JOURNAL of ANESTHESIA and PERIOPERATIVE MEDICINE

Review Article

# Impaired RISK-GSK3β Pathway is Responsible for Comorbidities by Suppressing the Conditioning Cardioprotection

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## ABSTRACT

**Aim of review:** This review elaborates the role of reperfusion injury salvage kinase and glycogen synthase kinase 3β (RISK-GSK3β) pathway in myocardial ischemia/reperfusion injury (IRI) and whether its impairment is responsible for comorbidities due to the suppression of the conditioning cardioprotection.

**Method:** We review the articles about RISK-GSK3β pathway in myocardial IRI published in the last two decades.

**Recent findings:** The RISK, including phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt) and extracellular signal regulated kinase 1/2 (ERK1/2), combines with the downstream target of GSK3β, which confers important role in the conditioning cardioprotection when activated specifically at the time of myocardial reperfusion. Unfortunately, the conditioning protection is weakened or abolished when equipped with comorbidities such as aging, diabetes, obesity, as well as heart diseases. It has been speculated that the pathological processes resulting in RISK-GSK3β pathway alterations may affect the development of IRI and the responses to conditioning.

**Summary:** The impairment of RISK-GSK3β pathway is responsible for the reduction of conditioning cardioprotection and any strategy that repairs the impairment may have the potential to restore the conditioning against the IRI in the heart in the state of comorbidities.


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**Citation: Shi-Yun Jin, Ye Zhang. Impaired RISK-GSK3β pathway is responsible for comorbidities by suppressing the conditioning cardioprotection. *J Anesth Perioper Med* 2016; 3: 209-19.**

Deprived blood flow followed by inadequate oxygen and nutrient supply in patients with ischemic heart disease may lead to myocardial stunning, cardiac dysfunction, arrhythmia and even lethal myocardial infarction. Timely restoring the myocardial perfusion is an effective method to alleviate myocardial infarction and improve the long-term survival. However, the reperfusion itself paradoxically even induces myocardial injury termed as ischemia/reperfusion injury (IRI) (Figure 1). For reduction of IRI as much as possible, investigators have harvested a lot of cardioprotective strategies including ischemic preconditioning(IPC), remote ischemic-conditioning, various pharmacological conditioning and the combination of each other in the last three decades, which are all termed as "conditioning" , and the conditioning protection has been confirmed in many species and IRI models (1, 2).

Extensive basic studies have been carried out for elucidating the signal transduction pathways in conditioning cardioprotection. Interestingly, these conditionings recruit a similar signaling pathway at the initial of myocardial reperfusion, comprising cell-surface receptors, a diverse array of protein kinase cascades including the reperfusion injury salvage kinase (RISK) pathway, survivor activating factor enhancement, glycogen synthase kinase 3β (GSK3β) and the ter-

minal point of mitochondrial permeability transition pore (mPTP) (1, 3). RISK pathway, including phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt) and extracellular signal regulated kinase 1/2 (ERK1/2), as well as the downstream targets of GSK3β and mPTP in a broad sense, is activated at the onset of reperfusion, which is one of the most important mechanisms in conditioning cardioprotection (2, 3). Despite these landmark results are consistently proved in experimental models, it is a pity that conditioning application is restricted and the protection is diminished in clinical settings such as cardiac operation and elective percutaneous coronary intervention. The main reason is that the aging population and the growing prevalence of comorbidities such as ageing (4), diabetes (5), hypercholesterolemia (6), and heart diseases (7, 8), which are not present in animal studies. It has been speculated that pathological processes resulting in fundamental signaling molecular alteration may affect the development of IRI and the responses to cardioprotective interventions. However, it is hopeful that selective intervention for target proteins in the RISK and GSK3β pathways by specific drugs can restore the conditioning protection in the situation of comorbidities (9). In this review, we will provide brief overview of the role of RISK-GSK3β pathway in the conditioning against IRI; and second, to emphasize that impairment of RISK-GSK3β pathway is responsible for the reduction of conditioning cardioprotection and any agent contributing to recovery will restore the protection.

## Reperfusion Injury Salvage Kinase Pathway

The reperfusion injury salvage kinase (RISK) pathway is one of the most active and popular signaling pathways in recent years which have been demonstrated to play an important role in conditioning against IRI in different species including rat (10), rabbit (11), mouse (12), porcine (13) as well as human (14). Since the late 1990s, with the gradually recognition of cell apoptosis playing an important role in the lethal reperfusion injury, investigators have found that the so-called pro-survival anti-apoptotic protein kinases including ERK1/2 and Akt can be activated at the initial of myocardial reperfusion, which promoted the RISK pathway emerging as a concept (15). Some reviews have systematically reviewed the original and evolution of RISK pathway, which not only included ERK1/2 and Akt but also PKC, activated protein kinase G (PKG) and GSK3β, and demonstrated the downstream effector mechanisms and the clinical application (16, 17). Among these pro-survival kinases, GSK3β may act as a point of convergence for a variety of pro-survival signaling pathways resulting in mPTP inhibition and plays an important role in transferring protective signals. Unlike most kinases, GSK3β is constitutively active and deactivated by phosphorylation. Inactivation of the signaling kinase GSK3β has been shown to be a key event in the intracellular signaling of conditioning (18). In normal state, exogenous drugs and autacoids including adenosine, opioids, and bradykinin induced by conditioning occupy their respective cellular receptor and activate PI3K and RAS-MEK1/2, which cause activation of Akt and ERK1/2, subsequently, the activation of Akt and ERK1/2 further elevate the phosphorylation of GSK3β and finally inhibit mPTP opening at the initial of reperfusion (As shown in Figure 2). It is very interesting that, as the important components of the RISK pathway, ERK1/2 and PI3K/Akt have a "cross-talk" which inhibits one kinase cascade up-regulate the activity of the other pathway at the initial of reperfusion. It will ensure that the signal for cellular protection is executed to act as a compensatory safeguard (19). With the continuous development of research, investigators comprehensively understand the RISK pathway in IRI. It is striking that MG53, a muscle-specific TRIM-family protein expressed exclusively in the heart and skeletal muscle, executes acute membrane repair and elicits the IPC cardiacprotection in response to IRI (20). This phenomenon depends on both the caveolin-3 and the downstream recruitment of the PI3K/Akt and ERK1/2 signaling cascades. However, the heart is more vulnerable to IRI and resistant to IPC due to lack of MG53 in the heart and RISK signaling deficiency. Note that, overexpression of MG53 enhances Akt, GSK3β, and ERK1/2 phosphorylation and provides protection (20). After that, numerous studies have shown that MG53 exhibits crucial roles in the cardioprotection by regulating

RISK pathway (21-23) (Figure 2). It will provide a novel cardioprotective therapeutic strategy for the treatment of acute myocardial infarction.

## Impaired RISK-GSK3β Pathway Affects the Conditioning Cardioprotection under the Comorbidities

Numerous preclinical studies have indicated that conditioning is effective against myocardial IRI (1, 2). However, it is disappointing that application of the conditioning has not achieved satisfactory results from bench to bedside. It may be related to the complexity in human with comorbidities such as aging, diabetes, obesity, hypercholesterolemia and heart diseases (3, 9). These comorbidities worsen the normal physiological activity of cardiomyocyte including ionic and energetic balance, pro-survival kinase and mitochondrial function etc., especially the pro-survival kinases mainly including RISK-GSK3β pathway take a major responsibility in the pathological changes. These changes may underpin the negative effects of comorbidities on IRI and responsiveness to conditioning. So, in the next, we will focus on the comorbidities interfering RISK-GSK3β pathway in conditioning against IRI, in addition, we also discuss whether some drugs interfering with the target proteins involved in RISK-GSK3β pathway can restore the conditioning cardioprotection under the comorbidities (Figure 1).

### Aging

Aging is a well-recognized risk factor for ischemic heart disease. Numerous studies indicate that age-related intolerance to IRI is associated with alterations of membrane-sensitive cytoprotective signaling and cardiac mitochondrial function(14, 24). Especially, RISK-GSK3β pathway altered in aged hearts will undertake the responsibility for the suppression of conditioning protection (Table 1) .

Akt is an established survival signal in the heart, which is one arm of RISK pathway. Over the last decade, studies have shown that aberrant activation of Akt is associated with cardiovascular disease and cardiac aging. Whittington et al. (25) published a study of IPC in aged diabetic rat hearts that the loss of cardioprotection

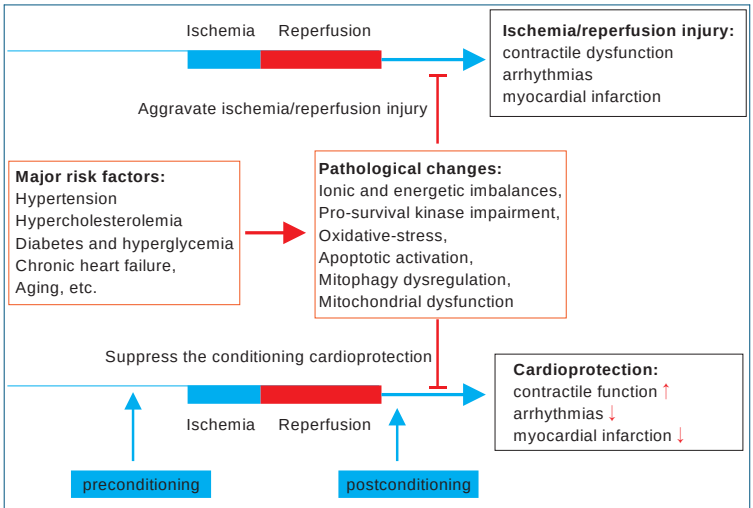


**Figure 1. Schematic of Ischemia/Reperfusion Injury and Conditioning Cardioprotection.**
Major risk factors may aggravate the ischemia/reperfusion injury(IRI) and suppress the conditioning protection. This figure was modified from pharmacological reviews (9).

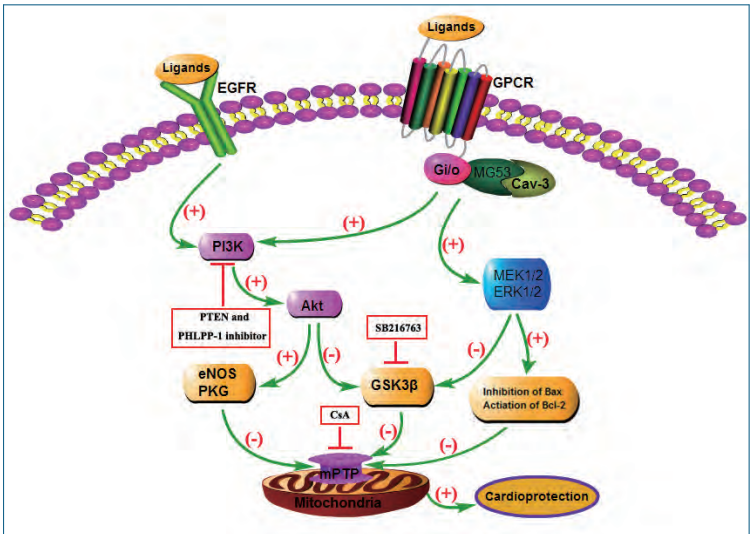


**Figure 2. RISK-GSK3β Pathway Signaling Transduction Involved in the Conditioning Cardioprotection.**
The figure shows a simplified overview of the RISK-GSK3β pathway signaling transduction involved in the conditioning and restoring the conditioning cardioprotection by interfering with some pro-survival kinases under the comorbidities. PTEN and PHLPP-1, the specific negative regulators of PI3K/Akt, are overexpressed in the heart under comorbidities, and inhibition of PTEN or PHLPP-1 will activate PI3K/Akt, subsequently inhibit GSK3β and mPTP opening to protect the heart against IRI. The inhibition of GSK3β and mPTP opening by their inhibitor, SB216763 and CsA, respectively, can also restore the conditioning protection under the comorbidities.
(+), activation; (-), inactivation; GSK3β, glycogen synthase kinase 3β; PI3K/Akt, phosphatidylinositol-3-kinase/protein kinase B; ERK1/2, extracellular signal regulated kinase 1/2; eNOS, endothelial nitric oxide synthase; PKG, activated protein kinase G; mPTP, mitochondrial permeability transition pore; CsA, cyclosporine A; PTEN, phosphatase and tensin homolog.

**Table 1. The List of the Main Pro-survival Kinases Involved in the Conditioning in Aged Hearts.**

| Conditioning | IRI model | Species | Pro-survival kinases involved | Main outcome |
|---|---|---|---|---|
| **Negative research** | | | | |
| Isoflurane post-conditioning (29) | 30 min RI and 60 min R | Wistar rats | ERK1/2, Akt, and GSK3β | Failed to reduce infarct size |
| Sevoflurane post-conditioning (30) | 30 min RI and 60 min R | Sprague-Dawley rats | ERK1/2 and Akt | Failed to reduce infarct size |
| Isoflurane pre-conditioning, CsA, SB-216763, and IPC (31-34) | 30 min RI and 60 min R | Fischer-344 rats | Akt/GSK3β and mPTP | Failed to prevent the mPTP opening and the fall in NAD+ levels |
| IpostC (28) | 30 min GI and 120 min R | middle-aged and old mice | Akt and GSK3β | Failed to reduce infarct size and Trx-1 |
| IPC (25) | 20 or 35 min RI and 60 min R | combined diabetic rats | Akt | Failed to reduce infarct size |
| **Positive research** | | | | |
| Hydrogen sulfide (37) | 40 min GI and 60 min R | Wistar rats | ERK1/2, JNK and p38 | Restored IpostC cardioprotection |
| Hydrogen sulfide (35, 36) | 40 min GI and 60 min R, 3h hypoxia and 6h reoxygenation | Wistar rats | PI3K, Akt, GSK3β, mPTP and Bcl-2 | Restored IpostC cardioprotection |
| PHLPP-1 knockdown Or exercise (27) | 30 min RI and 2, 4 or 24h R | Sprague-Dawley rats | Akt-1 | Reduction of apoptosis and infarct size and increased cardiac function |

RI, regional ischemia; GI, global ischemia; R, reperfusion.

was due to few extra Akt activation resulting from chronic Akt phosphorylation in the baseline, and continuous overexpression of Akt resulted in contractile dysfunction and susceptibility to cardiac injury (26). On the contrary, aging suppressed the conditioning protection due to insulin insensitiveness and overexpression of PHLPP-1(a specific negative regulator of Akt) during myocardial IRI, which subsequently limited Akt activity in the aged heart (27). In addition, a new study reported that ischemic post-conditioning (IPostC) failed to exert its cardioprotective effect owing to thioredoxin-1 degradation, a part of antioxidant system, and subsequently inactivated the Akt and GSK3β in middle-aged and old animals (28).

In addition to the impact of the Akt pathway, the aging also affects the ERK1/2 state in the situation of IRI. Chang et al. (29) demonstrated that brief administration of isoflurane 3 minutes before and 2 minutes after the initiation of early reperfusion failed to reduce infarct size associated with not only Akt, but also GSK3β and ERK1/2 in aged rat hearts. Another study also demonstrated that cardioprotection by sevoflurane post-conditioning in young rats is not effective in senescent rats, which may at least be the consequence of failing to activate Akt and ERK1/2, and inhibit mPTP opening ultimately (30).

In fact, the age-dependent impairment involves a wide signal transduction changes from the myocardial cell membrane receptors to mitochondrial targets (14), which was illustrated in a systematic and rigorous study by Peart et al. Similarly, Zhu et al. (31) demonstrated that the phosphorylation levels of Akt and GSK3β were remarkably elevated in the aged rats and isoflurane failed to perform cardioprotection related to incapable of further increasing the survival protein phosphorylation to reduce mPTP opening following IRI. In addition, they tried to use SB-216763 and cyclosporine A (CsA), GSK3β and mPTP inhibitor respectively, but it was a pity that both SB-216763 and CsA failed to reduce myocardial infarct size in the aged rats (32, 33). There is a deep reason that aging abolishes the conditioning protection associated with failing to reduce adenine nucleotide translocator-cyclophilin-D interactions and decrease GSK3β phosphorylation responsiveness to adenine nucleotide translocator, critical modulators of mPTP (34).

It is worthwhile to note that some drugs (Table 1) still preserve the cardioprotection by regu-

lating RISK-GSK3β pathway in the aged hearts and $H_2S$ is one of the most important members. In a series of studies, Li et al. had demonstrated that IpostC failed to protect the aged hearts against IRI related to impaired RISK-GSK3β pathway while exogenous $H_2S$ not only further enhanced the role of IPC in the young hearts but also recovered IpostC-induced cardioprotection via the inhibition of mPTP opening and oxidative stress by the activation of the ERK1/2, PI3K/Akt-GSK3β and PKC-ε-mitoKATP pathways in aging cardiomyocytes. These findings suggested that $H_2S$ might be a novel target for the treatment of aging cardiovascular diseases (35-37). Recently, a study showed that PHLPP-1 overexpression was responsible for the impairment of conditioning induced Akt activation in the aged hearts. However, PHLPP-1 knockdown or enhanced insulin sensitivity dramatically increased the phosphorylation of Akt and restored insulin induced cardioprotection (27).

Taken together, it is an indisputable fact that aging is associated with the loss or attenuation of conditioning protection. Some strategies have reported that the repair of RISK-GSK3β pathway may restore the conditioning protection in the aged hearts, but these findings have not been confirmed in clinical trials and studies to assess their effects on patients. Therefore, further clinical studies are warranted to understand the mechanisms of age-related myocardial changes, and to rationally evolve strategies to promote resistance to IRI.

### Hyperglycemia and Diabetes

Diabetes or hyperglycemia is important risk factor for the development of cardiovascular disorders. Treatment of patients with diabetes who have underlying ischemic heart disease is a challenge of the new millennium because of the complex pathophysiology and the poor prognosis of these comorbidities (3, 9, 18). Therefore, it is a very vigor and vitality field that investigators explore the relationship between cellular mechanisms changes in diabetes and the conditioning cardioprotection (Table 2).

Ischemic conditioning has been described impaired or suppressed in the diabetic hearts which greatly attributes to RISK pathway and this occurs through many mechanisms (3). An earlier paper reported that IPC still protected the diabetic hearts against IRI, but it appeared necessarily to increase the IPC stimulus compared with the non-diabetic hearts, which was attributed to demanding sufficient Akt phosphorylation to execute the IPC protective signal due to the threshold elevation (38). It is the same with aging that there is also chronic activation of PI3K/Akt in the diabetes hearts, and excessive insulin signaling to Akt is detrimental for IPC cardioprotection and could explain the failure of the diabetic myocardium to preconditioning (25, 26, 39). A recent study demonstrated that phosphatase and tensin homolog (PTEN), the negative regulator of PI3K/Akt, and overexpression in the diabetic rats were responsible for the loss of IpostC cardioprotection. But the effect that PTEN inhibition/gene knockdown mediated restoration of IpostC/hypoxic postconditioning (HpostC) cardioprotection, interestingly, was completely reversed by the PI3K inhibitor wortmannin (40). In addition, diabetes suppresses the cardioprotection induced by pharmacological conditioning via the RISK pathway. Kim et al. (41) performed a study that diabetes induced by the Streptozotocin mitigated remifentanil cardioprotection, which might be associated with reduction of the activities of proteins involved in anti-apoptotic pathways including ERK1/2 and the abnormal expression of sarcoplasmic reticulum genes as a result of IRI in rat hearts. What's more, hyperglycemia inhibited the protective effect of remifentanil pre-conditioning (42) or isoflurane post-conditioning (11) and this effect seemed to be mediated via modulation of Akt and eNOS.

Previous reviews have elaborated that the diabetes-mediated pathogenic effects are found to be mediated by inhibiting RISK and activating GSK3β pathway which undertakes the responsibility for impairment of ischemic or pharmacological conditioning in diabetic hearts (18). Hyperglycemia and hyper-insulinemia can both activate the GSK3β. The activated GSK3β can inhibit the myocardial transduction of insulin signaling and the utilization of glucose through the phosphorylation of IRS-1 which induces energy disarrangement and consequent pathological remodeling in the myocardium (43). The failure of cardioprotection by IpostC in diabetic hearts

may be attributed to the loss of GSK3β inactivation and thereby increasing oxidative stress (44). Therefore, it has become a very important target which is able to restore the conditioning cardioprotection by inhibiting the GSK3β activation in diabetic hearts. Two studies have shown diabetes abolished erythropoietin (EPO) or sufentanil- induced cardioprotection against IRI, while it was restored by the administration of GSK3β inhibitor SB216763(45, 46).

Although most of the studies show that there is a negative correlation between diabetes and myocardial IRI, but some other studies (47) also suggested that diabetes can change the mechanisms of anti- apoptosis of myocardial cells, inflammatory factors, increasing the energy utilization, and reducing the infarct size. Short- term diabetes promoted compensatory mechanisms that may provide cardioprotection against IRI, at least in part, by increasing antioxidants, the up-regulation of the pro-survival PI3K/Akt pathway, and the down- regulation of apoptotic genes and pro- inflammatory cytokine TNF- β (48). In addition, it is also reported that some agents restore the conditioning protection by interfering cell membrane receptors and the downstream signal transduction in diabetic hearts. A recent study performed by Rana et al. (49) demonstrated that diabetes mellitus caused attenuation of bioactive metabolism of membrane sphingolipids- sphingosine- 1- phosphate (S1P) and this may be a key mechanism of decreasing the IPC cardioprotection. It is interesting that S1P agonist FTY 720 combined with IPC prevents the diabetic hearts from IRI through inhibition of GSK3β and regulation of mPTP opening. Mokhtari et al. (50) indicated that troxerutin- treated by oral for 4 weeks could inactivate the GSK3β and reduce the apoptosis of IRI in the diabetic myocardium. Similarly, berberine treatment for 7 days prevented the diabetic hearts from IRI through AMPK activation, Akt phosphorylation, and GSK3β inhibition in the non- ischemic area of the diabetic hearts (51). What's more, it has been reported that EPO (52) and $Na_2S$ (12) therapy can protect the diabetic hearts against IRI by enhancing the EKR1/2 arm of the RISK pathway, and subsequently regulating the caspase-3 activity and oxidative stress (Table 2).

### Obesity

Obesity is a major risk factor for many diseases including dyslipidaemia, insulin resistance and hypertension. It has no effect on basal cardiac structure or functions but decreases myocardial tolerance to IRI and impedes the ability of conditioning cardioprotection by dys- regulation of kinase signaling pathways (53). However, some conditioning still preserves the cardioprotection in obesity, which is similar to aging and diabetes. Exercise is an important way to reduce obesity, induce cardioprotection against myocardial infarction, restore pro- survival signaling pathwayswith simultaneous increase in kinase phosphorylation including Akt, ERK1/2, p70S6K, AMPK and GSK3β, and increase resistance of mPTP opening, which has no relationship with improvement in obesity associated with comorbidities (54). In addition, investigators have creatively put forward that obesity is associated with better prognosis or tolerance to ischemic heart diseases, a phenomenon described as the "obesity paradox" since decade ago. Puzzling was that high fat diet reduced infarct size and cardiac dilation, as well as improved left ventricular function compared to control diet animals. Researchers clarified the mechanisms that these effects were associated with enhanced RISK pathway function in high fat diet- fed animals' hearts shown as increased Akt and GSK3β phosphorylation (55). Anyway, obesity has reached epidemic proportions and its association with the heart diseases may play a potential detrimental role in suppressing the conditioning cardioprotection.

### Hyperlipidemia and Hypercholesterolemia

Hypercholesterolemia is one type of hyperlipidemia and regarded as the most prevalent risk factor for coronary artery stenosis and suppressing the conditioning cardioprotection. Despite the cellular mechanisms of hyperlipidemia influencing the conditioning cardioprotection is unclear, but hypercholesterolemia- induced deterioration of RISK pathway can provide reference to elaborate the mechanisms. A study performed by Wu et al. (56) showed that hypercholesterolemia inhibited the phosphorylation of Akt and ERK1/2, and subsequently induced excessive apoptosis by down- regulating Bcl- 2 and up- regulating Bax,

**Table 2. The List of the Main Pro-survival Kinases Involved in the Conditioning in Diabetes Hearts.**

| Conditioning | IRI model | Species | Pro-survival kinases involved | Main outcome |
|---|---|---|---|---|
| **Negative research** | | | | |
| IpostC (44) | 30 min RI and 60 min R | Wistar rats | GSK3β | Failed to reduce infarct size |
| Sufentanil or erythropoietin (45, 46) | 25 min GI and 30 or 120 min R, 30 min RI and 60 min R | Sprague–Dawley rats or Wistar rats | ERK1/2, Akt and GSK3β | Failed to reduce infarct size |
| Isoflurane post-conditioning (11) | 40 min RI and 3 h R | Rabbits | Akt and eNOS | Failed to reduce infarct size and CK-MB |
| Remifentanil pre-conditioning (41, 42) | 30 min RI and 60 min R, 1h hypoxia and 5h reoxygenation | Sprague–Dawley rats | ERK1/2, Akt and Bax/Bcl-2 | Failed to reduce infarct size and apoptosis |
| IPC (25) | 20 or 35 min RI and 60 min R | combined aged rats | Akt | Failed to reduce infarct size |
| **Positive research** | | | | |
| IPC (38) | 35 min RI and 120 min R | Wistar rats | Akt | Only three cycles of IPC reduced infarction |
| SB-216763 (45, 46) | 25 min GI and 30 or 120 min R, 30 min RI and 60 min R | Sprague–Dawley rats or Wistar rats | ERK1/2, Akt and GSK3β | Reduction of infarct size |
| PTEN inhibitor and IPostC (40) | 30 min RI and 120 min R, 4h hypoxia and 2h reoxygenation | Sprague–Dawley rats and H9c2 cells | PI3K/Akt and GSK3β | Restoration of IpostC cardioprotecion |
| Hydrogen sulfide (12) | 45 min RI and 24 h R | Diabetic mice (db/db) | ERK1/2 and GSK3β | Reduction of infarct size and apoptosis |
| Erythropoietin (52) | 30 min RI and 4h R, 2h hypoxia and 4h reoxygenation | Sprague-Dawley rats and H9c2 cells | ERK1/2 and Bcl-2 | Reduction of infarct size and apoptosis |
| Berberine (51) | 30 min RI and 120 min R | Wistar rats | PI3K/Akt and GSK3β | Reduction of infarct size and arrhythmias |
| S1P agonist FTY720 (49) | 30 min GI and 120 min R | Wistar rats | GSK3β and mPTP | Reduction of infarct size, LDH and CK-MB |

RI, regional ischemia; GI, global ischemia; R, reperfusion.

cytochrome c, caspase 9 and caspase 3, and resulting in reversing the IpostC cardioprotection. It is exciting that a number of methods have been shown effective for preserving cardioprotection in the presence of hypercholesterolemia. Rho-kinase activity is involved in diverse cardiovascular diseases. Fasudil, inhibitor of Rho-kinase, preserved conditioning against myocardial IRI under hypercholesterolemia by up-regulating the PI3K/Akt/eNOS pathway and induced the the m KATP channel opening, but high dose of fasudil was needed (57). Furthermore, fasudil was also able to restore the IpostC cardioprotection in the hypercholesterolemic rat hearts through the activation of PI3K/Akt/eNOS signaling pathway (58). Recent studies have demonstrated that hyperchoesterolemia abrogated sevoflurane or ischemic-induced cardioprotection by alteration of upstream signaling of GSK3β, while it was reversed by GSK3β inhibitor SB216763. Furthermore, investigators showed that MG53, an upstream signaling cascade of Akt, contributed to acute membrane repair in cardiomyocytes and was impaired in hypercholesterolemic rat hearts, which was responsible for the deficit of Akt and ERK1/2 phosphorylation (22, 59). It will provide a new target for restoring the conditioning protection in hypercholesterolemic hearts.

## Uremic Heart

Uremia is one of the independent risk factors for cardiovascular mortality, which gives rise to a distinct cardiac pathology termed as uremic cardiomyopathy. Uremic cardiomyopathy is associated with a combination of structural and cellular remodeling, but it is controversial that whether the uremic heart suppresses the conditioning protection and is more susceptible to IRI. Semple et al. (60) established the rat uremia model induced by subtotal nephrectomy and uremia was associated with enhanced susceptibility to IRI. In the study, they also found that loss of insulin-mediated cardioprotection can be restored by administration of rosiglitazone. Altered Akt2 expression in uremic hearts after ischemia/reper-

fusion and impaired activation of the RISK pathway may underlie these findings. However, in another study, Uremia was induced by partial nephrectomy in male Wistar rats. The investigators demonstrated that uremia didn't aggravate myocardial infarct size and IPC protection was still preserved although uremia led to severe metabolic changes and mild myocardial dysfunction (61). A preclinical study reported by Byrne et al. (62) represented a comprehensive assessment of the effects of chronic uremia on ischemic conditioning and demonstrated that, unlike in diabetic and senescent animals, chronic uremia did not appear to attenuate the effect of ischemic conditioning. Analysis of the signaling mechanisms revealed that components of one arm of RISK Pathway, ERK1/2 was similarly up-regulated in both uremic and non-uremic animals after an IPC stimulus. While the researchers were unable to demonstrate a consistent result with respect to the phosphorylation of Akt in response to the preconditioning protocol (62). However, recent research results were inconsistent with their conclusion. Cardiomyocytes in uremia are characterized by a more oxidized mitochondrial network, with greater susceptibility to oxidant-induced cell death and enhanced vulnerability to calcium-induced mPTP formation (63). Mitochondrial is the downstream target of RISK pathway. Even if conditioning can activate the RISK pathway, it would be most unproductive if the mitochondrial function was compromised in the uremic hearts. However, further studies are needed to elucidate the abnormalities in well-established conditioning associated with RISK pathway and the downstream molecules involved in uremic hearts.

### Hypertension and Myocardial Hypertrophy

Myocardial hypertrophy is commonly defined as a pathological state characterized by modifications in cardiac size and shape, and changes in response to a variety of pathology including hypertension, renal disease and anemia, within which hypertension is the most common cause. The myocardial structural and signal transduction changes will render the heart more sensitive to IRI and suppress the effect of cardioprotective strategies. A very meaningful study performed by Andensern et al. (4) demonstrated that right ventricular hypertrophy increased IRI. Cardioprotection by IPC was abolished in the failing but not the compensated hypertrophic right ventricular of the rat hearts. But IPC cardioprotection was diminished in the compensated hypertrophic right ventricular due to the down-regulation of ERK1/2 phosphorylation in the baseline, and interestingly, the phosphorylation of Akt and GSK3β was unaltered in the failing and hypertrophic hearts, suggesting that the two proteins were not involved in the pathological process (5). Different from this, Ma et al. reported that ventricular hypertrophy abrogated sevoflurane or IpostC-induced cardioprotection against IRI by alteration of both Akt, ERK1/2, and GSK3β signals (64). Furthermore, the research team designed another study that intralipid was deprived of cardioprotection due to no capacity for up-regulating the phosphorylation of Akt, ERK1/2, and their downstream target of GSK3β in the hypertrophic myocardium, but GSK3β inhibitor SB216763 failed to restore the intralipid cardioprotection (7), and it coincided with another study performed by Oei et al. (65). On the contrary, some studies showed that GSK3β inhibitors imitated the cardioprotection afforded by ischemic conditioning and suggested that a decrease in mPTP by GSK3β/VDAC interaction was a crucial event (66). In addition, IpostC exerted cardioprotection in compensated hypertrophic myocardium induced by angiotensin II. Interestingly, they found that the Akt phosphorylation kinetic was paralleled with ERK1/2 activation, but Akt remained active in spite of PI3K dysfunction in compensated hypertrophic post-conditioning hearts, which was possibly due to a cross-talk between ERK1/2 and Akt (19, 67). Anyhow, it is a fact that the existence of a "threshold" in hypertensive myocardium needs much more stimulation in order to obtain the same cardioprotection as well as in the normal state.

### Post-infarct Myocardial Remodeling

A study performed by Miki et al. in 2000 for the first time reported that rabbit with post-infarct myocardial remodeling (PIMR) was refractory to IPC and suggested cardioprotective signals were impaired at the early stage of ventricular remodeling (8). Subsequently, they noted that, in contrast to IPC, EPO protected the

PIMR hearts against IRI in accordance with the normal myocardium, but the mechanisms were different by which activation of ERK1/2 compensated the lack of signal input from the activated PI3K/Akt pathway, sending signals to downstream mediators of cell protection in the PIMR hearts (68, 69). However, two studies showed that PI3K/Akt but not ERK1/2 was the predominant kinase in conditioning- mediated cardioprotection in PIMR. Conditioning increased phosphorylation of the PKB/Akt downstream targets of eNOS, GSK3β, and p70S6K in remodeled hearts (70, 71). It will provide a theoretical basis for clinical treatment of myocardial IRI after PIMR, though those basic researches come to diametrically opposite conclusion at the regulating mechanisms.

### Chronic Heart Failure

Chronic heart failure is the end-stage of various cardiovascular diseases, and even threats to the patients' life. A number of studies have shown that the failing hearts may be more vulnerable to IRI during cardiopulmonary bypass and even in the non-cardiac surgeries (72, 73). But the recent studies have shown that some drugs preserve the failing heart against IRI by the pro-survival kinases. Khaliulin et al. (74) indicated consecutive isoproterenol and adenosine treatment can protect the failing heart mediated by glycogen depletion and inhibition of mPTP. An important study conducted by Kondo et al. (75) demonstrated that $H_2S$ treatment preserved mitochondrial function, attenuated oxidative stress, and increased myocardial vascular density by activating a VEGF- Akt- eNOS- cGMP signaling pathway at the 6th week following the induction of pressure overload. It provided an important insight into the mechanism by which activation of Akt attenuated pressure overload- induced heart failure. On the contrary, Andersen et al. (5) found that right ventricular hypertrophy- induced heart failure abolished the protective effect of IPC. Compared with the normal condition, this change was mainly related to the decreased phosphorylation level of ERK1/2, but it had no relationship with Akt/GSK3β. Similarly, we have demonstrated that morphine preconditioning exerts cardioprotection via activation of ERK1/2 but not PI3K/Akt signaling pathway, and subsequently inhibits the downstream GSK3β activity in the failing hearts induced by doxorubicin (76). The roles of microRNAs (miRNAs) have been implicated in IRI and cardioprotection, recently, we revealed that morphine preconditioning can protect the adult rat cardiomyocytes against IRI by inducing differentially expressed miRNAs (77). It is known that miRNAs and the pro-survival kinase of ERK1/2 are inextricably linked, and in turn, it regulates the miRNAs and subsequent ERK1/2 activation, which may play an important role in the failing hearts in speculation (78). Although the experimental findings suggest a potential effect of protecting the failing heart against IRI, but further clinical researches are needed to be conducted to prove the effectiveness.

## Conclusion

Numerous studies have demonstrated that RISK-GSK3β pathway plays an important role in conditioning cardioprotection. Conditioning can effectively activate RISK pathway and subsequently inactivate GSK3β after a series of cascade activation reactions, inhibit mPTP opening, suppress cell apoptosis and improve the cardiac function. However, when combined with comorbidities including aging, diabetes, obesity, hypercholesterolemia and heart diseases, the pathological processes may cause pro-survival kinases over-activation or inactivation, mitochondrial dis- function as well as the pro- survival kinases negative regulator overexpression etc. It is natural that conditioning is unable to recruit the pro-survival kinases at the initial of reperfusion and thereby lacks the ability of cardioprotection under these changes. So, any strategy that repairs the impairment may have the potential to restore the conditioning against the IRI in the heart in the state of comorbidities. Studies more deeply and closely related to the clinical setting will provide pharmacological targets to limit IRI and reduce the mortality of ischemic heart disease.

This work was supported by the National Natural Science Foundation of China (81471145 to Zhang) and the Major Program of Natural Science Foundation of Higher Education Institutions of Anhui Province (KJ2014ZD16 to Zhang).
No potential conflict of interest relevant to this article was reported.

## References


1. Hausenloy DJ, Yellon DM. Ischaemic conditioning and reperfusion injury. *Nat Rev Cardiol* 2016; 13:193-209.
2. Headrick JP, See Hoe LE, Du Toit EF, Peart JN. Opioid receptors and cardioprotection- 'opioidergic conditioning' of the heart. *Br J Pharmacol* 2015;172: 2026-50.
3. Lejay A, Fang F, John R, Van JA, Barr M, Thaveau F, et al. Ischemia reperfusion injury, ischemic conditioning and diabetes mellitus. *J Mol Cell Cardiol* 2016; 91:11-22.
4. Andersen A, Povlsen JA, Botker HE, Nielsen-Kudsk JE. Right ventricular hypertrophy and failure abolish cardioprotection by ischaemic pre-conditioning. *Eur J Heart Fail* 2013;15:1208-14.
5. Przyklenk K. Efficacy of cardioprotective 'conditioning' strategies in aging and diabetic cohorts: the co-morbidity conundrum. *Drugs Aging* 2011;28:331-43.
6. Tang XL, Takano H, Xuan YT, Sato H, Kodani E, Dawn B, et al. Hypercholesterolemia abrogates late preconditioning via a tetrahydrobiopterin-dependent mechanism in conscious rabbits. *Circulation* 2005; 112:2149-56.
7. Ma LL, Ge HW, Kong FJ, Qian LB, Hu BC, Li Q, et al. Ventricular hypertrophy abrogates intralipid-induced cardioprotection by alteration of reperfusion injury salvage kinase/glycogen synthase kinase 3β signal. *Shock* 2014;41:435-42.
8. Miki T, Miura T, Tsuchida A, Nakano A, Hasegawa T, Fukuma T, et al. Cardioprotective mechanism of ischemic preconditioning is impaired by postinfarct ventricular remodeling through angiotensin II type 1 receptor activation. *Circulation* 2000;102:458-63.
9. Ferdinandy P, Schulz R, Baxter GF. Interaction of cardiovascular risk factors with myocardial ischemia/reperfusion injury, preconditioning, and postconditioning. *Pharmacol Rev* 2007;59: 418-58.
10. Manintveld OC, Sluiter W, Dekkers DH, te Lintel Hekkert M, Lamers JM, Verdouw PD, et al. Involvement of reperfusion injury salvage kinases in preconditioning depends critically on the preconditioning stimulus. *Exp Biol Med (Maywood)* 2011;236:874-82.
11. Raphael J, Gozal Y, Navot N, Zuo Z. Hyperglycemia inhibits anesthetic-induced postconditioning in the rabbit heart via modulation of phosphatidylinositol-3-kinase/Akt and endothelial nitric oxide synthase signaling. *J Cardiovasc Pharmacol* 2010;55:348-57.
12. Lambert JP, Nicholson CK, Amin H, Amin S, Calvert JW. Hydrogen sulfide provides cardioprotection against myocardial/ischemia reperfusion injury in the diabetic state through the activation of the RISK pathway. *Med Gas Res* 2014;4:20.
13. Santos-Gallego CG, Vahl TP, Goliasch G, Picatoste B, Arias T, Ishikawa K, et al. Sphingosine-1-phosphate receptor agonist fingolimod increases myocardial salvage and decreases adverse postinfarction left ventricular remodeling in a porcine model of ischemia/reperfusion. *Circulation* 2016;133:954-66.
14. Peart JN, Pepe S, Reichelt ME, Beckett N, See Hoe L, Ozberk V, et al. Dysfunctional survival-signaling and stress-intolerance in aged murine and human myocardium. *Exp Gerontol* 2014;50:72-81.
15. Zhao ZQ, Nakamura M, Wang NP, Wilcox JN, Shearer S, Ronson RS, et al. Reperfusion induces myocardial apoptotic cell death. *Cardiovasc Res* 2000;45: 651-60.
16. Hausenloy DJ, Yellon DM. Reperfusion injury salvage kinase signalling: taking a RISK for cardioprotection. *Heart Fail Rev* 2007;12:217-34.
17. Hausenloy DJ, Lecour S, Yellon DM. Reperfusion injury salvage kinase and survivor activating factor enhancement prosurvival signaling pathways in ischemic postconditioning: two sides of the same coin. *Antioxid Redox Signal* 2011;14:893-907.
18. Yin X, Zheng Y, Zhai X, Zhao X, Cai L. Diabetic inhibition of preconditioning- and postconditioning-mediated myocardial protection against ischemia/reperfusion injury. *Exp Diabetes Res* 2012;2012: 198048.
19. Hausenloy DJ, Mocanu MM, Yellon DM. Cross-talk between the survival kinases during early reperfusion: its contribution to ischemic preconditioning. *Cardiovasc Res* 2004;63:305-12.
20. Cao CM, Zhang Y, Weisleder N, Ferrante C, Wang X, Lv F, et al. MG53 constitutes a primary determinant of cardiac ischemic preconditioning. *Circulation* 2010;121:2565-74.
21. Zhang Y, Lv F, Jin L, Peng W, Song R, Ma J, et al. MG53 participates in ischaemic postconditioning through the RISK signalling pathway. *Cardiovasc Res* 2011;91:108-15.
22. Ma LL, Zhang FJ, Qian LB, Kong FJ, Sun JF, Zhou C, et al. Hypercholesterolemia blocked sevoflurane-induced cardioprotection against ischemia-reperfusion injury by alteration of the MG53/RISK/GSK3β signaling. *Int J Cardiol* 2013;168:3671-78.
23. Liu J, Zhu H, Zheng Y, Xu Z, Li L, Tan T, et al. Cardioprotection of recombinant human MG53 protein in a porcine model of ischemia and reperfusion injury. *J Mol Cell Cardiol* 2015;80:10-9.
24. Wohlgemuth SE, Calvani R, Marzetti E. The interplay between autophagy and mitochondrial dysfunction in oxidative stress-induced cardiac aging and pathology. *J Mol Cell Cardiol* 2014;71: 62-70.
25. Whittington HJ, Harding I, Stephenson CI, Bell R, Hausenloy DJ, Mocanu MM, et al. Cardioprotection in the aging, diabetic heart: the loss of protective Akt signalling. *Cardiovasc Res* 2013;99:694-704.
26. Taniyama Y, Ito M, Sato K, Kuester C, Veit K, Tremp G, et al. Akt3 overexpression in the heart results in progression from adaptive to maladaptive hypertrophy. *J Mol Cell Cardiol* 2005;38: 375-85.
27. Xing Y, Sun W, Wang Y, Gao F, Ma H. Mutual inhibition of insulin signaling and PHLPP-1 determines cardioprotective efficiency of Akt in aged heart. *Aging (Albany NY)* 2016; 8(5): 873-88.
28. Perez V, D Annunzio V, Mazo T, Marchini T, Caceres L, Evelson P, et al. Ischemic postconditioning confers cardioprotection and prevents reduction of Trx-1 in young mice, but not in middle-aged and old mice. *Mol Cell Biochem* 2016;415:67-76.
29. Chang DJ, Chang CH, Kim JS, Hong YW, Lee WK, Shim YH. Isoflurane-induced post-conditioning in senescent hearts is attenuated by failure to activate reperfusion injury salvage kinase pathway. *Acta Anaesthesiol Scand* 2012;56:896-903.
30. Li H, Zhou C, Chen D, Fang N, Yao Y, Li L. Failure to protect against myocardial ischemia-reperfusion injury with sevoflurane postconditioning in old rats in vivo. *Acta Anaesthesiol Scand* 2013;57:1024-31.
31. Zhu J, Rebecchi MJ, Tan M, Glass PS, Brink PR, Liu L. Age-associated differences in activation of Akt/GSK-3beta signaling pathways and inhibition of mitochondrial permeability transition pore opening in the rat heart. *J Gerontol A Biol Sci Med Sci* 2010;65:611-19.
32. Liu L, Zhu J, Brink PR, Glass PSA, Rebecchi MJ. Age-associated differences in the inhibition of mitochondrial permeability transition pore opening by cyclosporine A. *Acta Anaesthesiol Scand* 2011;55:622-30.
33. Zhu J, Rebecchi MJ, Glass PS, Brink PR, Liu L. Cardioprotection of the aged rat heart by GSK-3beta inhibitor is attenuated: age-related changes in mitochondrial permeability transition pore modulation. *Am J Physiol Heart Circ Physiol* 2011;300:H922-30.
34. Zhu J, Rebecchi MJ, Glass PS, Brink PR, Liu L. Interactions of GSK-3beta with mitochondrial permeability transition pore modulators during preconditioning: age-associated differences. *J Gerontol A Biol Sci Med Sci* 2013;68:395-403.
35. Li H, Wang Y, Wei C, Bai S, Zhao Y, Li H, et al. Mediation of exogenous hydrogen sulfide in recovery of ischemic post-conditioning-induced cardioprotection via down-regulating oxidative stress and up-regulating PI3K/Akt/GSK-3beta pathway in isolated aging rat hearts. *Cell Biosci* 2015;5:11.
36. Li H, Zhang C, Sun W, Li L, Wu B, Bai S, et al. Exogenous hydrogen sulfide restores cardioprotection of ischemic post-conditioning via inhibition of mPTP opening in the aging cardiomyocytes. *Cell Biosci* 2015;5:43.
37. Wei C, Zhao Y, Wang L, Peng X, Li H, Zhao Y, et al. H2S restores the cardioprotection from ischemic post-conditioning in isolated aged rat hearts. *Cell Biol Int* 2015;39:1173-76.
38. Tsang A, Hausenloy DJ, Mocanu MM, Carr RD, Yellon DM. Preconditioning the diabetic heart the importance of Akt phosphorylation. *Diabetes* 2005;54: 2360-64.
39. Fullmer TM, Pei S, Zhu Y, Sloan C, Manzanares R, Henrie B, et al. Insulin suppresses ischemic preconditioning-mediated cardioprotection through Akt-dependent mechanisms. *J Mol Cell Cardiol* 2013;64:20-9.
40. Xue R, Lei S, Xia ZY, Wu Y, Meng Q, Zhan L, et al. Selective inhibition of PTEN preserves ischaemic post-conditioning cardioprotection in STZ-induced Type 1 diabetic rats: role of the PI3K/Akt and JAK2/STAT3 pathways. *Clin Sci (Lond)* 2016;130:377-92.
41. Kim HS, Cho JE, Hwang KC, Shim YH, Lee JH, Kwak YL. Diabetes mellitus mitigates cardioprotective effects of remifentanil preconditioning in ischemia-reperfused rat heart in association with anti-apoptotic pathways of survival. *Eur J Pharmacol* 2010; 628:132-39.
42. Kim HS, Kim SY, Kwak YL, Hwang KC, Shim YH. Hyperglycemia attenuates myocardial preconditioning of remifentanil. *J Surg Res* 2012;174:231-37.
43. Wang Y, Feng W, Xue W, Tan Y, Hein DW, Li XK, et al. Inactivation of GSK-3beta by metallothionein prevents diabetes-related changes in cardiac energy metabolism, inflammation, nitrosative damage, and remodeling. *Diabetes* 2009;58:1391-402.
44. Badalzadeh R, Mohammadi M, Yousefi B, Farajnia S, Najafi M, Mohammadi S. Involvement of glycogen synthase kinase-3β and oxidation status in the loss of cardioprotection by postconditioning in chronic diabetic male rats. *Adv Pharm Bull* 2015;5:321-27.
45. Ghaboura N, Tamareille S, Ducluzeau PH, Grimaud L, Loufrani L, Croue A, et al. Diabetes mellitus abrogates erythropoietin-induced cardioprotection against ischemic-reperfusion injury by alteration of the RISK/GSK-3beta signaling. *Basic Res Cardiol* 2011;106:147-62.
46. Chen QL, Gu EW, Zhang L, Cao YY, Zhu Y, Fang WP. Diabetes mellitus abrogates the cardioprotection of sufentanil against ischaemia/reperfusion injury by altering glycogen synthase kinase-3beta. *Acta Anaesthesiol Scand* 2013;57:236-42.
47. Malfitano C, Alba Loureiro TC, Rodrigues B, Sirvente R, Salemi VM, Rabechi NB, et al. Hyperglycaemia protects the heart after myocardial infarction: aspects of programmed cell survival and cell death. *Eur J Heart Fail* 2010;12:659-67.
48. Korkmaz-Icoz S, Lehner A, Li S, Vater A, Radovits T, Hegedus P, et al. Mild type 2 diabetes mellitus reduces the susceptibility of the heart to ischemia/reperfusion injury: identification of underlying gene expression changes. *J Diabetes Res* 2015;2015:396414.
49. Rana A, Sharma S. Mechanism of sphingosine-1-phosphate induced cardioprotection against I/R injury in diabetic rat heart: possible involvement of glycogen synthase kinase 3β and mitochondrial permeability transition pore. *Clin Exp Pharmacol Physiol* 2016; 43:166-73.
50. Mokhtari B, Badalzadeh R, Alihemmati A, Mohammadi M. Phosphorylation of GSK-3β and reduction of apoptosis as targets of troxerutin effect on reperfusion injury of diabetic myocardium. *Eur J Pharmacol* 2015;765:316-21.
51. Chang W, Li K, Guan F, Yao F, Yu Y, Zhang M, et al. Berberine Pretreatment Confers Cardioprotection Against Ischemia-Reperfusion Injury in a Rat Model of Type 2 Diabetes. *J Cardiovasc Pharmacol Ther* 2016 Feb 4.
52. Jun JH, Jun NH, Shim JK, Shin EJ, Kwak YL. Erythropoietin protects myocardium against ischemia-reperfusion injury under moderate hyperglycemia. *Eur J Pharmacol* 2014;745:1-9.
53. Wensley I, Salaveria K, Bulmer AC, Donner DG, du Toit EF. Myocardial structure, function and ischaemic tolerance in a rodent model of obesity with insulin resistance. *Exp Physiol* 2013;98:1552-64.
54. Pons S, Martin V, Portal L, Zini R, Morin D, Berdeaux A, et al. Regular treadmill exercise restores cardioprotective signaling pathways in obese mice independently from improvement in associated co-mor-

bidities. *J Mol Cell Cardiol* 2013;54:82-89.
55. Poncelas M, Inserte J, Vilardosa U, Rodriguez-Sinovas A, Baneras J, Simo R, et al. Obesity induced by high fat diet attenuates postinfarct myocardial remodeling and dysfunction in adult B6D2F1 mice. *J Mol Cell Cardiol* 2015;84:154-61.
56. Wu N, Zhang X, Jia P, Jia D. Hypercholesterolemia abrogates the protective effect of ischemic postconditioning by induction of apoptosis and impairment of activation of reperfusion injury salvage kinase pathway. *Biochem Biophys Res Commun* 2015; 458:148-53.
57. Wu N, Zhang X, Jia D. Highdose fasudil preconditioning and postconditioning attenuate myocardial ischemiareperfusion injury in hypercholesterolemic rats. *Mol Med Rep* 2014;9:560-66.
58. Wu N, Li W, Shu W, Lv Y, Jia D. Inhibition of Rho-kinase by fasudil restores the cardioprotection of ischemic postconditioninng in hypercholesterolemic rat heart. *Mol Med Rep* 2014;10:2517-24.
59. Xu Y, Ma LL, Zhou C, Zhang FJ, Kong FJ, Wang WN, et al. Hypercholesterolemic myocardium is vulnerable to ischemia-reperfusion injury and refractory to sevoflurane-induced protection. *PLoS One* 2013;8: e76652.
60. Semple DJ, Bhandari S, Seymour AM. Uremic cardiomyopathy is characterized by loss of the cardioprotective effects of insulin. *Am J Physiol Renal Physiol* 2012;303:F1275-86.
61. Kocsis GF, Sarkozy M, Bencsik P, Pipicz M, Varga ZV, Paloczi J, et al. Preconditioning protects the heart in a prolonged uremic condition. *Am J Physiol Heart Circ Physiol* 2012;303:H1229-36.
62. Byrne CJ, McCafferty K, Kieswich J, Harwood S, Andrikopoulos P, Raftery M, et al. Ischemic conditioning protects the uremic heart in a rodent model of myocardial infarction. *Circulation* 2012;125:1256-65.
63. Taylor D, Bhandari S, Seymour AM. Mitochondrial dysfunction in uremic cardiomyopathy. *Am J Physiol Renal Physiol* 2015;308:F579-87.
64. Ma LL, Zhang FJ, Kong FJ, Qian LB, Ma H, Wang JA, et al. Hypertrophied myocardium is refractory to sevoflurane-induced protection with alteration of reperfusion injury salvage kinase/glycogen synthase kinase 3β signals. *Shock* 2013;40:217-21.
65. Oei GT, Huhn R, Heinen A, Hollmann MW, Schlack WS, Preckel B, et al. Helium-induced cardioprotection of healthy and hypertensive rat myocardium in vivo. *Eur J Pharmacol* 2012;684:125-31.
66. Gonzalez Arbelaez LF, Perez Nunez IA, Mosca SM. Gsk-3βinhibitors mimic the cardioprotection mediated by ischemic pre- and postconditioning in hypertensive rats. *Biomed Res Int* 2013;2013:317456.
67. Hernández-Reséndiz S, Roldán FJ, Correa F, Martínez-Abundis E, Osorio-Valencia G, Ruíz-de-Jesús O, et al. Postconditioning protects against reperfusion injury in hypertensive dilated cardiomyopathy by activating MEK/ERK1/2 signaling. *J Card Fail* 2013;19:135-46.
68. Miki T, Miura T, Yano T, Takahashi A, Sakamoto J, Tanno M, et al. Alteration in erythropoietin-induced cardioprotective signaling by postinfarct ventricular remodeling. *J Pharmacol Exp Ther* 2006;317: 68-75.
69. Miki T, Miura T, Tanno M, Nishihara M, Naitoh K, Sato T, et al. Impairment of cardioprotective PI3K-Akt signaling by post-infarct ventricular remodeling is compensated by an ERK-mediated pathway. *Basic Res Cardiol* 2007;102:163-70.
70. Zhu M, Feng J, Lucchinetti E, Fischer G, Xu L, Pedrazzini T, et al. Ischemic postconditioning protects remodeled myocardium via the PI3K-PKB/Akt reperfusion injury salvage kinase pathway. *Cardiovasc Res* 2006;72:152-62.
71. Feng J FG, Lucchinetti E, Zhu M, Bestmann L, Jegger D, Arras M, et al. Infarct-remodeled myocardium is receptive to protection by isoflurane postconditioning: role of protein kinase B/Akt signaling. *Anesthesiology* 2006;104:1004-14.
72. Hausenloy DJ, Boston-Griffiths E, Yellon DM. Cardioprotection during cardiac surgery. *Cardiovasc Res* 2012;94:253-65.
73. Devereaux PJ, Chan MT, Alonso-Coello P, Walsh M, Berwanger O, Villar JC, et al. Association between postoperative troponin levels and 30-day mortality among patients undergoing noncardiac surgery. *JAMA* 2012;307:2295-304.
74. Khaliulin I, Halestrap AP, Bryant SM, Dudley DJ, James AF, Suleiman MS. Clinically-relevant consecutive treatment with isoproterenol and adenosine protects the failing heart against ischaemia and reperfusion. *J Transl Med* 2014;12:139.
75. Kondo K, Bhushan S, King AL, Prabhu SD, Hamid T, Koenig S, et al. H2S Protects Against Pressure Overload-Induced Heart Failure via Upregulation of Endothelial Nitric Oxide Synthase. *Circulation* 2013; 127:1116-27.
76. He SF, Jin SY, Wu H, Wang B, Wu YX, Zhang SJ, et al. Morphine preconditioning confers cardioprotection in doxorubicin-induced failing rat hearts via ERK/GSK-3βpathway independent of PI3K/Akt. *Toxicol Appl Pharmacol* 2015;288:349-58.
77. He SF, Zhu HJ, Han ZY, Wu H, Jin SY, Irwin MG, et al. MicroRNA-133b-5p Is Involved in Cardioprotection of Morphine Preconditioning in Rat Cardiomyocytes by Targeting Fas. *Can J Cardiol* 2015; Oct 30.
78. Sanchez-Simon FM, Zhang XX, Loh HH, Law PY, Rodriguez RE. Morphine regulates dopaminergic neuron differentiation via miR-133b. *Mol Pharmacol* 2010;78:935-42.