Tani *et al.*, 2001; Lesnefsky *et al.*, 2006; Peart *et al.*, 2007) although other mechanisms, such as dysfunctional calcium homeostasis (Swynghedauw *et al.*, 1995), have been suggested.

Ageing also results in remodelling of mitochondria in terms of lipid content and membrane integrity (Pepe, 2005), defects in respiratory chain components (Lesnefsky *et al.*, 2001) and increased oxidant stress (Hagen, 2003) which ultimately lead to reduced capacity to exclude calcium, generate ATP and limit injury mediated by ROS (Jahangir *et al.*, 2001; Lakatta and Sollott, 2002; Lesnefsky and Hoppel, 2008). All of these factors may affect mPTP function. The molecular composition of the mPTP is controversial. Potential components of the mPTP have included the adenine nucleotide transporter (ANT) on the inner mitochondrial membrane and the voltage-dependent anion channel on the outer membrane, although genetic inactivation studies have suggested that neither of these components is necessary for mitochondrial permeability transition to occur (Bernardi, 2013). Cyclophilin D in the mitochondrial matrix also is thought to play a role in response to stress and the formation of the mPTP (Javadov and Karmazyn, 2007). A benzodiazepine receptor, hexokinase, and creatine kinase have also been proposed as regulators of the pore. Recent work has suggested the F_0F_1 ATP synthase forms a channel with properties similar to the functioning mPTP (Bernardi, 2013). It is unclear if cardiac protective agents act by inhibiting the opening of a preformed mPTP complex, a particular subunit of the complex, or the assembly or organization of the complex. Work in a model of protection has shown that increased phosphorylation of glycogen synthase kinase (GSK)-3β reduces the affinity of the ANT for cyclophilin D, suggesting that assembly of the complex is targeted by protective signals to limit mPTP opening (Nishihara *et al.*, 2007). Importantly, regulation of the pore is diminished with age (Jahangir *et al.*, 2001; Seo *et al.*, 2008), but the precise mechanism of modulation is unknown (Di Lisa and Bernardi, 2005). Age-related changes may involve, as with cellular signalling, altered organization and function of the mPTP leading to inefficiency and dysfunction. Data from *Drosophila* indicate that the only protein that shows age-associated increases in carbonyl modifications (an index of oxidative injury) is ANT, a change that results in loss of mPTP function, which is accelerated by pro-oxidant stimuli (Yan and Sohal, 1998).

The function of mitochondria is intimately connected to mitochondrial dynamics. Mitochondria are in equilibrium between fusion and fission events to maintain their morphology and function. When fusion is inhibited, mitochondria become fragmented resulting in reduced glucose oxidation, respiration and loss of mitochondrial membrane potential (Olichon *et al.*, 2003; Griparic *et al.*, 2004). When fission is inhibited, mitochondria become tubular and elongated (Stojanovski *et al.*, 2004). Fission is important for segregating irreversibly damaged mitochondria targeted for degradation. Excessive fission and lack of fusion result in loss of mitochondrial DNA, increased generation of ROS and loss of the mitochondrial network (Yaffe, 1999). Aged hearts have fewer mitochondria suggesting defects in fusion/fission.

Is caveolin a therapeutic target for reduced ischaemic tolerance with ageing?

The discussion thus far leads to two separate possibilities: (i) there is a potential ageing deficit that leads to an altered cardiac phenotype with age or (ii) the cellular environment created by ageing limits normal processes. Importantly, these are not mutually exclusive. Treatments aimed at restoring ischaemic tolerance in the aged myocardium must address the 'ageing deficit' and/or recreate a 'young environment' to alter not only cellular signalling but also restore dysfunctional mitochondria. From an experimental perspective, the only known external intervention to extend life in a number of species and reduce disease risk associated with ageing in primates and humans is caloric restriction (CR), an idea first conceptualized in 1935 (McCay *et al.*, 1935). CR is likely to activate or deactivate a number of pathways to extend lifespan and to enhance protective and repair processes. These pathways include mitochondrial function, dynamics and autophagy (Masoro, 2009). Interestingly, two reports suggest that CR prevents an age-related decline in Cav-1 expression in hepatic sinusoids (Jamieson *et al.*, 2007) and maternal CR elevates message for caveolin in the fetal cardiac left ventricle (Han *et al.*, 2004). Could these findings somehow indicate a role for caveolin in longevity?

Little is known regarding caveolin expression and ageing. Early studies of ageing in isolated senescent cells showed increases in caveolin expression (Volonte *et al.*, 2002; Cho and Park, 2005). However, in such studies, the concept of 'ageing' is contrived, as it is dependent on passage number. The concept that senescence is equivalent to ageing is flawed, as senescence is defined as an inability of cells to divide.

Cardiac myocytes contain high levels of caveolin and are senescent cells by definition (they do not undergo significant cell division) but not necessarily aged. Therefore, the role of caveolin in ageing must be considered from a cell-type and organ-specific perspective. Animal studies reveal organ-specific patterns of changes in caveolin expression with age. Importantly, a decrease in the expression of cardiac Cav-3 (Kawabe *et al.*, 2001) is observed as a function of age, a result we confirm in our preliminary data. Ageing results in dissociation of Cav-1 and Cav-3 from membrane caveolae (Ratajczak *et al.*, 2003). Cav-3 KO mice develop a progressive cardiomyopathy (Woodman *et al.*, 2002) and are also resistant to cardiac protective stimuli (Horikawa *et al.*, 2008). Cav-1-deficient mice show reduced lifespan and increased cardiac dysfunction (Park *et al.*, 2003) and are resistant to cardiac protective stimuli (Patel *et al.*, 2007). We have recently shown that ischaemic tolerance is reduced in human atrial tissue (Peart *et al.*, 2014). This observation was paralleled with the observation that Cav-3 is decreased in aged, compared with young, mouse hearts. We, furthermore, have indications that Cav-3 decreases with age in human hearts (unpublished data).

Caveolae are dynamic entities that form and dissipate in response to various stimuli (Tsutsumi *et al.*, 2008) and serve as a clathrin-independent mechanism for the endocytosis of plasma membrane constituents. Caveolae-mediated endocytosis facilitates transport of vesicles to other cellular regions and across the cell (transcytosis) (Mukherjee *et al.*, 2006; Ge *et al.*, 2008). Co-expression of flotillins 1 and 2 in caveolae enhances the accumulation of intracellular vesicles (Frick *et al.*, 2007). The fate of such vesicles is unknown. Recent data indicate that caveolae forms contacts with other cellular compartments to communicate membrane-derived signals to other organelles and regions of the cells. For example, smooth muscle cells have 'nanocontacts' between caveolae and the endoplasmic reticulum (Gherghiceanu and Popescu, 2007). Caveolins are found in cells and intracellular regions lacking caveolae, suggesting roles for caveolins in non-sarcolemmal locations (Head and Insel, 2007; Fridolfsson *et al.*, 2014). We have recently shown that there is a stress-adaptive transfer of caveolin to mitochondria which is facilitated by IPC that leads to protection of the heart from ischaemia-reperfusion injury and that this is a generalized protective pathway active in cancer and *Caenorhabditis elegans* and involves the activation of GPCR signalling and survival kinases (Fridolfsson *et al.*, 2012; Wang *et al.*, 2014). It is possible that a loss of caveolin expression with age affects the ability of the membrane not only to house and regulate survival kinases but also limits the ability of the cell to modulate mitochondrial function during stress.

Diabetes

Why is the diabetic heart dysfunctional?

According to the American Diabetes Association in the United States, there are nearly 26 million individuals, adults and children, with diabetes. In addition, there may be as many as 79 million individuals who are prediabetic. In 2007, diabetes was listed as the underlying cause of >70 000 deaths and a contributing factor of an additional 160 000 deaths. Those aged 65 years or older represent an ever-growing population facing the consequences of diabetes. In 2004, the most recent year for which statistics are available, heart disease was noted in nearly 70% of diabetes-related deaths among people 65 years or older and adults with diabetes have heart disease mortality rates that are two to four times higher than adults without diabetes.

Controversy exists as to whether cardiac events associated with diabetes are a consequence of underlying coronary artery disease and hypertension. Growing evidence suggests that diabetes results in altered cardiac structure and function independent of vascular pathology, supporting the existence of a 'diabetic cardiomyopathy'. Diabetes in animal models results in both diastolic (i.e. prolongation of relaxation and increased left ventricular end diastolic pressure) (Joffe *et al.*, 1999) and systolic (i.e. heart rate, systolic BP and fractional shortening) (Joffe *et al.*, 1999) dysfunction and such findings are also observed in humans (Poirier *et al.*, 2001). Structural changes also have been observed in the diabetic heart that include perivascular and interstitial fibrosis, possibly as a result of replacement of myocyte loss, altered mitochondrial structure and altered cardiac ultrastructure (Eto *et al.*, 1987; Warley *et al.*, 1995; Mizushige *et al.*, 2000). The molecular mechanisms proposed for diabetic cardiomyopathy are diverse and may include impaired calcium handling, altered substrate supply and utilization, altered energy generation with mitochondrial dysfunction, altered ion channel function, myocyte apoptosis, endothelial dysfunction, cardiac insulin resistance and activation of the renin-angiotensin system (Zhang and Chen, 2012). Additionally, diabetic hearts are refractory to protective interventions that limit ischaemia-reperfusion injury, suggesting major defects in survival kinase signalling (Balakumar and Sharma, 2012a). Such findings suggest that diabetic cardiomyopathy is a complex disease that is manifested with many cellular alterations that may or may not have a common control point of regulation that can be targeted therapeutically.

Are caveolins potential regulators of diabetes?

Cav-3 KO mice have a variety of deleterious phenotypes, such as muscle degeneration (Hagiwara *et al.*, 2000), insulin resistance (Oshikawa *et al.*, 2004) and progressive cardiomyopathy with age (Woodman *et al.*, 2002). Knockdown of Cav-1 in adipocytes results in loss of insulin receptor signalling as a result of decreased insulin receptor and glucose transporter 4 (GLUT4) expression (Gonzalez-Munoz *et al.*, 2009). Although hearts of Cav-1 and Cav-3 KO mice develop cardiomyopathy, they appear to have normal substrate utilization (Augustus *et al.*, 2008). In H9C2 cardiomyoblasts, Cav-1 knockdown has been shown to inhibit signalling by insulin-like growth factors (Salani *et al.*, 2008) and insulin signalling directly coupled to Akt and glucose transport (Ha and Pak, 2005). Importantly, Cav-3 was a positive regulator of insulin signalling (Yamamoto *et al.*, 1998) and caveolin gene transfer to the liver improved glucose metabolism in diabetic mice (Otsu *et al.*, 2009). Recently, our group has shown that Cav-3 overexpression in the heart leads to enhanced Akt phosphorylation that results in protection of the heart from ischaemia-reperfusion injury (Tsutsumi *et al.*, 2008). Other studies reveal that compounds such as resveratrol, which are

polyphenols shown to have lifespan-expanding properties (Frojdo *et al.*, 2008), also recruit GLUT4 to caveolae and up-regulate Akt signalling in the setting of type I diabetes (Penumathsa *et al.*, 2008). Caveolae also are major regulators of calcium storage and influx which may be an added cellular regulatory feature important to limiting diabetic cardiomyopathy (Shaul and Anderson, 1998).

Diabetes results in altered cardiac mitochondrial function with respect to complex activity, generation of ATP and activation of the mPTP, a key feature leading to cellular apoptosis (Oliveira *et al.*, 2003; Boudina *et al.*, 2007). Recent evidence suggests that caveolin-deficient stromal cells have compromised mitochondrial function (Pavlides *et al.*, 2010) and mitochondria from Cav-1-KO fibroblasts accumulate cholesterol and have severe dysfunction; such cells adapt poorly to nutrient starvation and are predisposed to apoptosis (Bosch *et al.*, 2011). Loss of caveolin leads to altered mitochondrial function in adipose tissue, suggesting a link between caveolin and metabolism (Wernstedt Asterholm *et al.*, 2012).

Caveolins also may play a role in pathologies associated with diabetes including metabolic syndrome, as recently reviewed by Zhang (2014). Specifically, the association between GLUT4 transporters and caveolin plays an important role in the development of insulin resistance (Kabayama *et al.*, 2007; Liu *et al.*, 2008b). Clinical studies utilizing caveolin as a marker or protein of interest in the setting of insulin resistance are rare and primarily address insulin resistance in the context of caveolinopathies (Mendez-Gimenez *et al.*, 2014). Some authors argue in favour of the importance of the caveolar structure, rather than the loss of either Cav-1 or Cav-3 (Mendez-Gimenez *et al.*, 2014). In a translational approach, Cav-1 polymorphisms have been linked to insulin resistance and hypertension in Caucasian and Hispanic patients (Pojoga *et al.*, 2011). In diabetes mellitus patients that underwent flow-mediated dilation of coronary arterioles during heart surgery, membrane localized Cav-1 was significantly reduced. This reduction was attributed to peroxynitrite, which contributes to microvascular dysfunction in diabetes mellitus (Cassuto *et al.*, 2014).

A major confounding factor in the translation of protective strategies to patients with diabetes is that many pharmacological agents that patients are prescribed may negate cardioprotection. Most diabetic patients receiving oral medications will be taking a sulfonylurea that blocks K_{ATP} channels, which in the pancreas increases insulin secretion but, in the heart, the same drug results in the attenuation of cardiac protection (Gross and Auchampach, 1992). Most diabetics and elderly patients are also on statins to maintain low blood cholesterol. Although statins have been shown to have many effects that have potential to protect the heart in specific settings, there is growing concerns that diabetics and individual with other pathophysiologies may not benefit as much as previously thought (Gullestad *et al.*, 2007; Drummond *et al.*, 2010; Schilling *et al.*, 2014). Reduction of caveolin through statin treatment in endothelial cells could affect protection indirectly, through modifying eNOS signalling (Balakumar *et al.*, 2012b). Conversely, one could argue that statin inhibition of the cholesterol pathway and a consequent decrease in Cav-3 in cardiac myocytes could result in impaired survival kinase signalling. The proof of this concept in the heart, specifically under long-term treatment, is still under investigation, while some effects of statin treatment on Cav-1 and the development of diabetes in preclinical models have been recently reviewed (Brault *et al.*, 2014).

In both the ageing and the diabetic heart, the two central features of the pathology are loss of effective signalling networks and compromised ultrastructure, the two perquisites Murry, Jennings and Reimer described early on, as being critical to the induction of IPC. Central to this dysfunction appears to be the loss of caveolin in the heart.

Caveolins in myopathies

Cav-3 interacts with signalling molecules involved in cardiac hypertrophy, remodelling and the progression of heart failure (Fujita *et al.*, 2001; Krajewska and Maslowska, 2004). Cav-3 KO mice exhibit reduced cardiac function and cardiomyopathy (Woodman *et al.*, 2002). These results suggest a potential role for Cav-3 in heart failure. Expression of cardiac Cav-3 is changed in models of heart failure and patients with cardiomyopathy, although there are inconsistencies in the findings (Hare *et al.*, 2000; Damy *et al.*, 2004; Hayashi *et al.*, 2004; Ruiz-Hurtado *et al.*, 2007). In the heart, mutations of Cav-3 can lead to familial hypertrophic cardiomyopathy (Hayashi *et al.*, 2004) as well as arrhythmias such as the congenital long-QT syndrome (Vatta *et al.*, 2006; Balijepalli and Kamp, 2008). In the models and patients examined, the variability of the results may be due to species differences or the stage of heart failure development and ventricular dysfunction. Recently, Feiner *et al.* (2011) reported reduced levels of Cav-3 in two well-established models of heart failure in mice, overexpression of the adenosine A_1 receptor or TNFα. They found significantly reduced levels of Cav-3 protein and mRNA in the mice with heart failure and showed a significant correlation between the reduced levels of Cav-3 and reduced cardiac function. In addition, these investigators found a significant correlation between the reduced levels of Cav-3 in failing human heart samples and the levels of the sarcoplasmic-endoplasmic reticulum calcium ATPase, a marker of heart failure. Our group has shown that cardiac myocyte-specific overexpression of Cav-3 limits the hypertrophic response to transverse aortic constriction and improves survival (Horikawa *et al.*, 2011). Such data indicate a role for Cav-3 as a therapeutic protein in heart failure. Cav-3 also plays a role in muscular dystrophies (Woodman *et al.*, 2004) including limb girdle muscular dystrophy type 1C (Angelini, 2004). The pathogenesis involved in these diseases involves a failure to traffic Cav-3 from the Golgi network to the plasma membrane (Woodman *et al.*, 2004).

Translational approaches to increasing caveolin

From the data presented, it is evident that heart-specific decreases in caveolin are detrimental to the heart, whereas up-regulation of caveolin may be beneficial to the heart. Our laboratory is currently developing a gene therapy-based approach to overexpress caveolin in a cell type-specific manner using selective promoters and regulatory elements.

Although this approach has clinical potential, translation is likely to be far in the future. Therefore, we need to find other natural means to increase caveolin expression. In one study on 14 male pentathlon athletes, exercise increased Cav-1, Cav-3, GLUT4 and the insulin receptor-β in samples from the vastus lateralis muscle after a 1500 m swim trial (Kim *et al.*, 2009). In another experiment, the manipulation of pre-exercise muscle glycogen storage was assessed. Here, an increase in the baseline levels of Cav-1 after recovery from initial glycogen depletion exercise was noted (Roepstorff *et al.*, 2004). Furthermore, in an exercise countermeasure during 12 weeks of bed rest, exercise altered NOS2/Cav-3 co-immunostaining patterns in vastus lateralis and soleus myofibres (Rudnick *et al.*, 2004). Additionally, in an intensive care unit model in rats, immobilization results in distinct alterations in gene expression and down-regulation of Cav-3 expression (Llano-Diez *et al.*, 2011).

Conclusion

Caveolae and caveolins are comparatively new players in a relatively saturated field of ischaemia-reperfusion injury. Given the data provided here, it should be clear that there is a central role for caveolin expression in the protection of the heart, and potentially other organs, from ischaemia-reperfusion injury and cell stress in general. It is intriguing that conditions in which protection is lost show marked loss of caveolin expression coupled to decreased survival kinase signalling and dysfunctional myocyte ultrastructure. Mice with cardiac specific overexpression have dramatic cardiac stress adaptation in a variety of disease settings and provide hope that caveolin may serve as a critical mediator and potential therapeutic target to provide protection from ischaemia-reperfusion injury in humans.

Acknowledgements

This work was supported by grants from the National Institutes of Health HL091071 (H. H. P.), HL107200 (H. H. P.), HL066941 (D. M. R. and H. H. P.) and HL115933 (D. M. R.), and VA Merit BX001963 (H. H. P.) and BX000783 (D. M. R.).

Conflict of interest

There are no competing interests or disclosures.

References

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL Spedding M *et al.* (2013a). The Concise Guide to PHARMACOLOGY 2013/14: Enzymes. Br J Pharmacol 170: 1797–1867.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL Spedding M *et al.* (2013b). The Concise Guide to PHARMACOLOGY 2013/14: Transporters. Br J Pharmacol 170: 1706–1796.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL Spedding M *et al.* (2013c). The Concise Guide to PHARMACOLOGY 2013/14: Catalytic Receptors. Br J Pharmacol 170: 1676–1705.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Catterall WA *et al.* (2013d). The Concise Guide to PHARMACOLOGY 2013/14: Ion Channels. Br J Pharmacol 170: 1607–1651.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL Spedding M *et al.* (2013e). The Concise Guide to PHARMACOLOGY 2013/14: G Protein-Coupled Receptors. Br J Pharmacol 170: 1459–1581.

Angelini C (2004). Limb-girdle muscular dystrophies: heterogeneity of clinical phenotypes and pathogenetic mechanisms. Acta Myol 23: 130–136.

Armstrong SC, Latham CA, Shivell CL, Ganote CE (2001). Ischemic loss of sarcolemmal dystrophin and spectrin: correlation with myocardial injury. J Mol Cell Cardiol 33: 1165–1179.

Auchampach JA, Grover GJ, Gross GJ (1992). Blockade of ischaemic preconditioning in dogs by the novel ATP dependent potassium channel antagonist sodium 5-hydroxydecanoate. Cardiovasc Res 26: 1054–1062.

Augustus AS, Buchanan J, Addya S, Rengo G, Pestell RG, Fortina P *et al.* (2008). Substrate uptake and metabolism are preserved in hypertrophic caveolin-3 knockout hearts. Am J Physiol Heart Circ Physiol 295: H657–H666.

Balakumar P, Sharma NK (2012a). Healing the diabetic heart: does myocardial preconditioning work? Cell Signal 24: 53–59.

Balakumar P, Kathuria S, Taneja G, Kalra S, Mahadevan N (2012b). Is targeting eNOS a key mechanistic insight of cardiovascular defensive potentials of statins? J Mol Cell Cardiol 52: 83–92.

Balijepalli RC, Kamp TJ (2008). Caveolae, ion channels and cardiac arrhythmias. Prog Biophys Mol Biol 98: 149–160.

Ballard-Croft C, Locklar AC, Kristo G, Lasley RD (2006). Regional myocardial ischemia induced activation of MAPKs is associated with subcellular redistribution of caveolin and cholesterol. Am J Physiol Heart Circ Physiol 291: H658–H667.

Becher A, McIlhinney RA (2005). Consequences of lipid raft association on G-protein-coupled receptor function. Biochem Soc Symp 72: 151–164.

Becker LB, VandenHoek TL, Shao Z-H, Li C-Q, Schumacker PT (1999). Generation of superoxide in cardiomyocytes during ischemia before reperfusion. Am J Physiol Heart Circ Physiol 277: H2240–H2246.

Bernardi P (2013). The mitochondrial permeability transition pore: a mystery solved? Front Physiol 4: 95.

Boersma E, Pieper KS, Steyerberg EW, Wilcox RG, Chang WC, Lee KL *et al.* (2000). Predictors of outcome in patients with acute coronary syndromes without persistent ST-segment elevation. Results from an international trial of 9461 patients. The PURSUIT Investigators. Circulation 101: 2557–2567.

Bosch M, Mari M, Herms A, Fernandez A, Fajardo A, Kassan A *et al.* (2011). Caveolin-1 deficiency causes cholesterol-dependent mitochondrial dysfunction and apoptotic susceptibility. Curr Biol 21: 681–686.

Boudina S, Sena S, Theobald H, Sheng X, Wright JJ, Hu XX *et al.* (2007). Mitochondrial energetics in the heart in obesity-related diabetes: direct evidence for increased uncoupled respiration and activation of uncoupling proteins. Diabetes 56: 2457–2466.

Brault M, Ray J, Gomez YH, Mantzoros CS, Daskalopoulou SS (2014). Statin treatment and new-onset diabetes: a review of proposed mechanisms. Metabolism 63: 735–745.

Cao CM, Zhang Y, Weisleder N, Ferrante C, Wang X, Lv F *et al.* (2011). MG53 constitutes a primary determinant of cardiac ischemic preconditioning. Circulation 121: 2565–2574.

Capozza F, Cohen AW, Cheung MW, Sotgia F, Schubert W, Battista M *et al.* (2005). Muscle-specific interaction of caveolin isoforms: differential complex formation between caveolins in fibroblastic vs. muscle cells. Am J Physiol Cell Physiol 288: C677–C691.

Cassuto J, Dou H, Czikora I, Szabo A, Patel VS, Kamath V *et al.* (2014). Peroxynitrite disrupts endothelial caveolae leading to eNOS uncoupling and diminished flow-mediated dilation in coronary arterioles of diabetic patients. Diabetes 63: 1381–1393.

Chini B, Parenti M (2004). G-protein coupled receptors in lipid rafts and caveolae: how, when and why do they go there? J Mol Endocrinol 32: 325–338.

Cho KA, Park SC (2005). Caveolin-1 as a prime modulator of aging: a new modality for phenotypic restoration? Mech Ageing Dev 126: 105–110.

Chun M, Liyanage UK, Lisanti MP, Lodish HF (1994). Signal transduction of a G protein-coupled receptor in caveolae: colocalization of endothelin and its receptor with caveolin. Proc Natl Acad Sci U S A 91: 11728–11732.

Cohen AW, Hnasko R, Schubert W, Lisanti MP (2004). Role of caveolae and caveolins in health and disease. Physiol Rev 84: 1341–1379.

Collins BM, Davis MJ, Hancock JF, Parton RG (2012). Structure-based reassessment of the caveolin signaling model: do caveolae regulate signaling through caveolin-protein interactions? Dev Cell 23: 11–20.

Damy T, Ratajczak P, Shah AM, Camors E, Marty I, Hasenfuss G *et al.* (2004). Increased neuronal nitric oxide synthase-derived NO production in the failing human heart. Lancet 363: 1365–1367.

Di Lisa F, Bernardi P (2005). Mitochondrial function and myocardial aging. A critical analysis of the role of permeability transition. Cardiovasc Res 66: 222–232.

Dietzen DJ, Hastings WR, Lublin DM (1995). Caveolin is palmitoylated on multiple cysteine residues. Palmitoylation is not necessary for localization of caveolin to caveolae. J Biol Chem 270: 6838–6842.

Donaldson JG, Porat-Shliom N, Cohen LA (2009). Clathrin-independent endocytosis: a unique platform for cell signaling and PM remodeling. Cell Signal 21: 1–6.

Drummond JC, Head BP, Patel PM (2010). Statins might contribute to postoperative delirium. Anesthesiology 113: 500–501.

Engelman JA, Chu C, Lin A, Jo H, Ikezu T, Okamoto T *et al.* (1998). Caveolin-mediated regulation of signaling along the p42/44 MAP kinase cascade *in vivo*. A role for the caveolin-scaffolding domain. FEBS Lett 428: 205–211.

Eto M, Watanabe K, Sekiguchi M, Iwashima Y, Morikawa A, Oshima E *et al.* (1987). Metabolic and morphological changes of the heart in Chinese hamsters (CHAD strain) with spontaneous long-term diabetes. Diabetes Res Clin Pract 3: 297–305.

Fang J, Wu L, Chen L (2008). Postconditioning attenuates cardiocyte ultrastructure injury and apoptosis by blocking mitochondrial permeability transition in rats. Acta Cardiol 63: 377–387.

Fecchi K, Volonte D, Hezel MP, Schmeck K, Galbiati F (2006). Spatial and temporal regulation of GLUT4 translocation by flotillin-1 and caveolin-3 in skeletal muscle cells. FASEB J 20: 705–707.

Feiner EC, Chung P, Jasmin JF, Zhang J, Whitaker-Menezes D, Myers V *et al.* (2011). Left ventricular dysfunction in murine models of heart failure and in failing human heart is associated with a selective decrease in the expression of caveolin-3. J Card Fail 17: 253–263.

Feron O, Balligand JL (2006). Caveolins and the regulation of endothelial nitric oxide synthase in the heart. Cardiovasc Res 69: 788–797.

Feron O, Kelly RA (2001). The caveolar paradox: suppressing, inducing, and terminating eNOS signaling. Circ Res 88: 129–131.

Feron O, Saldana F, Michel JB, Michel T (1998). The endothelial nitric-oxide synthase-caveolin regulatory cycle. J Biol Chem 273: 3125–3128.

Frick M, Bright NA, Riento K, Bray A, Merrified C, Nichols BJ (2007). Coassembly of flotillins induces formation of membrane microdomains, membrane curvature, and vesicle budding. Curr Biol 17: 1151–1156.

Fridolfsson HN, Kawaraguchi Y, Ali SS, Panneerselvam M, Niesman IR, Finley JC *et al.* (2012). Mitochondria-localized caveolin in adaptation to cellular stress and injury. FASEB J 26: 4637–4649.

Fridolfsson HN, Roth DM, Insel PA, Patel HH (2014). Regulation of intracellular signaling and function by caveolin. FASEB J 28: 3823–3831.

Frojdo S, Durand C, Pirola L (2008). Metabolic effects of resveratrol in mammals – a link between improved insulin action and aging. Curr Aging Sci 1: 145–151.

Fuhs SR, Insel PA (2011). Caveolin-3 undergoes SUMOylation by the SUMO E3 ligase PIASy: sumoylation affects G-protein-coupled receptor desensitization. J Biol Chem 286: 14830–14841.

Fujimoto T (1993). Calcium pump of the plasma membrane is localized in caveolae. J Cell Biol 120: 1147–1157.

Fujimoto T, Nakade S, Miyawaki A, Mikoshiba K, Ogawa K (1992). Localization of inositol 1,4,5-trisphosphate receptor-like protein in plasmalemmal caveolae. J Cell Biol 119: 1507–1513.

Fujita T, Toya Y, Iwatsubo K, Onda T, Kimura K, Umemura S *et al.* (2001). Accumulation of molecules involved in alpha1-adrenergic signal within caveolae: caveolin expression and the development of cardiac hypertrophy. Cardiovasc Res 51: 709–716.

Ge S, Song L, Serwanski DR, Kuziel WA, Pachter JS (2008). Transcellular transport of CCL2 across brain microvascular endothelial cells. J Neurochem 104: 1219–1232.

Geshi E, Ishioka H, Nomizo A, Nakatani M, Katagiri T (1998). Biochemical and ultrastructural evaluations of the effect of ischemic preconditioning on ischemic myocardial injury – role of the adenosine triphosphate-sensitive potassium channel. Jpn Circ J 62: 915–924.

Gherghiceanu M, Popescu LM (2007). Electron microscope tomography: further demonstration of nanocontacts between caveolae and smooth muscle sarcoplasmic reticulum. J Cell Mol Med 11: 1416–1418.

Giovanardi RO, Rhoden EL, Cerski CT, Salvador M, Kalil AN (2009). Ischemic preconditioning protects the pig liver by preserving the mitochondrial structure and downregulating caspase-3 activity. J Invest Surg 22: 88–97.

Gonzalez-Munoz E, Lopez-Iglesias C, Calvo M, Palacin M, Zorzano A, Camps M (2009). Caveolin-1 loss of function accelerates glucose transporter 4 and insulin receptor degradation in 3T3-L1 adipocytes. Endocrinology 150: 3493–3502.

Gottlieb RA, Gustafsson AB (2011). Mitochondrial turnover in the heart. Biochim Biophys Acta 1813: 1295–1301.

Grande-Garcia A, Del Pozo MA (2008). Caveolin-1 in cell polarization and directional migration. Eur J Cell Biol 87: 641–647.

Grande-Garcia A, Echarri A, de Rooij J, Alderson NB, Waterman-Storer CM, Valdivielso JM *et al.* (2007). Caveolin-1 regulates cell polarization and directional migration through Src kinase and Rho GTPases. J Cell Biol 177: 683–694.

Griparic L, van der Wel NN, Orozco IJ, Peters PJ, van der Bliek AM (2004). Loss of the intermembrane space protein Mgm1/OPA1 induces swelling and localized constrictions along the lengths of mitochondria. J Biol Chem 279: 18792–18798.

Gross GJ, Auchampach JA (1992). Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs. Circ Res 70: 223–233.

Gullestad L, Oie E, Ueland T, Yndestad A, Aukrust P (2007). The role of statins in heart failure. Fundam Clin Pharmacol 21: 35–40.

Ha H, Pak Y (2005). Modulation of the caveolin-3 and Akt status in caveolae by insulin resistance in H9c2 cardiomyoblasts. Exp Mol Med 37: 169–178.

Hagen TM (2003). Oxidative stress, redox imbalance, and the aging process. Antioxid Redox Signal 5: 503–506.

Hagiwara Y, Sasaoka T, Araishi K, Imamura M, Yorifuji H, Nonaka I *et al.* (2000). Caveolin-3 deficiency causes muscle degeneration in mice. Hum Mol Genet 9: 3047–3054.

Hagiwara Y, Nishina Y, Yorifuji H, Kikuchi T (2002). Immunolocalization of caveolin-1 and caveolin-3 in monkey skeletal, cardiac and uterine smooth muscles. Cell Struct Funct 27: 375–382.

Han HC, Austin KJ, Nathanielsz PW, Ford SP, Nijland MJ, Hansen TR (2004). Maternal nutrient restriction alters gene expression in the ovine fetal heart. J Physiol 558: 111–121.

Hare JM, Lofthouse RA, Juang GJ, Colman L, Ricker KM, Kim B *et al.* (2000). Contribution of caveolin protein abundance to augmented nitric oxide signaling in conscious dogs with pacing-induced heart failure. Circ Res 86: 1085–1092.

Hausenloy DJ, Yellon DM (2006). Survival kinases in ischemic preconditioning and postconditioning. Cardiovasc Res 70: 240–253.

Hausenloy DJ, Yellon DM (2010). Cell membrane repair as a mechanism for ischemic preconditioning? Circulation 121: 2547–2549.

Hausenloy DJ, Tsang A, Yellon DM (2005). The reperfusion injury salvage kinase pathway: a common target for both ischemic preconditioning and postconditioning. Trends Cardiovasc Med 15: 69–75.

Hayashi T, Arimura T, Ueda K, Shibata H, Hohda S, Takahashi M *et al.* (2004). Identification and functional analysis of a caveolin-3 mutation associated with familial hypertrophic cardiomyopathy. Biochem Biophys Res Commun 313: 178–184.

Head BP, Insel PA (2007). Do caveolins regulate cells by actions outside of caveolae? Trends Cell Biol 17: 51–57.

Head BP, Patel HH, Roth DM, Lai NC, Niesman IR, Farquhar MG *et al.* (2005). G-protein-coupled receptor signaling components localize in both sarcolemmal and intracellular caveolin-3-associated microdomains in adult cardiac myocytes. J Biol Chem 280: 31036–31044.

Head BP, Patel HH, Roth DM, Murray F, Swaney JS, Niesman IR *et al.* (2006). Microtubules and actin microfilaments regulate lipid raft/caveolae localization of adenylyl cyclase signaling components. J Biol Chem 281: 26391–26399.

Headrick JP, Willems L, Ashton KJ, Holmgren K, Peart J, Matherne GP (2003). Ischaemic tolerance in aged mouse myocardium: the role of adenosine and effects of A1 adenosine receptor overexpression. J Physiol 549: 823–833.

Hill MM, Bastiani M, Luetterforst R, Kirkham M, Kirkham A, Nixon SJ *et al.* (2008). PTRF-Cavin, a conserved cytoplasmic protein required for caveola formation and function. Cell 132: 113–124.

Horikawa YT, Patel HH, Tsutsumi YM, Jennings MM, Kidd MW, Hagiwara Y *et al.* (2008). Caveolin-3 expression and caveolae are required for isoflurane-induced cardiac protection from hypoxia and ischemia/reperfusion injury. J Mol Cell Cardiol 44: 123–130.

Horikawa YT, Panneerselvam M, Kawaraguchi Y, Tsutsumi YM, Ali SS, Balijepalli RC *et al.* (2011). Cardiac-specific overexpression of caveolin-3 attenuates cardiac hypertrophy and increases natriuretic Peptide expression and signaling. J Am Coll Cardiol 57: 2273–2283.

Jahangir A, Ozcan C, Holmuhamedov EL, Terzic A (2001). Increased calcium vulnerability of senescent cardiac mitochondria: protective role for a mitochondrial potassium channel opener. Mech Ageing Dev 122: 1073–1086.

Jamieson HA, Hilmer SN, Cogger VC, Warren A, Cheluvappa R, Abernethy DR *et al.* (2007). Caloric restriction reduces age-related pseudocapillarization of the hepatic sinusoid. Exp Gerontol 42: 374–378.

Javadov S, Karmazyn M (2007). Mitochondrial permeability transition pore opening as an endpoint to initiate cell death and as a putative target for cardioprotection. Cell Physiol Biochem 20: 1–22.

Joffe II, Travers KE, Perreault-Micale CL, Hampton T, Katz SE, Morgan JP *et al.* (1999). Abnormal cardiac function in the streptozotocin-induced non-insulin-dependent diabetic rat: noninvasive assessment with doppler echocardiography and contribution of the nitric oxide pathway. J Am Coll Cardiol 34: 2111–2119.

Jones KA, Jiang X, Yamamoto Y, Yeung RS (2004). Tuberin is a component of lipid rafts and mediates caveolin-1 localization: role of TSC2 in post-Golgi transport. Exp Cell Res 295: 512–524.

Juhaszova M, Zorov DB, Kim SH, Pepe S, Fu Q, Fishbein KW *et al.* (2004). Glycogen synthase kinase-3beta mediates convergence of protection signaling to inhibit the mitochondrial permeability transition pore. J Clin Invest 113: 1535–1549.

Kabayama K, Sato T, Saito K, Loberto N, Prinetti A, Sonnino S *et al.* (2007). Dissociation of the insulin receptor and caveolin-1 complex by ganglioside GM3 in the state of insulin resistance. Proc Natl Acad Sci U S A 104: 13678–13683.

Kamoun WS, Karaa A, Kresge N, Merkel SM, Korneszczuk K, Clemens MG (2006). LPS inhibits endothelin-1-induced endothelial NOS activation in hepatic sinusoidal cells through a negative feedback involving caveolin-1. Hepatology 43: 182–190.

Kawabe JI, Grant BS, Yamamoto M, Schwencke C, Okumura S, Ishikawa Y (2001). Changes in caveolin subtype protein expression in aging rat organs. Mol Cell Endocrinol 176: 91–95.

Kido M, Otani H, Kyoi S, Sumida T, Fujiwara H, Okada T *et al.* (2004). Ischemic preconditioning-mediated restoration of membrane dystrophin during reperfusion correlates with protection against contraction-induced myocardial injury. Am J Physiol Heart Circ Physiol 287: H81–H90.

Kim HA, Kim KH, Lee RA (2006). Expression of caveolin-1 is correlated with Akt-1 in colorectal cancer tissues. Exp Mol Pathol 80: 165–170.

Kim HS, Kim HJ, Kim YS, Park SC, Harris R, Kim CK (2009). Caveolin, GLUT4 and insulin receptor protein content in human arm and leg muscles. Eur J Appl Physiol 106: 173–179.

Krajewska WM, Maslowska I (2004). Caveolins: structure and function in signal transduction. Cell Mol Biol Lett 9: 195–220.

Lakatta EG, Sollott SJ (2002). The 'heartbreak' of older age. Mol Interv 2: 431–446.

Lasley R, Prakash N, Uittenbogaard A, Smart E (2000). Activated cardiac adenosine A1 receptors translocate out of caveolae. J Biol Chem 275: 4417–4421.

Lesnefsky EJ, Hoppel CL (2008). Cardiolipin as an oxidative target in cardiac mitochondria in the aged rat. Biochim Biophys Acta 1777: 1020–1027.

Lesnefsky EJ, Gudz TI, Migita CT, Ikeda-Saito M, Hassan MO, Turkaly PJ *et al.* (2001). Ischemic injury to mitochondrial electron transport in the aging heart: damage to the iron-sulfur protein subunit of electron transport complex III. Arch Biochem Biophys 385: 117–128.

Lesnefsky EJ, He D, Moghaddas S, Hoppel CL (2006). Reversal of mitochondrial defects before ischemia protects the aged heart. FASEB J 20: 1543–1545.

Li S, Seitz R, Lisanti MP (1996). Phosphorylation of caveolin by src tyrosine kinases. The alpha-isoform of caveolin is selectively phosphorylated by v-Src *in vivo*. J Biol Chem 271: 3863–3868.

Li WP, Liu P, Pilcher BK, Anderson RG (2001). Cell-specific targeting of caveolin-1 to caveolae, secretory vesicles, cytoplasm or mitochondria. J Cell Sci 114: 1397–1408.

Lisanti MP, Scherer PE, Tang Z, Sargiacomo M (1994). Caveolae, caveolin and caveolin-rich membrane domains: a signalling hypothesis. Trends Cell Biol 4: 231–235.

Liu L, Pilch PF (2008a). A critical role of cavin (polymerase I and transcript release factor) in caveolae formation and organization. J Biol Chem 283: 4314–4322.

Liu L, Brown D, McKee M, Lebrasseur NK, Yang D, Albrecht KH *et al.* (2008b). Deletion of Cavin/PTRF causes global loss of caveolae, dyslipidemia, and glucose intolerance. Cell Metab 8: 310–317.

Llano-Diez M, Gustafson AM, Olsson C, Goransson H, Larsson L (2011). Muscle wasting and the temporal gene expression pattern in a novel rat intensive care unit model. BMC Genomics 12: 602.

Masoro EJ (2009). Caloric restriction-induced life extension of rats and mice: a critique of proposed mechanisms. Biochim Biophys Acta 1790: 1040–1048.

McCay C, Crowell M, Maynard L (1935). The effect of retarded growth upon the length of life and upon the ultimate body size. J Nutr 10: 63–79.

Mendez-Gimenez L, Rodriguez A, Balaguer I, Fruhbeck G (2014). Role of aquaglyceroporins and caveolins in energy and metabolic homeostasis. Mol Cell Endocrinol 397: 78–92.

Mio Y, Bienengraeber MW, Marinovic J, Gutterman DD, Rakic M, Bosnjak ZJ *et al.* (2008). Age-related attenuation of isoflurane preconditioning in human atrial cardiomyocytes: roles for mitochondrial respiration and sarcolemmal adenosine triphosphate-sensitive potassium channel activity. Anesthesiology 108: 612–620.

Miyamae M, Fujiwara H, Tanaka M, Yokota R, Takemura G, Itoh S *et al.* (2002). Oxygen radicals mediate ultrastructural and metabolic protection of preconditioning *in vivo* in pig hearts. Exp Clin Cardiol 7: 173–179.

Mizushige K, Yao L, Noma T, Kiyomoto H, Yu Y, Hosomi N *et al.* (2000). Alteration in left ventricular diastolic filling and accumulation of myocardial collagen at insulin-resistant prediabetic stage of a type II diabetic rat model. Circulation 101: 899–907.

Moolman JA, Genade S, Winterbach R, Harper IS, Williams K, Lochner A (1995). Preconditioning with a single short episode of global ischemia in the isolated working rat heart: effect on structure, mechanical function, and energy metabolism for various durations of sustained global ischemia. Cardiovasc Drugs Ther 9: 103–115.

Mousley CJ, Yuan P, Gaur NA, Trettin KD, Nile AH, Deminoff SJ *et al.* (2012). A sterol-binding protein integrates endosomal lipid metabolism with TOR signaling and nitrogen sensing. Cell 148: 702–715.

Mukherjee S, Tessema M, Wandinger-Ness A (2006). Vesicular trafficking of tyrosine kinase receptors and associated proteins in the regulation of signaling and vascular function. Circ Res 98: 743–756.

Murry CE, Jennings RB, Reimer KA (1986). Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 74: 1124–1136.

Murry CE, Richard VJ, Reimer KA, Jennings RB (1990). Ischemic preconditioning slows energy metabolism and delays ultrastructural damage during a sustained ischemic episode. Circ Res 66: 913–931.

Nichols B (2003). Caveosomes and endocytosis of lipid rafts. J Cell Sci 116: 4707–4714.

Niesman IR, Zemke N, Fridolfsson HN, Haushalter KJ, Levy K, Grove A *et al.* (2013). Caveolin isoform switching as a molecular, structural, and metabolic regulator of microglia. Mol Cell Neurosci 56: 283–297.

Nishihara M, Miura T, Miki T, Tanno M, Yano T, Naitoh K *et al.* (2007). Modulation of the mitochondrial permeability transition pore complex in GSK-3beta-mediated myocardial protection. J Mol Cell Cardiol 43: 564–570.

Olichon A, Baricault L, Gas N, Guillou E, Valette A, Belenguer P *et al.* (2003). Loss of OPA1 perturbates the mitochondrial inner membrane structure and integrity, leading to cytochrome c release and apoptosis. J Biol Chem 278: 7743–7746.

Oliveira PJ, Seica R, Coxito PM, Rolo AP, Palmeira CM, Santos MS *et al.* (2003). Enhanced permeability transition explains the reduced calcium uptake in cardiac mitochondria from streptozotocin-induced diabetic rats. FEBS Lett 554: 511–514.

Oshikawa J, Otsu K, Toya Y, Tsunematsu T, Hankins R, Kawabe J *et al.* (2004). Insulin resistance in skeletal muscles of caveolin-3-null mice. Proc Natl Acad Sci U S A 101: 12670–12675.

Otsu K, Toya Y, Oshikawa J, Kurotani R, Yazawa T, Sato M *et al.* (2009). Caveolin gene transfer improves glucose metabolism in diabetic mice. Am J Physiol Cell Physiol 298: C450–C456.

Pain T, Yang X-M, Critz SD, Yue Y, Nakano A, Liu GS *et al.* (2000). Opening of mitochondrial K_{ATP} channels triggers the preconditioned state by generating free radicals. Circ Res 87: 460–466.

Palade G (1953). Fine structure of blood capillaries. J Appl Phys 24: 1424.

Park DS, Cohen AW, Frank PG, Razani B, Lee H, Williams TM *et al.* (2003). Caveolin-1 null (-/-) mice show dramatic reductions in life span. Biochemistry 42: 15124–15131.

Park SC, Cho KA, Jang IS, Kim KT, Ryu SJ (2004). Functional efficiency of the senescent cells: replace or restore? Ann N Y Acad Sci 1019: 309–316.

Parton RG, Way M, Zorzi N, Stang E (1997). Caveolin-3 associates with developing T-tubules during muscle differentiation. J Cell Biol 136: 137–154.

Patel HH, Head BP, Petersen HN, Niesman IR, Huang D, Gross GJ *et al*. (2006). Protection of adult rat cardiac myocytes from ischemic cell death: role of caveolar microdomains and delta-opioid receptors. Am J Physiol Heart Circ Physiol 291: H344–H350.

Patel HH, Tsutsumi YM, Head BP, Niesman IR, Jennings M, Horikawa Y *et al*. (2007). Mechanisms of cardiac protection from ischemia/reperfusion injury: a role for caveolae and caveolin-1. FASEB J 21: 1565–1574.

Pavlides S, Tsirigos A, Vera I, Flomenberg N, Frank PG, Casimiro MC *et al*. (2010). Loss of stromal caveolin-1 leads to oxidative stress, mimics hypoxia and drives inflammation in the tumor microenvironment, conferring the 'reverse Warburg effect': a transcriptional informatics analysis with validation. Cell Cycle 9: 2201–2219.

Pawson AJ, Sharman JL, Benson HE, Faccenda E, Alexander SP, Buneman OP *et al*.; NC-IUPHAR (2014). The IUPHAR/BPS Guide to PHARMACOLOGY: an expert-driven knowledge base of drug targets and their ligands. Nucleic Acids Res 42 (Database Issue): D1098–1106.

Peart JN, Gross ER, Headrick JP, Gross GJ (2007). Impaired p38 MAPK/HSP27 signaling underlies aging-related failure in opioid-mediated cardioprotection. J Mol Cell Cardiol 42: 972–980.

Peart JN, Pepe S, Reichelt ME, Beckett N, See Hoe L, Ozberk V *et al*. (2014). Dysfunctional survival-signaling and stress-intolerance in aged murine and human myocardium. Exp Gerontol 50: 72–81.

Peng Y, Akmentin W, Connelly MA, Lund-Katz S, Phillips MC, Williams DL (2004). Scavenger receptor BI (SR-BI) clustered on microvillar extensions suggests that this plasma membrane domain is a way station for cholesterol trafficking between cells and high-density lipoprotein. Mol Biol Cell 15: 384–396.

Penna C, Perrelli MG, Raimondo S, Tullio F, Merlino A, Moro F *et al*. (2009). Postconditioning induces an anti-apoptotic effect and preserves mitochondrial integrity in isolated rat hearts. Biochim Biophys Acta 1787: 794–801.

Penumathsa SV, Thirunavukkarasu M, Zhan L, Maulik G, Menon VP, Bagchi D *et al*. (2008). Resveratrol enhances GLUT-4 translocation to the caveolar lipid raft fractions through AMPK/Akt/eNOS signalling pathway in diabetic myocardium. J Cell Mol Med 12: 2350–2361.

Pepe S (2005). Effect of dietary polyunsaturated fatty acids on age-related changes in cardiac mitochondrial membranes. Exp Gerontol 40: 751–758.

Pike LJ (2003). Lipid rafts: bringing order to chaos. J Lipid Res 44: 655–667.

Pike LJ (2005). Growth factor receptors, lipid rafts and caveolae: an evolving story. Biochim Biophys Acta 1746: 260–273.

Poirier P, Bogaty P, Garneau C, Marois L, Dumesnil JG (2001). Diastolic dysfunction in normotensive men with well-controlled type 2 diabetes: importance of maneuvers in echocardiographic screening for preclinical diabetic cardiomyopathy. Diabetes Care 24: 5–10.

Pojoga LH, Underwood PC, Goodarzi MO, Williams JS, Adler GK, Jeunemaitre X *et al*. (2011). Variants of the caveolin-1 gene: a translational investigation linking insulin resistance and hypertension. J Clin Endocrinol Metab 96: E1288–E1292.

Quarrie R, Lee DS, Steinbaugh G, Cramer B, Erdahl W, Pfeiffer DR *et al*. (2012). Ischemic preconditioning preserves mitochondrial membrane potential and limits reactive oxygen species production. J Surg Res 178: 8–17.

Raikar LS, Vallejo J, Lloyd PG, Hardin CD (2006). Overexpression of caveolin-1 results in increased plasma membrane targeting of glycolytic enzymes: the structural basis for a membrane associated metabolic compartment. J Cell Biochem 98: 861–871.

Ralston E, Ploug T (1999). Caveolin-3 is associated with the T-tubules of mature skeletal muscle fibers. Exp Cell Res 246: 510–515.

Ratajczak P, Damy T, Heymes C, Oliviero P, Marotte F, Robidel E *et al*. (2003). Caveolin-1 and -3 dissociations from caveolae to cytosol in the heart during aging and after myocardial infarction in rat. Cardiovasc Res 57: 358–369.

Razzaq TM, Ozegbe P, Jury EC, Sembi P, Blackwell NM, Kabouridis PS (2004). Regulation of T-cell receptor signalling by membrane microdomains. Immunology 113: 413–426.

Rodgers W, Farris D, Mishra S (2005). Merging complexes: properties of membrane raft assembly during lymphocyte signaling. Trends Immunol 26: 97–103.

Roepstorff C, Vistisen B, Roepstorff K, Kiens B (2004). Regulation of plasma long-chain fatty acid oxidation in relation to uptake in human skeletal muscle during exercise. Am J Physiol Endocrinol Metab 287: E696–E705.

Rothberg KG, Heuser JE, Donzell WC, Ying YS, Glenney JR, Anderson RG (1992). Caveolin, a protein component of caveolae membrane coats. Cell 68: 673–682.

Rudnick J, Puttmann B, Tesch PA, Alkner B, Schoser BG, Salanova M *et al*. (2004). Differential expression of nitric oxide synthases (NOS 1–3) in human skeletal muscle following exercise countermeasure during 12 weeks of bed rest. FASEB J 18: 1228–1230.

Ruiz-Hurtado G, Fernandez-Velasco M, Mourelle M, Delgado C (2007). LA419, a novel nitric oxide donor, prevents pathological cardiac remodeling in pressure-overloaded rats via endothelial nitric oxide synthase pathway regulation. Hypertension 50: 1049–1056.

Rybin VO, Grabham PW, Elouardighi H, Steinberg SF (2003). Caveolae-associated proteins in cardiomyocytes: caveolin-2 expression and interactions with caveolin-3. Am J Physiol Heart Circ Physiol 285: H325–H332.

Salani B, Briatore L, Garibaldi S, Cordera R, Maggi D (2008). Caveolin-1 down-regulation inhibits insulin-like growth factor-I receptor signal transduction in H9C2 rat cardiomyoblasts. Endocrinology 149: 461–465.

Sargiacomo M, Scherer PE, Tang Z, Kübler E, Song KS, Sanders MC *et al*. (1995). Oligomeric structure of caveolin: implications for caveolae membrane organization. Proc Natl Acad Sci U S A 92: 9407–9411.

Scherer PE, Lewis RY, Volonte D, Engelman JA, Galbiati F, Couet J *et al*. (1997). Cell-type and tissue-specific expression of caveolin-2. Caveolins 1 and 2 co-localize and form a stable hetero-oligomeric complex *in vivo*. J Biol Chem 272: 29337–29346.

Schilling JM, Cui W, Godoy JC, Risbrough VB, Niesman IR, Roth DM *et al*. (2014). Long-term atorvastatin treatment leads to alterations in behavior, cognition, and hippocampal biochemistry. Behav Brain Res 267: 6–11.

Scriven DR, Klimek A, Lee KL, Moore ED (2002). The molecular architecture of calcium microdomains in rat cardiomyocytes. Ann N Y Acad Sci 976: 488–499.

Scriven DR, Klimek A, Asghari P, Bellve K, Moore ED (2005). Caveolin-3 is adjacent to a group of extradyadic ryanodine receptors. Biophys J 89: 1893–1901.

Seo AY, Xu J, Servais S, Hofer T, Marzetti E, Wohlgemuth SE *et al.* (2008). Mitochondrial iron accumulation with age and functional consequences. Aging Cell 7: 706–716.

Shaul PW, Anderson RG (1998). Role of plasmalemmal caveolae in signal transduction. Am J Physiol Lung Cell Mol Physiol 275: L843–L851.

Song KS, Scherer PE, Tang Z, Okamoto T, Li S, Chafel M *et al.* (1996). Expression of caveolin-3 in skeletal, cardiac, and smooth muscle cells. Caveolin-3 is a component of the sarcolemma and co-fractionates with dystrophin and dystrophin-associated glycoproteins. J Biol Chem 271: 15160–15165.

Steinberg SF, Brunton LL (2001). Compartmentation of g protein-coupled signaling pathways in cardiac myocytes. Annu Rev Pharmacol Toxicol 41: 751–773.

Stojanovski D, Koutsopoulos OS, Okamoto K, Ryan MT (2004). Levels of human Fis1 at the mitochondrial outer membrane regulate mitochondrial morphology. J Cell Sci 117: 1201–1210.

Swynghedauw B, Besse S, Assayag P, Carre F, Chevalier B, Charlemagne D *et al.* (1995). Molecular and cellular biology of the senescent hypertrophied and failing heart. Am J Cardiol 76: 2D–7D.

Tang Z, Scherer PE, Okamoto T, Song K, Chu C, Kohtz DS *et al.* (1996). Molecular cloning of caveolin-3, a novel member of the caveolin gene family expressed predominantly in muscle. J Biol Chem 271: 2255–2261.

Tani M, Honma Y, Hasegawa H, Tamaki K (2001). Direct activation of mitochondrial KATP channels mimics preconditioning but protein kinase C activation is less effective in middle-aged rat hearts. Cardiovasc Res 49: 56–68.

Tsutsumi YM, Horikawa YT, Jennings MM, Kidd MW, Niesman IR, Yokoyama U *et al.* (2008). Cardiac-specific overexpression of caveolin-3 induces endogenous cardiac protection by mimicking ischemic preconditioning. Circulation 118: 1979–1988.

Tsutsumi YM, Kawaraguchi Y, Niesman IR, Patel HH, Roth DM (2010a). Opioid-induced preconditioning is dependent on caveolin-3 expression. Anesth Analg 111: 1117–1121.

Tsutsumi YM, Kawaraguchi Y, Horikawa YT, Niesman IR, Kidd MW, Chin-Lee B *et al.* (2010b). Role of caveolin-3 and glucose transporter-4 in isoflurane-induced delayed cardiac protection. Anesthesiology 112: 1136–1145.

VandenHoek TL, Becker LB, Shao Z, Li C, Schumacker PT (1998). Reactive oxygen species released from mitochondria during brief hypoxia induce preconditioning in cardiomyocytes. J Biol Chem 273: 18092–18098.

VandenHoek TL, Becker LB, Shao Z-H, Li C-Q, Schumacker PT (2000). Preconditioning in cardiomyocytes protects by attenuating oxidant stress at reperfusion. Circ Res 86: 541–548.

Vatta M, Ackerman MJ, Ye B, Makielski JC, Ughanze EE, Taylor EW *et al.* (2006). Mutant caveolin-3 induces persistent late sodium current and is associated with long-QT syndrome. Circulation 114: 2104–2112.

Vinten J, Johnsen AH, Roepstorff P, Harpoth J, Tranum-Jensen J (2005). Identification of a major protein on the cytosolic face of caveolae. Biochim Biophys Acta 1717: 34–40.

Volonte D, Zhang K, Lisanti MP, Galbiati F (2002). Expression of caveolin-1 induces premature cellular senescence in primary cultures of murine fibroblasts. Mol Biol Cell 13: 2502–2517.

Wang J, Schilling JM, Niesman IR, Headrick JP, Finley JC, Kwan E *et al.* (2014). Cardioprotective trafficking of caveolin to mitochondria is Gi-protein dependent. Anesthesiology 121: 538–548.

Warley A, Powell JM, Skepper JN (1995). Capillary surface area is reduced and tissue thickness from capillaries to myocytes is increased in the left ventricle of streptozotocin-diabetic rats. Diabetologia 38: 413–421.

Wernstedt Asterholm I, Mundy DI, Weng J, Anderson RG, Scherer PE (2012). Altered mitochondrial function and metabolic inflexibility associated with loss of caveolin-1. Cell Metab 15: 171–185.

Willems L, Zatta A, Holmgren K, Ashton KJ, Headrick JP (2005). Age-related changes in ischemic tolerance in male and female mouse hearts. J Mol Cell Cardiol 38: 245–256.

Williams TM, Lisanti MP (2004). The caveolin proteins. Genome Biol 5: 214.

Woodman SE, Park DS, Cohen AW, Cheung M, Chandra M, Shirani J *et al.* (2002). Caveolin-3 knock-out mice develop a progressive cardiomyopathy and show hyperactivation of the p42/44 MAP kinase cascade. J Biol Chem 277: 38988–38997.

Woodman SE, Sotgia F, Galbiati F, Minetti C, Lisanti MP (2004). Caveolinopathies: mutations in caveolin-3 cause four distinct autosomal dominant muscle diseases. Neurology 62: 538–543.

Yaffe MP (1999). The machinery of mitochondrial inheritance and behavior. Science 283: 1493–1497.

Yamabhai M, Anderson RG (2002). Second cysteine-rich region of epidermal growth factor receptor contains targeting information for caveolae/rafts. J Biol Chem 277: 24843–24846.

Yamamoto M, Toya Y, Schwencke C, Lisanti MP, Myers MG Jr, Ishikawa Y (1998). Caveolin is an activator of insulin receptor signaling. J Biol Chem 273: 26962–26968.

Yan LJ, Sohal RS (1998). Mitochondrial adenine nucleotide translocase is modified oxidatively during aging. Proc Natl Acad Sci U S A 95: 12896–12901.

Yang KC, Rutledge CA, Mao M, Bakhshi FR, Xie A, Liu H *et al.* (2014). Caveolin-1 modulates cardiac gap junction homeostasis and arrhythmogenecity by regulating cSrc tyrosine kinase. Circ Arrhythm Electrophysiol 7: 701–710.

Yao Z, Tong J, Tan X, Li C, Shao Z, Kim WC *et al.* (1999). Role of reactive oxygen species in acetylcholine-induced preconditioning in cardiomyocytes. Am J Physiol Heart Circ Physiol 277: H2504–H2509.

Young LH, Ikeda Y, Lefer AM (2001). Caveolin-1 peptide exerts cardioprotective effects in myocardial ischemia-reperfusion via nitric oxide mechanism. Am J Physiol Heart Circ Physiol 280: H2489–H2495.

Zhang WZ (2014). An association of metabolic syndrome constellation with cellular membrane caveolae. Pathobiol Aging Age Relat Dis 4: 23866. doi: 10.3402/pba.v4.23866; eCollection 2014.

Zhang X, Chen C (2012). A new insight of mechanisms, diagnosis and treatment of diabetic cardiomyopathy. Endocrine 41: 398–409.

Zhong N, Zhang Y, Zhu HF, Zhou ZN (2000). Intermittent hypoxia exposure prevents mtDNA deletion and mitochondrial structure damage produced by ischemia/reperfusion injury. Sheng Li Xue Bao 52: 375–380.