BBA - Molecular Basis of Disease 1865 (2019) 822–830

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

Review

Is there an effect of ischemic conditioning on myocardial contractile function following acute myocardial ischemia/reperfusion injury?☆

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ARTICLE INFO

Keywords:
Myocardial ischemic conditioning
Contractile function
Mitochondria
Post-translational modifications

ABSTRACT

Ischemic conditioning induces cardioprotection; the final infarct size following a myocardial ischemic event is reduced. However, whether ischemic conditioning has long-term beneficial effects on myocardial contractile function following such an ischemic event needs further elucidation. To date, *ex vivo* studies have shown that ischemic conditioning improves the contractile recovery of isolated ventricular papillary muscle or atrial trabeculae following simulated ischemia. However, *in vivo* animal studies and studies in patients undergoing elective cardiac surgery show conflicting results. At the subcellular level, it is known that ischemic conditioning improved energy metabolism, preserved mitochondrial respiration, ATP production, and Ca^{2+} homeostasis in isolated mitochondria from the myocardium. Ischemic conditioning also presents with post-translational modifications of proteins in the contractile machinery of the myocardium. The beneficial effects on myocardial contractile function need further elucidation. This article is part of a Special Issue entitled: The power of metabolism: Linking energy supply and demand to contractile function edited by Torsten Doenst, Michael Schwarzer and Christine Des Rosiers.

1. Protection against myocardial ischemia/reperfusion injury

1.1. Myocardial hibernation and stunning

Subsequent to a myocardial infarction, the final infarct size determines left ventricular (LV) function, remodeling and the long-term outcome of patients surviving the myocardial infarction [1]. Although early survival following ST segment elevation myocardial infarction has improved during the last few decades [2], the long-term risk of heart failure remains persistently high [3,4]. Cardiomyocyte death, in short term myocardial contractile dysfunction and in more long term left ventricular remodeling are the principal causes of heart failure following myocardial infarction [5].

The myocardium tolerates transient periods of 20–40 min of severe ischemia without cardiomyocyte loss [6,7]. However, also surviving cardiomyocytes develop post-ischemic contractile dysfunction. Such dysfunction may be reversible and includes myocardial hibernation, which develops during short-term (minutes to hours) or repetitive (over days to weeks) ischemia, and is completely reversible upon revascularization [8,9]. Myocardial hibernation describes the relationship between regional coronary flow reduction and contractile dysfunction of the myocardium [10]. Such an adaptation to ischemia maintains myocardial integrity and viability [9,10]. In patients, chronic myocardial hibernation occurs in unstable and stable angina, acute myocardial infarction, left ventricular dysfunction and congestive heart failure [7].

Reversible myocardial contractile dysfunction can also develop upon reperfusion, when blood flow is fully or almost fully restored, referred to as myocardial stunning [7]. Timely reperfusion is mandatory to salvage the ischemic myocardium from infarction [11,12]; however, reperfusion is associated with reperfusion injury. This post-ischemic contractile dysfunction of viable myocardium requires hours or days to recover [13]. In patients, myocardial stunning occurs following global ischemia/reperfusion (I/R) of hearts, such as in cardiac

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https://doi.org/10.1016/j.bbadis.2018.12.020
Received 30 May 2018; Received in revised form 18 December 2018; Accepted 19 December 2018
Available online 17 January 2019

arrest, during cardiac surgery, or in regional I/R as in percutaneous coronary intervention, thrombolysis, unstable angina or in stress or exercise-induced angina [14]. The complex relationship between regional myocardial blood flow and function during myocardial hibernation and stunning is depicted in Heusch [10].

In reversible myocardial contractile dysfunction, the cardiomyocytes are dysfunctional but eventually recover [15]. Mechanisms for reversible myocardial contractile dysfunction include a disruption in cardiomyocyte calcium (Ca^{2+}) homeostasis which includes decreased responsiveness of the myofilaments to Ca^{2+}, intracellular Ca^{2+} overload, and disturbed excitation-contraction uncoupling due to sarcoplasmic reticulum (SR) dysfunction [9,16,17]. Myofilament structural alterations, biochemical modifications and degradation of myofilament proteins, such as troponin I (TnI) alters Ca^{2+} responsiveness [16]. In a pig model of myocardial hibernation with a sustained and moderate reduction of regional coronary flow for 24 h, a reduction in sarcomeres was observed [18]. Examination of biopsies from patients with myocardial hibernation revealed a reduction in the expression, and disorganization of the myofilament proteins actin, myosin and titin [19]. In pig models of chronic hibernation, there was downregulation of mitochondrial proteins involved in the citric acid cycle and electron transport chain (complexes I–V), therefore limiting mitochondrial oxygen consumption [20,21].

When myocardial ischemia occurs beyond 20–40 min, necrosis ensues and contractile function is lost as the myocardium is irreversibly damaged [7]. During ischemia, gap junction-mediated communication between the cardiomyocytes is loosed [22], and during reperfusion, gap junctions propagate cardiomyocyte hypercontracture which contributes to contraction band necrosis [22]. Moreover, gap junctions in the non-ischemic regions of the surviving/remote myocardium are fewer and smaller and their distribution is disrupted [23].

I/R injury also induces an inflammatory response that leads to LV dysfunction. Increased inflammatory mediator expression, including cytokines and chemokines, and an infiltration of inflammatory cells in the infarct region contribute to scar formation and LV remodeling [24]. Scar formation is mediated by cardiac fibroblast proliferation, migration and differentiation into cardiac myofibroblasts, which synthesize and secrete extracellular matrix proteins [25]. The fibrotic tissue expands to areas remote to the infarct zone and contributes to altered ventricular compliance and increased stiffness [26]. This results in LV dysfunction in both the infarcted and remote myocardium [27,28].

1.2. Ischemic conditioning

Ischemic conditioning, brief repeated episodes of I/R performed locally at the heart or in tissue or organs remote from the heart (remote ischemic conditioning), induces cardioprotection and reduces I/R injury and therefore the final infarct size in experimental animal models but also in patients [29–31]. Ischemic pre- and perconditioning, respectively, refer to brief repeated episodes of I/R immediately before and during the sustained I/R, while postconditioning refers to a conditioning manoeuvre performed immediately at the onset of reperfusion [29,30]. Whereas local ischemic conditioning is only applicable as ischemic pre- and postconditioning, remote ischemic conditioning includes ischemic pre-, per- or postconditioning [30]. Remote ischemic conditioning is straightforward in clinical application as it can be induced by repetitive inflation and deflation of a blood pressure cuff placed on a limb [30]. Several proof-of-concept studies have demonstrated that local and remote ischemic conditioning reduce myocardial damage/infarct size, specifically a reduction in infarct size as determined by biomarker (creatine kinase–muscle brain, or troponin) release, in patients undergoing interventional reperfusion for acute myocardial infarction or cardiovascular surgery under ischemic cardioplegic arrest [32]. Ischemic conditioning reduces I/R injury in most, but not all studies in patients with acute myocardial infarction and those undergoing cardiovascular surgery. Different single-center studies suggest improved clinical outcome, a significant reduction in all-cause mortality and major adverse cardiac and cerebrovascular events rate, of patients after ischemic conditioning, however, no phase III study is available which has reported improved clinical outcomes as the primary end point for any of the ischemic conditioning manoeuvres [32]. Recently, the first prospective trial on remote ischemic conditioning in patients with acute myocardial infarction reported improved clinical outcome as the primary endpoint (cardiac death and hospitalization) over a minimum follow-up period of 12 months [33].

In the following sections, we focus on experimental results on the effects of myocardial ischemic conditioning on short term myocardial contractile dysfunction following I/R. A Pubmed search with the keywords “ischemic pre- per- or postconditioning,” and “ischemia/reperfusion injury”, “atrial trabeculae” or “ventricular papillary muscle”, “isolated perfused heart”, “left ventricular end-diastolic pressure”, “left ventricular developed pressure”, and “Ca^{2+} homeostasis”, “contractile proteins” or “mitochondria” was done. Articles on ischemic conditioning up to October 2018 in English, or with an abstract in English, that provided data in humans or animals have been reviewed. Relevant reference articles from within these have been selected for further discussion.

2. Effects of ischemic conditioning on myocardial contractile function

2.1. Ex vivo

In *ex vivo* models of isolated ventricular papillary muscle from animals [34,35] or atrial trabeculae from patients [36–38], ischemic pre- [35–37,39] or postconditioning [38] can be simulated by brief and repetitive changes from glucose-free hypoxic buffer with reduced pH and gassed with 95% N_2/5% CO_2, to reoxygenation buffer (with glucose, physiological pH and gassed with 95% O_2/5% CO_2) before or after the sustained simulated ischemia. Before, during and after the sustained simulated ischemia, the developed force of isometric contraction was determined as a measurement of contractile function [35–40]. The contractile function of isolated ventricular papillary muscles from rats [40], rabbits [35] and guinea pigs [39] was improved by *ex vivo* preconditioning [35,39] or remote ischemic preconditioning performed *in vivo* on hindlimbs before tissue collection [40]. In right atrial trabeculae isolated from right atrial appendages of patients who underwent elective coronary artery bypass graft surgery, ischemic conditioning was simulated *ex vivo,* pre- (4 min simulated ischemia followed by 16 min reoxygenation) [36,37] or postconditioning (4 cycles of 30 s or 60 s simulated ischemia/reoxygenation) [38] also resulted in improved contractile function following 90 min of sustained simulated ischemia. Although more accessible in human studies than ventricular samples, atrial trabeculae are not necessarily an indicator of ventricular contractile function. Indeed, baseline force of contraction was lower in right atrial trabeculae than in right ventricular trabeculae, and a prostacyclin analogue increased the force of contraction in right atrial trabeculae and had no effect on the force of contraction of right ventricular trabeculae isolated from pigs [41]. Furthermore, excitation-contraction coupling differs between atrial and ventricular cardiomyocytes in pigs and rodents [42].

In isolated perfused heart models myocardial contractile function can be assessed with left ventricular end-diastolic pressure (LVEDP) and left ventricular developed pressure (LVDP), obtained by subtracting the end-diastolic pressure from the end-systolic pressure. In isolated rat hearts, local ischemic preconditioning by 1, 2 or 3 cycles of 3 or 5 min global I/R before 30–45 min sustained ischemia improved postischemic left ventricular diastolic function by reducing LVEDP and improving LVDP recovery [43,44]. In the long term when hearts were excised 7 days after *in vivo* I/R, LVDP only recovered better with local ischemic pre- but not with postconditioning [45]. These data hint towards a difference between the impact of ischemic pre- and postconditioning on

functional recovery. In mice, remote ischemic preconditioning by 6 cycles of 5 min I/R on both hind limbs 24 h before [46], and in rats 4 cycles of 5 min I/R on one limb immediately before [47,48] the 30–40 min sustained global ischemia on the isolated heart improved postischemic left ventricular diastolic function by reducing LVEDP and improving LVDP recovery. Similarly, pre-treatment with plasma dialysate from healthy human volunteers [49] or rabbits [50] who received remote ischemic preconditioning resulted in improved LVDP and LVEDP in isolated perfused mouse [49] and rabbit hearts [50] with global I/R, respectively. Improved left ventricular function in isolated perfused mouse, rat and rabbit hearts coincided with smaller infarct sizes [46–50]. However, the isolated heart is not a suitable model to examine the effects of ischemic preconditioning on myocardial stunning. Myocardial stunning has been shown to occur in the isolated perfused rat heart following 20 min of ischemia [51]. In this study on myocardial stunning, at 60 min of reperfusion, cardiac output reached 85% of the maximum [51]. However, in the isolated heart model cardiac output deteriorates as contractile and chronotropic function decline 5–10% per hour [52].

2.2. In vivo

In vivo animal models show conflicting results regarding the effects of ischemic conditioning on myocardial contractile function. Again, studies have been performed in models where myocardial stunning occurs. Local ischemic preconditioning of the left anterior descending coronary artery in conscious sheep (6 cycles of 5 min I/R before 12 min sustained ischemia) [53,54], or open-chest rabbits (1 min of ischemia and 6 min reperfusion before 10 min sustained ischemia) [55], reduced myocardial stunning during reperfusion determined by a recovery of wall thickening fraction [53–55]. In the sheep model, the ischemic area was less than 20% of the LV mass in both controls and ischemic preconditioned groups, and there was no report of myocardial infarction [53,54]. As in myocardial stunning, the sustained ischemic period in this model was too brief (12 min) to cause myocardial infarction [53,54]. Consequently, it can be assumed that the reduction in myocardial stunning was independent from a reduction in infarct size in this model [53,54]. However, local ischemic preconditioning of the left anterior descending coronary artery in open-chest dogs (2.5/5 min ischemia and 5 min reperfusion) and pigs (2 cycles of 5 min I/R) before 15 min sustained ischemia, did not improve myocardial stunning during reperfusion, and there was no evidence of myocardial infarction in these models in controls and ischemic preconditioned groups [56,57]. Differences between these models include species differences, variations in the sustained ischemic period, and the number and repetitions of the cycles of ischemic preconditioning, may explain the discrepancies in the effects of ischemic preconditioning on myocardial stunning [53–57].

Importantly, in patients who underwent elective percutaneous coronary intervention, local ischemic preconditioning induced protection against postischemic left ventricular diastolic dysfunction [58]. However, remote ischemic preconditioning did not improve ischemic left ventricular dysfunction during reperfusion in patients who underwent elective percutaneous coronary intervention [59]. In both studies, however, there was no clear evidence that ischemic preconditioning was cardioprotective [58,59]. In one study, 60% of patients who received remote ischemic preconditioning had increased cardiac troponin I [59], while the other study did not report biomarkers of myocardial injury, or infarct size by imaging techniques [58].

In isolated papillary muscle, atrial trabeculae, whole heart preparations and in the *in vivo* setting it is not possible to determine whether improved myocardial contractile function is a direct consequence of ischemic conditioning or due to improved myocardial viability associated with a reduction in I/R injury. For example, a negative correlation between infarct size and postischemic LVDP in a model of global ischemia in isolated perfused rabbit hearts, indicated that the improved LVDP was only due to a reduction in infarct size by ischemic preconditioning and not due to a direct effect on myocardial contractile function [60].

2.3. Effects of ischemic conditioning at the subcellular level

Nonetheless, evidence indicates that there may be direct effects on myocardial contractile function independent of infarct size. The underlying signaling, however, is not well understood. Various experimental models suggest, that myocardial signaling of local and remote ischemic conditioning is similar but it seems to be species specific [30]. However, independently of the involved myocardial signaling, mitochondria are viewed as end-effectors of cardioprotective strategies in all species tested so far. Ischemic conditioning preserved mitochondrial respiration and thus ATP production and improved Ca^{2+} homeostasis. Alterations of mitochondrial function clearly not only improve myocardial contractile function but also cardiomyocyte viability [61]. In addition to mitochondrial function, ischemic conditioning induces, *via* myocardial signaling, post-translational modifications and changes in the expression of proteins of the contractile machinery and thus also induces changes in myocardial contractile function. Post-translational protein modifications are mechanisms of acute alterations in protein function, whereas changes in protein expression are most likely associated with long-term outcomes.

Therefore, in the following sections we focus on the effects of ischemic conditioning on cardiomyocyte energy metabolism, alterations in cardiomyocyte Ca^{2+} homeostasis and ROS production – all known to be relevant for myocardial contractile function. Known post-translational modifications of contractile proteins, which seem to be associated with myocardial contractile function, are also discussed.

3. Energy metabolism, Ca^{2+} homeostasis and oxidative stress

3.1. During ischemia/reperfusion (I/R)

ATP is mandatory to maintain myocardial contractile function and more than 95% of this ATP is generated by mitochondrial oxidative phosphorylation under normoxic conditions [62]. Myocardial contractile function involves thin filament proteins, actin, tropomyosin and the troponins, as well as thick filament proteins, myosin light chain 1 and myosin light chain 2 (regulatory light chain). During myocardial contraction, when myosin binds to actin to form cross-bridges during contraction, and when the bond is broken during relaxation, ATP is utilized [63]. Through cytosolic influx and release through the ryanodine channel (RyR), Ca^{2+} diffuses to the contractile machinery to initiate contraction by activating the actin-myosin interaction [63]. During relaxation, Ca^{2+} is rapidly removed from the cytosol by ATP-dependent processes.

I/R injury results in mitochondrial damage which contributes to impaired energy metabolism and cardiomyocyte contractile dysfunction [64,65]. During ischemia, inadequate blood flow through the coronary arteries results in low oxygen and nutrient (such as glucose and fatty acid) supply to the surrounding cardiomyocytes and subcellular mitochondria. The reduced oxygen supply disrupts oxidative phosphorylation in the mitochondrial respiratory chain, and high-energy phosphates, mainly ATP and phosphocreatine, are reduced [66–68]. Cellular ATP levels are depleted, and ATP is synthesized at a lower rate mainly through anaerobic glycolysis which also results in reduced nicotinamide adenine dinucleotide (NADH) accumulation [69,70]. Cytosolic Ca^{2+} and Na^{+}, and mitochondrial Ca^{2+} are overloaded [70] and utilization of the available Ca^{2+} for myocardial contractile function is decreased [71]. Reperfusion restores oxygen delivery, and mitochondrial respiratory activity and ATP synthesis are partially recovered [72]. Phosphocreatine recovers rapidly whereas ATP recovers more slowly [73].

During reperfusion, however, the recovery of ATP synthesis

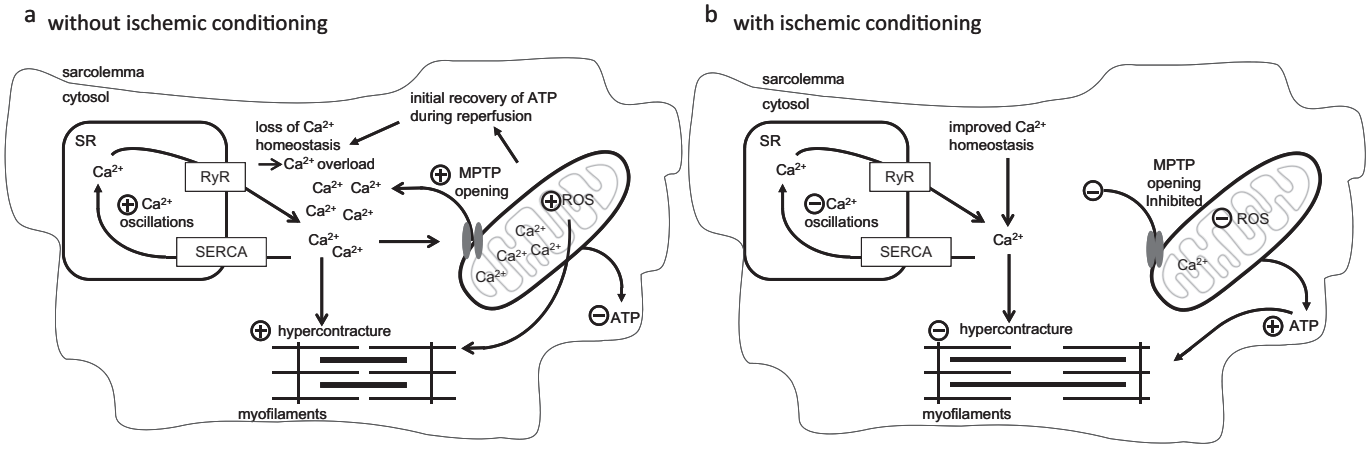

Fig. 1. Key signaling steps on subcellular level, influencing short term myocardial contractile function during ischemia and reperfusion without (a) and with (b) ischemic conditioning. (+) denotes increase; (−) denotes decrease; ATP-adenosine trisphosphate; Ca^{2+}-calcium; I/R-ischemia/reperfusion MPTP-mitochondrial permeability transition pore; ROS-reactive oxygen species; RyR-ryanodine receptor; SERCA-sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase; SR-sarcoplasmic reticulum.

contributed to hypercontracture of isolated rat cardiomyocytes [74]. The initial availability of ATP induced Ca^{2+} oscillations, *i.e.* Ca^{2+} uptake by the sarcoplasmic reticulum through sarcoendoplasmic reticulum calcium transport ATPase (SERCA) and Ca^{2+} release into the cytosol by ryanodine receptor calcium release channels, and an accumulation of Ca^{2+} in the mitochondria of isolated rat cardiomyocytes [75,76]. This resulted in contraction/hypercontracture and MPTP opening of isolated rat cardiomyocytes [75,76]. Alternatively, other studies showed that secondary to MPTP opening, Ca^{2+} was released from the mitochondria into the cytosol, which may have caused hypercontracture in rat cardiomyocytes [77,78] (Fig. 1a). I/R injury was also associated with reduced Ca^{2+}-contraction coupling and contractile response to Ca^{2+} in isolated guinea pig hearts [79].

Reperfusion is also associated with an increased formation of cellular ROS. ROS was produced by the mitochondria in isolated rat cardiomyocytes following I/R [75]. ROS directly induced hypercontracture in adult rabbit cardiomyocytes [80] and in rat cardiomyocytes, treatment with an antioxidant prevented ROS formation, hypercontracture and MPTP opening during reperfusion [81] (Fig. 1a). Together, cellular ROS, hypercontracture and opening of the MPTP contribute to reperfusion-induced death of cardiomyocytes [82].

3.2. Ischemia/reperfusion and ischemic conditioning

Regardless of the stimulus site (local or remote ischemic conditioning), in response to intracellular activated signaling cascades, signal transduction of ischemic conditioning converges onto the mitochondria [30]. Local and remote ischemic pre- and postconditioning preserved mitochondrial respiration [72,83,84], ATP production [72,84,85] and mitochondrial membrane potential [86] directly after the conditioning manoeuvre in human right atrial appendages [84] and during reperfusion in rat [72,85–87] and pig hearts [83]. A reduction in infarct size accompanied preserved mitochondrial respiration and ATP production in isolated perfused rat hearts subjected to global I/R and pre-treated with dialysate from pigs which received remote ischemic pre- or perconditioning [31,72]. A reduction in infarct size also accompanied preserved mitochondrial membrane potential in isolated perfused hearts from rats which received remote ischemic preconditioning [86]. In patients who underwent cardiac surgery, remote ischemic preconditioning preserved mitochondrial respiration *in situ* in left ventricle biopsies and right atrial appendages [84,88]. Phosphocreatine is involved in the transport of ATP from the mitochondria to ATPases, *i.e.* sites of utilization in the cytosol [89]. ATP, produced by the mitochondria, generates adenosine diphosphate (ADP) and phosphocreatine, a reaction catalyzed by creatine kinase, which then enter the cytosol to generate ATP and creatine [90]. Ischemic preconditioning in rat hearts resulted in higher levels of phosphocreatine during reperfusion [87,91]. Exogenous phosphocreatine administration in rats had beneficial effects during ischemia by restoring ATP levels [92].

Ischemic preconditioning in isolated perfused rat hearts improved the viability of cardiomyocytes which were subsequently isolated [61]. In mitochondria or cardiomyocytes isolated from rat [31,61,72,93] or rabbit hearts [94], local and remote ischemic pre- or perconditioning improved Ca^{2+} homeostasis [61], inhibited MPTP opening [31,72,93,94] and reduced hypercontracture [61] (Fig. 1b). Local [95] and remote [31,72,84] ischemic conditioning was associated with reduced ROS production by the mitochondria isolated from rat hearts and human right atrial appendages. As such, ROS-induced hypercontracture and MPTP opening may also be attenuated by ischemic conditioning (Fig. 1b). Mitochondrial function is essential for a proper cardiomyocyte contraction and as such may also underlie an improved contractile function seen in association with improved mitochondrial function in trabeculae of human right atrial appendages with RIPC [84].

4. Effects of ischemic conditioning on cardiomyocyte contractile proteins

Myocardial I/R injury causes numerous adaptational changes in the surviving cardiomyocytes, and thereby initiates a cascade of remodeling processes that eventually progress to heart failure. Early remodeling processes include post-translational modification and accelerated degradation of proteins involved in myocardial contractile function [96–101]. These processes are critically triggered by oxidative stress [102], which has been reported to modify the activity of numerous redox sensitive kinases and phosphatases [103]. Potentially oxidized proteins in myocardial muscle include actin, tropomyosin, and myosin heavy chain [104]. Importantly, oxidative stress is elevated in infarcted as well as in remote regions of hearts with myocardial I/R injury [105].

There are different post-translational modifications and changes in transcription rates of contractile proteins in response to ischemic conditioning [106–110]. Protein kinases and protein phosphatases catalyze the most frequent protein modification: the reversible and thus short-acting phosphorylation of specific amino acids, particularly Ser, Thr and Tyr residues. The regulation of the phosphorylation status of a protein is a potent mechanism to regulate its biological activity [111].

Cardiac myosin binding protein c (cMyBPc), a thick filament protein

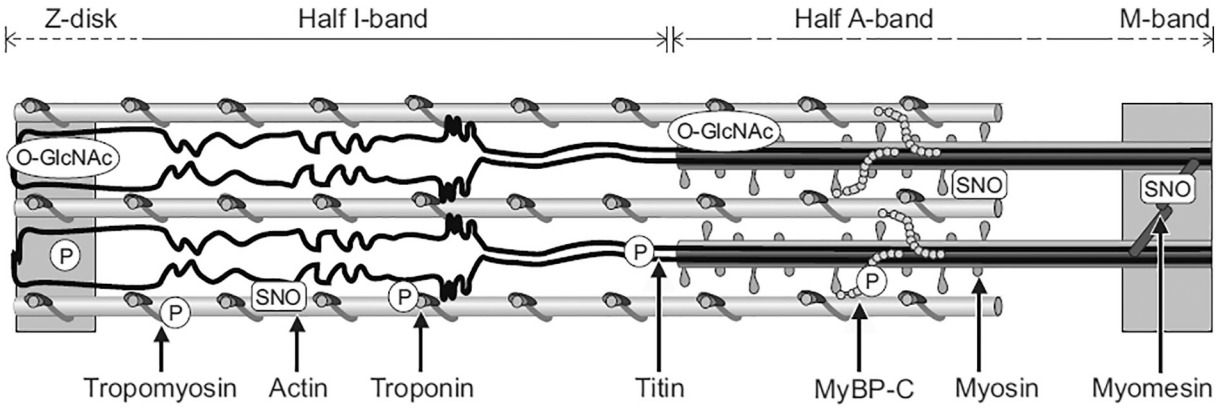

Fig. 2. Short term post-translational modifications by ischemic conditioning of proteins in the cardiomyocyte sarcomere. The sarcomeric *Z*-disk defines the lateral borders of the sarcomere. The troponin complex consists 3 protein subunits, the Ca^{2+}-binding troponin C, the actomyosin ATPase inhibitory troponin I and the tropomyosin-binding troponin T. Cardiac myosin binding protein c is a thick filament protein that interacts with titin, myosin and actin, and regulates sarcomeric assembly, structure and function as well as cross-bridge formation, *i.e.* binding of actin to myosin, through phosphorylation by protein kinase A, protein kinase C or Ca^{2+}/calmodulin-dependent kinase. S-nitrosylation of myosin, actin and the M-band protein myomesin occurs in ischemic preconditioning or ischemic post-conditioning. Remote ischemic preconditioning is associated with phosphorylation of troponin I, tropomyosin and certain Z-disc proteins. A decrease in cardiac myosin binding protein c phosphorylation occurs in response to ischemic preconditioning. Ischemic preconditioning is associated with decreased expression in the myosin light chain kinase gene. cMyBPc-cardiac myosin binding protein c; P-phosphorylation; SNO-S-nitrosylation; TnC-troponin C; TnI-troponin I; TnT-troponin T.

(Fig. 2), can regulate cross-bridge formation by dynamic phosphorylation by protein kinase A, protein kinase C and Ca^{2+}-calmodulin-activated kinase, indicating that cMyBPc phosphorylation is an important modulator of myocardial contractile function. Following I/R injury, total phosphorylation of cMyBPc is decreased and results in increased cleavage of cMyBPc, which is associated with thick filament disruption, reduction in actomyosin cross-bridges and contractile dysfunction [112]. Transgenic mice with myocardial-specific expression of a cMyBPc mutant, which mimics constitutive phosphorylation, were shown to be resistant to I/R injury [113], and hearts from transgenic mice with nonphosphorylatable cMyBPc develop contractile dysfunction and heart failure [114,115]. Myocardial stunning in a rat model was associated with increased phosphorylation of cMyBPc, which was abolished by ischemic preconditioning [110]. It was speculated that this decreased phosphorylation of cMyBPc may play a role in cardioprotection by ischemic preconditioning [110]. However, these results are surprising as basal levels of cMyBPc phosphorylation are considered to be necessary for maintaining thick filament orientation, dynamic regulation and contractile mechanics, and phosphorylation of cMyBPc is associated with decreased infarct size and enhanced myocardial relaxation in mice [113,116]. In isolated perfused rat hearts, ischemic preconditioning upregulated the gene expression of cMyBPc in comparison to I/R [20]. The consequences of upregulated gene expression of cMyBPc on myocardial contractile function in ischemic preconditioning are unclear and most likely associated with long-term outcomes. Taken together, there is evidence that ischemic conditioning induces cMyBPc dephosphorylation and increased gene expression; however, its exact role in ischemic conditioning needs further elucidation.

The sarcomeric *Z*-disk (Fig. 2) plays a role in mechanosensation and mechanotransduction, and provides a molecular link to the t-tubular system and the sarcoplasmic reticulum [117]. Signaling molecules, such as protein kinase A and protein kinase C epsilon are preferentially localized at the *Z*-disks [118], which may increase the likelihood of transmitting ischemia-induced alterations to the contractile apparatus. Phosphoproteomic analysis provided evidence that remote ischemic preconditioning is associated with increased phosphorylation of Z-disk proteins, including myozenin-2, obscurin, and myopalladin [106]. However, the functional implications of these modifications for myocardial contractile function and a possible role of the Z-disk in cardioprotection are currently unknown.

Troponin I (TnI) (Fig. 2) is a key regulator of contraction and relaxation, and an increased phosphorylation of TnI has been shown to accelerate myocardial relaxation [119,120]. By returning to its myosin blocking position for myofilament interaction during relaxation, tropomyosin also plays a central role in both contraction and relaxation. Phosphorylation of tropomyosin enhances actin activated myosin S-1 ATPase activity, whereas depressed phosphorylation of tropomyosin is associated with impaired myofilament function and decreased myocardial ejection fraction [74,121]. It was postulated that tropomyosin is phosphorylated as a compensatory mechanism to augment myocardial contractile function during periods of oxidative stress when myosin function is impaired [122]. Remote ischemic preconditioning in mouse hearts is associated with increased phosphorylation of TnI and tropomyosin (2) (Fig. 2).

In pigs with remote ischemic preconditioning, increased phosphorylation of proteins involved in myocardial contractile function was observed [123]. These include F-actin capping protein beta subunit variant II, xin actin-binding repeat-containing protein 1 which is involved in protection of actin filaments during depolymerization, actin-binding LIM protein 1, and nexilin which is a filamentous actin-binding protein [123].

During prolonged ischemia and reperfusion, nitric oxide (NO) synthase activity is decreased, [124] possibly by a shift within the arginine metabolism [125]. NO synthesis and availability are increased in response to ischemic conditioning suggesting NO to be an important mediator of cardioprotection [126,127]. Increased NO availability is associated with S-nitrosylation, a NO-mediated nitrosylation of a thiol group [128]. Local ischemic pre- [108] and postconditioning [109] of isolated perfused mouse hearts increases S-nitrosylation levels of myomesin, cardiac α-myosin heavy chain [108], myosin light chain 1 and α cardiac muscle actin [109] (Fig. 2) and were associated with improved postischemic myocardial LVDP [108] and the rate-pressure product [109]. The functional relevance of S-nitrosylation in cardioprotection is further supported by findings showing that exposure of isolated perfused rat hearts to the S-nitrosylating agent S-nitrocysteine before ischemia, increased levels of cardiac intracellular S-nitrosothiols and improved the rate-pressure product during reperfusion [129]. The effects of S-nitrosylation may alter function of the contractile apparatus. In cardiomyocytes and cardiac skinned fibers from papillary muscle isolated from mice, contractile proteins such as myosin heavy chain, myosin light chain 3, actin, tropomyosin, TnI and TnC, cMyBPc, and titin were S-nitrosylated by treatment with the S-nitrosylating agents, S-nitrocysteine and S-nitrosoglutathione. [130]. Low levels of S-

nitrosylation desensitized the myofilaments to Ca^{2+} and decreased maximal force development, whereas higher levels of S-nitrosylation impaired the relaxation rate and cross-bridge turnover [130]. Low levels of S-nitrosylation, *e.g.* during oxidative stress, down-regulated myocardial contractile function (Fig. 2) [130].

Ischemic preconditioning was associated with increased O-linked beta-*N*-acetylglucosamine (O-GlcNAc) glycosylation of myocardial proteins. O-GlcNAc post-translationally modifies serine or threonine residues of nuclear and cytoplasmic proteins by a sugar modification of the hydroxyl group, a form of protein glycosylation [131]. Isolated atrial trabeculae from patients who underwent elective heart surgery exposed to dialysate from healthy volunteers who had remote ischemic preconditioning, had improved myocardial contractile function following the hypoxic stimulus and this improved function was associated with an increased expression of O-GlcNAc [132]. In skinned rat ventricular myocardial trabeculae, the contractile proteins actin, myosin heavy chain, myosin light chain 1 and 2, and TnI, were modified by O-GlcNAc glycosylation. Such O-GlcNAc glycosylation decreased myocardial myofilament Ca^{2+} sensitivity [133], thus O-GlcNAc glycosylation may therefore contribute to the mechanisms of ischemic conditioning.

Ischemic preconditioning in isolated perfused rats hearts was associated with a decreased expression of the gene encoding myosin light chain kinase (MLCK) after I/R compared to hearts subjected to only I/R [107]. MLCK is a Ca^{2+}/calmodulin-dependent protein kinase that phosphorylates ventricular and atrial myosin light chains [134,135]. By phosphorylating ventricular myosin light chain, MLCK regulates sarcomere reassembly and left ventricular function [134,135]. The functional relevance for altered MLCK expression in ischemic conditioning is largely unclear and is more likely associated with long-term outcomes of myocardial contractile function.

Nonetheless, in the long-term, altered protein turnover may be an important mechanism of cardioprotection. I/R injury results in a substantial loss of ventricular myocytes, which, especially prior to completed scar formation, implies a significant increase in mechanical stress to the remaining viable tissue. This increased mechanical demand of the remote myocardium requires an increased turnover of proteins of the contractile machinery, including the elastic filament protein titin [136]. In isolated hearts ischemic preconditioning protected the function of the ubiquitin proteasome system by diminishing oxidative damage to 19S regulatory subunits by allowing this complex to facilitate degradation of proapoptotic proteins [137]. Preserving or even improving the function of the proteasomal system after I/R injury may therefore be an important goal for the development of novel treatment strategies.

5. Conclusion

Ischemic conditioning reduces infarct size in animal models and patients, but evidence indicates a parallel improvement on postischemic myocardial contractile function. Ischemic conditioning has a direct effect on postischemic myocardial contractile function by changes in cardiomyocyte energy metabolism and post-translational modifications of contractile proteins. Future studies should be carried out to unravel these additional effects of ischemic conditioning in patients. Meaningful evaluation could include the assessment of myocardial contractile function following elective cardiac surgery when infarct size remains constant. Improving postischemic contractile dysfunction, may translate into improved long-term clinical outcomes.

Sources of funding

Supported by the German Research Foundation (SFB 1116 A02, B08).

Transparency document

The Transparency document associated with this article can be found, in online version.

References

[1] J.F. Viles-Gonzalez, V. Fuster, J.J. Badimon, Atherothrombosis: a widespread disease with unpredictable and life-threatening consequences, Eur. Heart J. 25 (2004) 1197–1207.
[2] P. American College of Emergency, A. Society for Cardiovascular, Interventions, P.T. O'Gara, F.G. Kushner, D.D. Ascheim, D.E. Casey Jr., M.K. Chung, J.A. de Lemos, S.M. Ettinger, J.C. Fang, F.M. Fesmire, B.A. Franklin, C.B. Granger, H.M. Krumholz, J.A. Linderbaum, D.A. Morrow, L.K. Newby, J.P. Ornato, N. Ou, M.J. Radford, J.E. Tamis-Holland, C.L. Tommaso, C.M. Tracy, Y.J. Woo, D.X. Zhao, J.L. Anderson, A.K. Jacobs, J.L. Halperin, N.M. Albert, R.G. Brindis, M.A. Creager, D. DeMets, R.A. Guyton, J.S. Hochman, R.J. Kovacs, F.G. Kushner, E.M. Ohman, W.G. Stevenson, C.W. Yancy, ACCF/AHA guideline for the management of ST-elevation myocardial infarction: executive summary: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, J. Am. Coll. Cardiol. 61 (2013) (2013) 485–510.
[3] Y. Gerber, S.A. Weston, M. Enriquez-Sarano, C. Berardi, A.M. Chamberlain, S.M. Manemann, R. Jiang, S.M. Dunlay, V.L. Roger, Mortality associated with heart failure after myocardial infarction: a contemporary community perspective, Circ. Heart Fail. 9 (2016) e002460.
[4] R.S. Velagaleti, M.J. Pencina, J.M. Murabito, T.J. Wang, N.I. Parikh, R.B. D'Agostino, D. Levy, W.B. Kannel, R.S. Vasan, Long-term trends in the incidence of heart failure after myocardial infarction, Circulation 118 (2008) 2057–2062.
[5] M.F. Minicucci, P.S. Azevedo, B.F. Polegato, S.A. Paiva, L.A. Zornoff, Heart failure after myocardial infarction: clinical implications and treatment, Clin. Cardiol. 34 (2011) 410–414.
[6] K.A. Reimer, J.E. Lowe, M.M. Rasmussen, R.B. Jennings, The wavefront phenomenon of ischemic cell death. 1. Myocardial infarct size vs duration of coronary occlusion in dogs, Circulation 56 (1977) 786–794.
[7] G. Heusch, R. Schulz, Characterization of hibernating and stunned myocardium, Eur. Heart J. 18 (Suppl.D) (1997) D102–D110.
[8] G. Heusch, R. Schulz, Hibernating myocardium. New answers, still more questions!, Circ. Res. 91 (2002) 863–865.
[9] G. Heusch, R. Schulz, S.H. Rahimtoola, Myocardial hibernation: a delicate balance, Am. J. Physiol. Heart Circ. Physiol. 288 (2005) H984–H999.
[10] G. Heusch, The regional myocardial flow-function relationship: a framework for an understanding of acute ischemia, hibernation, stunning and coronary microembolization, Circ. Res. 112 (2013) 1535–1537.
[11] G.M. Frohlich, P. Meier, S.K. White, D.M. Yellon, D.J. Hausenloy, Myocardial reperfusion injury: looking beyond primary PCI, Eur. Heart J. 34 (2013) 1714–1722.
[12] D.M. Yellon, D.J. Hausenloy, Myocardial reperfusion injury, N. Engl. J. Med. 357 (2007) 1121–1135.
[13] R.A. Kloner, R.B. Jennings, Consequences of brief ischemia: stunning, preconditioning, and their clinical implications. Part I, Circulation 104 (2001) 2981–2989.
[14] S. Verma, P.W. Fedak, R.D. Weisel, J. Butany, V. Rao, A. Maitland, R.K. Li, B. Dhillon, T.M. Yau, Fundamentals of reperfusion injury for the clinical cardiologist, Circulation 105 (2002) 2332–2336.
[15] R. Bolli, Mechanism of myocardial "stunning", Circulation 82 (1990) 723–738.
[16] R. Bolli, E. Marban, Molecular and cellular mechanisms of myocardial stunning, Physiol. Rev. 70 (1999) 609–634.
[17] A. Kronenbitter, F. Funk, K. Hackert, S. Gorressen, D. Glaser, P. Boknik, G. Poschmann, K. Stuhler, M. Isic, M. Kruger, J.P. Schmitt, Impaired Ca(2+) cycling of nonischemic myocytes contributes to sarcomere dysfunction early after myocardial infarction, J. Mol. Cell. Cardiol. 119 (2018) 28–39.
[18] C. Chen, L. Chen, J.T. Fallon, L. Ma, L. Li, L. Bow, D. Knibbs, R. McKay, L.D. Gillam, D.D. Waters, Functional and structural alterations with 24-hour myocardial hibernation and recovery after reperfusion. A pig model of myocardial hibernation, Circulation 94 (1996) 507–516.
[19] A. Elsässer, M. Schlepper, W.-P. Klövekorn, W.-j. Cai, R. Zimmermann, K.-D. Müller, R. Strasser, S. Kostin, C. Gagel, B. Münkel, W. Schaper, J. Schaper, Hibernating myocardium. An incomplete adaptation to ischemia, Circulation 96 (1997) 2920–2931.
[20] H.K. Kim, S.W. Kang, S.H. Jeong, N. Kim, J.H. Ko, H. Bang, W.S. Park, T.H. Choi, Y.R. Ha, Y.S. Lee, J.B. Youm, K.S. Ko, B.D. Rhee, J. Han, Identification of potential target genes of cardioprotection against ischemia-reperfusion injury by express sequence tags analysis in rat hearts, J. Cardiol. 60 (2012) 98–110.
[21] B. Page, R. Young, V. Iyer, G. Suzuki, M. Lis, L. Korotchkina, M.S. Patel, K.M. Blumenthal, J.A. Fallavollita, J.M. Canty Jr., Persistent regional down-regulation in mitochondrial enzymes and upregulation of stress proteins in swine with chronic hibernating myocardium, Circ. Res. 102 (2008) 103–112.
[22] D. Garcia-Dorado, M. Ruiz-Meana, F. Padilla, A. Rodriguez-Sinovas, M. Mirabet, Gap junction-mediated intercellular communication in ischemic preconditioning, Cardiovasc. Res. 55 (2002) 456–465.
[23] N.S. Peters, Myocardial gap junction organization in ischemia and infarction, Microsc. Res. Tech. 31 (1995) 375–386.
[24] J. Liu, H. Wang, J. Li, Inflammation and inflammatory cells in myocardial infarction and reperfusion injury: a double-edged sword, Clin. Med. Insights Cardiol.

10 (2016) 79–84.
[25] F.A. van Nieuwenhoven, N.A. Turner, The role of cardiac fibroblasts in the transition from inflammation to fibrosis following myocardial infarction, Vasc. Pharmacol. 58 (2013) 182–188.
[26] V. Talman, H. Ruskoaho, Cardiac fibrosis in myocardial infarction-from repair and remodeling to regeneration, Cell Tissue Res. 365 (2016) 563–581.
[27] J. Bogaert, H. Bosmans, A. Maes, P. Suetens, G. Marchal, F.E. Rademakers, Remote myocardial dysfunction after acute anterior myocardial infarction: impact of left ventricular shape on regional function: a magnetic resonance myocardial tagging study, J. Am. Coll. Cardiol. 35 (2000) 1525–1534.
[28] M. Jessup, S. Brozena, Heart failure, N. Engl. J. Med. 348 (2003) 2007–2018.
[29] G. Heusch, Critical issues for the translation of cardioprotection, Circ. Res. 120 (2017) 1477–1486.
[30] P. Kleinbongard, A. Skyschally, G. Heusch, Cardioprotection by remote ischemic conditioning and its signal transduction, Pflugers Arch. - Eur. J. Physiol. 469 (2017) 159–181.
[31] A. Skyschally, P. Kleinbongard, H.R. Lieder, N. Gedik, L. Stoian, G. Amanakis, E. Elbers, G. Heusch, Humoral transfer and intra-myocardial signal transduction of protection by remote ischemic perconditioning in pigs, rats, and mice, Am. J. Physiol. Heart Circ. Physiol. 315 (2018) H159–H172.
[32] G. Heusch, T. Rassaf, Time to give up on cardioprotection? A critical appraisal of clinical studies on ischemic pre-, post-, and remote conditioning, Circ. Res. 119 (2016) 676–695.
[33] A. Gaspar, A.P. Lourenco, M.A. Pereira, P. Azevedo, R. Roncon-Albuquerque Jr., J. Marques, A.F. Leite-Moreira, Randomized controlled trial of remote ischaemic conditioning in ST-elevation myocardial infarction as adjuvant to primary angioplasty (RIC-STEMI), Basic Res. Cardiol. 113 (2018) 14.
[34] C.-M. Yu, J. Wing-Hong Fung, Q. Zhang, C.-K. Chan, Y.-S. Chan, H. Lin, L.C.C. Kum, S.-L. Kong, Y. Zhang, J.E. Sanderson, Tissue Doppler imaging is superior to strain rate imaging and postsystolic shortening on the prediction of reverse remodeling in both ischemic and nonischemic heart failure after cardiac resynchronization therapy, Circulation 110 (2004) 66–73.
[35] D.M. Walker, M.S. Marber, J.M. Walker, D.M. Yellon, Preconditioning in isolated superfused rabbit papillary muscles, Am. J. Phys. 266 (1994) H1534–H1540.
[36] V. Sivaraman, D.J. Hausenloy, S. Kolvekar, M. Hayward, J. Yap, D. Lawrence, S.C. Di, D.M. Yellon, The divergent roles of protein kinase C epsilon and delta in simulated ischaemia-reperfusion injury in human myocardium, J. Mol. Cell. Cardiol. 46 (2009) 758–764.
[37] V. Sivaraman, D.J. Hausenloy, A.M. Wynne, D.M. Yellon, Preconditioning the diabetic human myocardium, J. Cell. Mol. Med. 14 (2010) 1740–1746.
[38] V. Sivaraman, N.R. Mudalgiri, S.C. Di, S. Kolvekar, M. Hayward, J. Yap, B. Keogh, D.J. Hausenloy, D.M. Yellon, Postconditioning protects human atrial muscle through the activation of the RISK pathway, Basic Res. Cardiol. 102 (2007) 453–459.
[39] R. Dworakowski, D. Dworakowska, I. Kocic, T. Wirth, M. Gruchala, M. Kaminski, R. Ray, J. Petrusewicz, S. Yla-Herttuala, A. Rynkiewicz, Experimental hyperlipidaemia does not prevent preconditioning and it reduces ischemia-induced apoptosis, Int. J. Cardiol. 126 (2008) 62–67.
[40] S.M. Davidson, P. Selvaraj, D. He, C. Boi-Doku, R.L. Yellon, J.M. Vicencio, D.M. Yellon, Remote ischaemic preconditioning involves signalling through the SDF-1alpha/CXCR4 signalling axis, Basic Res. Cardiol. 108 (2013) 377.
[41] S. Holmboe, A. Andersen, R.V. Jensen, H.H. Kimose, L.B. Ilkjaer, L. Shen, L.H. Clapp, J.E. Nielsen-Kudsk, Prostacyclins have no direct inotropic effect on isolated atrial strips from the normal and pressure-overloaded human right heart, Pulm. Circ. 7 (2017) 339–347.
[42] H. Tanaka, T. Kawanishi, K. Shigenobu, Optical bioimaging: from living tissue to a single molecule: atrio-ventricular difference in myocardial excitation-contraction coupling - sequential versus simultaneous activation of SR Ca^{2+} release units, J. Pharmacol. Sci. 93 (2003) 248–252.
[43] G.K. Asimakis, K. Inners-McBride, G. Medellin, V.R. Conti, Ischemic preconditioning attenuates acidosis and postischemic dysfunction in isolated rat heart, Am. J. Physiol. Heart Circ. Physiol. 263 (1992) H887–H894.
[44] A.C. Cave, G.L. Horowitz, C.S. Apstein, Can ischemic preconditioning protect against hypoxia-induced damage? Studies of contractile function in isolated perfused rat hearts, J. Mol. Cell. Cardiol. 26 (1994) 1471–1486.
[45] R. Schreckenberg, P. Bencsik, M. Weber, Y. Abdallah, C. Csonka, K. Gomori, K. Kiss, J. Paloczi, J. Pipis, M. Sarkozy, P. Ferdinandy, R. Schulz, K.D. Schluter, Adverse effects on beta-adrenergic receptor coupling: ischemic postconditioning failed to preserve long-term cardiac function, J. Am. Heart Assoc. 6 (2017) e006809.
[46] G. Li, F. Labruto, A. Sirsjo, F. Chen, J. Vaage, G. Valen, Myocardial protection by remote preconditioning: the role of nuclear factor kappa-B p105 and inducible nitric oxide synthase, Eur. J. Cardiothorac. Surg. Off. J. Eur. Assoc. Cardiothorac. Surg. 26 (2004) 968–973.
[47] R. Sharma, P.K. Randhawa, N. Singh, A.S. Jaggi, Possible role of thromboxane A2 in remote hind limb preconditioning-induced cardioprotection, Naunyn Schmiedeberg's Arch. Pharmacol. 389 (2016) 1–9.
[48] B. Singh, P.K. Randhawa, N. Singh, A.S. Jaggi, Investigations on the role of leukotrienes in remote hind limb preconditioning-induced cardioprotection in rats, Life Sci. 152 (2016) 238–243.
[49] H.A. Hildebrandt, V. Kreienkamp, S. Gent, P. Kahlert, G. Heusch, P. Kleinbongard, Kinetics and signal activation properties of circulating factor(s) from healthy volunteers undergoing remote ischemic pre-conditioning, JACC. Basic Transl. Sci. 1 (2016) 3–13.
[50] T. Steensrud, J. Li, X. Dai, C. Manlhiot, R.K. Kharbanda, M. Tropak, A. Redington, Pretreatment with the nitric oxide donor SNAP or nerve transection blocks humoral preconditioning by remote limb ischemia or intra-arterial adenosine, Am. J. Physiol. Heart Circ. Physiol. 299 (2010) H1598–H1603.
[51] E.F. Du Toit, L.H. Opie, Modulation of severity of reperfusion stunning in the isolated rat heart by agents altering calcium flux at the onset of reperfusion, Circ. Res. 70 (1992) 960–967.
[52] R.M. Bell, M.M. Mocanu, D.M. Yellon, Retrograde heart perfusion: the Langendorff technique of isolated heart perfusion, J. Mol. Cell. Cardiol. 50 (2011) 940–950.
[53] H.F. del Valle, E.C. Lascano, J.A. Negroni, A.J. Crottogini, Absence of ischemic preconditioning protection in diabetic sheep hearts: role of sarcolemmal KATP channel dysfunction, Mol. Cell. Biochem. 249 (2003) 21–30.
[54] E.C. Lascano, J.A. Negroni, H.F. del Valle, Early preconditioning protection against stunning in conscious sheep. Role of KATP channels, Mol. Cell. Biochem. 331 (2009) 247–257.
[55] K. Urabe, T. Miura, T. Iwamoto, T. Ogawa, M. Goto, J. Sakamoto, O. Iimuara, Preconditioning enhances myocardial resistance to postischemic myocardial stunning via adenosine activation, Cardiovasc. Res. 27 (1993) 657–662.
[56] M. Miyamae, H. Fujiwara, M. Kida, R. Yokota, M. Tanaka, M. Katsuragawa, K. Hasegawa, M. Ohura, K. Koga, Y. Yabuuchi, S. Sasayama, Preconditioning improves energy metabolism during reperfusion but does not attenuate myocardial stunning in porcine hearts, Circulation 88 (1993) 223–234.
[57] M. Ovize, K. Przyklenk, S.L. Hale, R.A. Kloner, Preconditioning does not attenuate myocardial stunning, Circulation 85 (1992) 2247–2254.
[58] E. Hyodo, K. Shimada, S. Fukuda, T. Takeda, T. Saito, T. Hasegawa, N. Ichiba, K. Tamita, S. Ehara, T. Muro, M. Yoshiyama, J. Yoshikawa, Effect of preconditioning on post-ischemic left ventricular diastolic stunning in patients with coronary artery disease undergoing percutaneous coronary intervention, Circulation 126 (2016) A13855.
[59] S.P. Hoole, S.N. Khan, P.A. White, P.M. Heck, R.K. Kharbanda, C.G. Densem, S.C. Clarke, L.M. Shapiro, P.M. Schofield, M. O'Sullivan, D.P. Dutka, Remote ischaemic pre-conditioning does not attenuate ischaemic left ventricular dysfunction in humans, Eur. J. Heart Fail. 11 (2009) 497–505.
[60] D.P. Jenkins, W.B. Bugsley, D.M. Yellon, Ischaemic preconditioning in a model of global ischaemia: infarct size limitation, but no reduction of stunning, J. Mol. Cell. Cardiol. 27 (1995) 1623–1632.
[61] G.C. Rodrigo, N.J. Samani, Ischemic preconditioning of the whole heart confers protection on subsequently isolated ventricular myocytes, Am. J. Physiol. Heart Circ. Physiol. 294 (2008) H524–H531.
[62] G.D. Lopaschuk, J.R. Ussher, C.D. Folmes, J.S. Jaswal, W.C. Stanley, Myocardial fatty acid metabolism in health and disease, Physiol. Rev. 90 (2010) 207–258.
[63] C.J. Fearnley, H.L. Roderick, M.D. Bootman, Calcium signaling in cardiac myocytes, Cold Spring Harb. Perspect. Biol. 3 (2011) a004242.
[64] E.J. Lesnefsky, S. Moghaddas, B. Tandler, J. Kerner, C.L. Hoppel, Mitochondrial dysfunction in cardiac disease: ischemia–reperfusion, aging, and heart failure, J. Mol. Cell. Cardiol. 33 (2001) 1065–1089.
[65] P. Rosenberg, Mitochondrial dysfunction and heart disease, Mitochondrion 4 (2004) 621–628.
[66] A.C. Cave, J.S. Ingwall, J. Friedrich, R. Lioa, K.W. Saupe, C.S. Apstein, F.R. Eberli, ATP synthesis during low-flow ischemia. Influence of increased glycolytic substrate, Circulation 101 (2000) 2090–2096.
[67] E.J. Lesnefsky, B. Tandler, J. Ye, T.J. Slabe, J. turkaly, C.L. Hoppel, Myocardial ischemia decreases oxidative phosphorylation through cytochrome oxidase in subsarcolemmal mitochondria, Am. J. Physiol. Heart Circ. Physiol. 273 (1997) H1544–H1554.
[68] T. Zhou, C.C. Chuang, L. Zuo, Molecular characterization of reactive oxygen species in myocardial ischemia-reperfusion injury, Biomed. Res. Int. 2015 (2015) 864946.
[69] G.D. Lopaschuk, Metabolic modulators in heart disease: past, present, and future, Can. J. Cardiol. 33 (2017) 838–849.
[70] S.G. Varadarajan, J. An, E. Novalija, S.C. Smart, D.F. Stowe, Changes in [Na(+)](i), compartmental [Ca(2+)], and NADH with dysfunction after global ischemia in intact hearts, Am. J. Physiol. Heart Circ. Physiol. 280 (2001) H280–H293.
[71] S.S. Rhodes, A.K. Camara, K.M. Ropella, S.H. Audi, M.L. Riess, P.S. Pagel, D.F. Stowe, Ischemia reperfusion dysfunction changes model-estimated kinetics of myofilament interaction due to inotropic drugs in isolated hearts, Biomed. Eng. Online 5 (2006) 16.
[72] N. Gedik, L. Maciel, C. Schulte, A. Skyschally, G. Heusch, P. Kleinbongard, Cardiomyocyte mitochondria as targets of humoral factors released by remote ischemic preconditioning, Arch. Med. Sci. 13 (2016) 448–458.
[73] S.G. Ellis, C.I. Henschke, T. Sandor, J. Wynne, E. Braunwald, R.A. Kloner, Time course of functional and biochemical recovery of myocardium salvaged by reperfusion, J. Am. Coll. Cardiol. 1 (1983) 1047–1055.
[74] G.C. Rodrigo, N.B. Standen, Role of mitochondrial re-energization and Ca^{2+} influx in reperfusion injury of metabolically inhibited cardiac myocytes, Cardiovasc. Res. 67 (2005) 291–300.
[75] Y. Abdallah, S.A. Kasseckert, W. Iraqi, M. Said, T. Shahzad, A. Erdogan, C. Neuhof, D. Gunduz, K.D. Schluter, H. Tillmanns, H.M. Piper, H.P. Reusch, Y. Ladilov, Interplay between Ca^{2+} cycling and mitochondrial permeability transition pores promotes reperfusion-induced injury of cardiac myocytes, J. Cell. Mol. Med. 15 (2011) 2478–2485.
[76] M. Ruiz-Meana, A. Abellan, E. Miro-Casas, E. Agullo, D. Garcia-Dorado, Role of sarcoplasmic reticulum in mitochondrial permeability transition and cardiomyocyte death during reperfusion, Am. J. Physiol. Heart Circ. Physiol. 297 (2009) H1281–H1289.
[77] D.J. Hausenloy, J.A. Barrabes, H.E. Bøtker, S.M. Davidson, F. Di Lisa, J. Downey, T. Engstrom, P. Ferdinandy, H.A. Carbrera-Fuentes, G. Heusch, B. Ibanez, E.K. Iliodromitis, J. Inserte, R. Jennings, N. Kalia, R. Kharbanda, S. Lecour,

M. Marber, T. Miura, M. Ovize, M.A. Perez-Pinzon, H.M. Piper, K. Przyklenk, M.R. Schmidt, A. Redington, M. Ruiz-Meana, G. Vilahur, J. Vinten-Johansen, D.M. Yellon, D. Garcia-Dorado, Ischaemic conditioning and targeting reperfusion injury: a 30 year voyage of discovery, Basic Res. Cardiol. 111 (2016) 70.
[78] M. Ruiz-Meana, A. Abellan, E. Miro-Casas, D. Garcia-Dorado, Opening of mitochondrial permeability transition pore induces hypercontracture in Ca^{2+} overloaded cardiac myocytes, Basic Res. Cardiol. 102 (2007) 542–552.
[79] S.S. Rhodes, K.M. Ropella, A.K. Camara, Q. Chen, M.L. Riess, P.S. Pagel, D.F. Stowe, Ischemia-reperfusion injury changes the dynamics of Ca^{2+}-contraction coupling due to inotropic drugs in isolated hearts, J. Appl. Physiol. 100 (2006) 940–950.
[80] S. Wagner, T. Seidler, E. Picht, L.S. Maier, V. Kazanski, N. Teucher, W. Schillinger, B. Pieske, G. Isenberg, G. Hasenfuss, H. Kögler, Na^+-Ca^{2+} exchanger overexpression prediposes to reactive oxygen species-induced injury, Cardiovasc. Res. 60 (2003) 404–412.
[81] J.-S. Kim, Y. Jin, J.J. Lemasters, Reactive oxygen species, but not Ca^{2+} overloading, trigger pH- and mitochondrial permeability transition-dependent death of adult rat after ischemia-reperfusion, Am. J. Physiol. Heart Circ. Physiol. 290 (2006) H2024–H2034.
[82] D.J. Hausenloy, D.M. Yellon, Myocardial ischemia-reperfusion injury: a neglected therapeutic target, J. Clin. Invest. 123 (2013) 92–100.
[83] G. Heusch, J. Musiolik, N. Gedik, A. Skyschally, Mitochondrial STAT3 activation and cardioprotection by ischemic postconditioning in pigs with regional myocardial ischemia/reperfusion, Circ. Res. 109 (2011) 1302–1308.
[84] P. Kleinbongard, N. Gedik, M. Kirca, L. Stoian, U. Frey, A. Zandi, M. Thielmann, H. Jakob, J. Peters, M. Kamler, G. Heusch, Mitochondrial and contractile function of human right atrial tissue in response to remote ischemic conditioning, J. Am. Heart Assoc. 7 (2018) e009540.
[85] D.W. Green, H.N. Muurray, P.G. Sleph, F.L. Wang, A.J. Bair, W.L. Rogers, G.J. Grover, Preconditioning in rat hearts is independent of mitochondrial F1F0 ATPase inhibition, Am. J. Phys. 274 (1998) H90–H97.
[86] Y. Cao, S.Z. Zhang, S.Q. Zhao, I.C. Bruce, The mitochondrial Ca(2+)-activated K(+) channel contributes to cardioprotection by limb remote ischemic preconditioning in rat, Life Sci. 88 (2011) 1026–1030.
[87] M. Kobara, T. Tatsumi, S. Matoba, Y. Yamahara, C. Nakagawa, B. Ohta, T. Matsumoto, D. Inoue, J. Asayama, M. Nakagawa, Effect of ischemic preconditioning on mitochondrial oxidative phosphorylation and high energy phosphates in rat hearts, J. Mol. Cell. Cardiol. 28 (1996) 417–428.
[88] K.H. Slagsvold, O. Rognmo, M. Hoydal, U. Wisloff, A. Wahba, Remote ischemic preconditioning preserves mitochondrial function and influences myocardial microRNA expression in atrial myocardium during coronary bypass surgery, Circ. Res. 114 (2014) 851–859.
[89] F. Joubert, J.L. Mazet, P. Mateo, J.A. Hoerter, 31P NMR detection of subcellular creatine kinase fluxes in the perfused rat heart: contractility modifies energy transfer pathways, J. Biol. Chem. 277 (2002) 18469–18476.
[90] L. Guimaraes-Ferreira, Role of the phosphocreatine system on energetic homeostasis in skeletal and cardiac muscles, Einstein. 12 (2014) 126–131.
[91] T. Tatsumi, S. Matoba, M. Kobara, N. Keira, A. Kawahara, K. Tsuruyama, T. Tanaka, M. Katamura, C. Nakagawa, B. Ohta, Y. Yamahara, J. Asayama, M. Nakagawa, Energy metabolism after ischemic preconditioning in streptozotocin-induced diabetic rat hearts, J. Am. Coll. Cardiol. 31 (1998) 707–715.
[92] G. Prabhakar, L. Vona-Davis, D. Murray, P. Lakhani, G. Murray, Phosphocreatine restores high-energy phosphates in ischemic myocardium: implication for off-pump cardiac revascularization, J. Am. Coll. Surg. 197 (2003) 786–791.
[93] S.A. Javadov, S. Clarke, M. Das, E.J. Griffiths, K.H. Lim, A.P. Halestrap, Ischaemic preconditioning inhibits opening of mitochondrial permeability transition pores in the reperfused rat heart, J. Physiol. 549 (2003) 513–524.
[94] L. Argaud, O. Gateau-Roesch, L. Chalabreysse, L. Gomez, J. Loufouat, F. Thivolet-Bejui, D. Robert, M. Ovize, Preconditioning delays Ca^{2+}-induced mitochondrial permeability transition, Cardiovasc. Res. 61 (2004) 115–122.
[95] T. Andrienko, P. Pasdois, A. Rossbach, A.P. Halestrap, Real-time fluorescence measurements of ROS and [Ca^{2+}] in ischemic/reperfused rat hearts: detectable increases occur only after mitochondrial pore opening and are attenuated by ischemic preconditioning, PLoS One 11 (2016) e0167300.
[96] V.J. Cadete, J. Sawicka, J.S. Jaswal, G.D. Lopaschuk, R. Schulz, D. Szczesna-Cordary, G. Sawicki, Ischemia/reperfusion-induced myosin light chain 1 phosphorylation increases its degradation by matrix metalloproteinase 2, FEBS J. 279 (2012) 2444–2454.
[97] R.S. Decker, S. Nakamura, M.L. Decker, M. Sausamuta, S. Sinno, K. Harris, F.J. Klocke, I. Kulikovskaya, S. Winegrad, The dynamic role of cardiac myosin binding protein-C during ischemia, J. Mol. Cell. Cardiol. 52 (2012) 1145–1154.
[98] L. Gao, Y.J. Zheng, S.S. Gu, J.L. Tan, C. Paul, Y.G. Wang, H.T. Yang, Degradation of cardiac myosin light chain kinase by matrix metalloproteinase-2 contributes to myocardial contractile dysfunction during ischemia/reperfusion, J. Mol. Cell. Cardiol. 77 (2014) 102–112.
[99] Y.S. Han, O. Ogut, Force relaxation and thin filament protein phosphorylation during acute myocardial ischemia, Cytoskeleton. 68 (2011) 18–31.
[100] J.L. McDonough, K. Arrell, J.E. van Eyk, Troponin I degradation and covalent complex formation accompanies myocardial ischemia/reperfusion injury, Circ. Res. 84 (1999) 9–20.
[101] G. Sawicki, Synergistic effect of inhibitors of MMPs and ROS-dependent modifications of contractile proteins on protection hearts subjected to oxidative stress, Curr. Pharm. Des. 20 (2014) 1345–1348.
[102] S.F. Steinberg, Oxidative stress and sarcomeric proteins, Circ. Res. 112 (2013) 393–405.
[103] P.H. Sugden, A. Clerk, Oxidative stress and growth-regulating intracellular signaling pathways in cardiac myocytes, Antioxid. Redox Signal. 8 (2006) 2111–2124.
[104] M. Canton, I. Neverova, R. Menabó, J. Van Eyk, F. Di Lisa, Evidence of myofibrillar protein oxidation induced by postischemic reperfusion in isolated rat hearts, Am. J. Physiol. Heart Circ. Physiol. 286 (2004) H870–H877.
[105] Y. Sun, Myocardial repair/remodelling following infarction: roles of local factors, Cardiovasc. Res. 81 (2009) 482–490.
[106] S. Abdul-Ghani, K.J. Heesom, G.D. Angelini, M.S. Suleiman, Cardiac phosphoproteomics during remote ischemic preconditioning: a role for the sarcomeric Z-disk proteins, Biomed. Res. Int. 2014 (2014) 767812.
[107] A. Onody, A. Zvara, L. Hackler Jr., L. Vigh, P. Ferdinandy, L.G. Puskas, Effect of classic preconditioning on the gene expression pattern of rat hearts: a DNA microarray study, FEBS Lett. 536 (2003) 35–40.
[108] J. Sun, M. Morgan, R.F. Shen, C. Steenbergen, E. Murphy, Preconditioning results in S-nitrosylation of proteins involved in regulation of mitochondrial energetics and calcium transport, Circ. Res. 101 (2007) 1155–1163.
[109] G. Tong, A.M. Aponte, M.J. Kohr, C. Steenbergen, E. Murphy, J. Sun, Postconditioning leads to an increase in protein S-nitrosylation, Am. J. Physiol. Heart Circ. Physiol. 306 (2014) H825–H832.
[110] C. Yuan, Y. Guo, R. Ravi, K. Przyklenk, N. Shilkofski, R. Diez, R.N. Cole, A.M. Murphy, Myosin binding protein C is differentially phosphorylated upon myocardial stunning in canine and rat hearts– evidence for novel phosphorylation sites, Proteomics 6 (2006) 4176–4186.
[111] J.A. Ubersax, J.E. Ferrell, Jr, Mechanisms of specificity in protein phosphorylation, Nat. Rev. Mol. Cell Biol. 8 (2007) 530–541.
[112] R.S. Decker, M.L. Decker, I. Kulikovskaya, S. Nakamura, D.C. Lee, K. Harris, F.J. Klocke, S. Winegrad, Myosin-binding protein C phosphorylation, myofibril structure, and contractile function during low-flow ischemia, Circulation 111 (2005) 906–912.
[113] S. Sadayappan, H. Osinska, R. Klevitsky, J.N. Lorenz, M. Sargent, J.D. Molkentin, C.E. Seidman, J.G. Seidman, J. Robbins, Cardiac myosin binding protein c phosphorylation is cardioprotective, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 16918–16923.
[114] S. Sadayappan, J. Gulick, H. Osinska, L.A. Martin, H.S. Hahn, G.W. Dorn 2nd, R. Klevitsky, C.E. Seidman, J.G. Seidman, J. Robbins, Cardiac myosin-binding protein-C phosphorylation and cardiac function, Circ. Res. 97 (2005) 1156–1163.
[115] C.W. Tong, J.E. Stelzer, M.L. Greaser, P.A. Powers, R.L. Moss, Acceleration of crossbridge kinetics by protein kinase a phosphorylation of cardiac myosin binding protein C modulates cardiac function, Circ. Res. 103 (2008) 974–982.
[116] P.C. Rosas, Y. Liu, M.I. Abdalla, C.M. Thomas, D.T. Kidwell, G.F. Dusio, D. Mukhopadhyay, R. Kumar, K.M. Baker, B.M. Mitchell, P.A. Powers, D.P. Fitzsimons, B.G. Patel, C.M. Warren, R.J. Solaro, R.L. Moss, C.W. Tong, Phosphorylation of cardiac Myosin-binding protein-C is a critical mediator of diastolic function, Circ. Heart Fail. 8 (2015) 582–594.
[117] R. Knöll, B. Buyandelger, M. Lab, The sarcomeric Z-disc and Z-discopathies, J. Biomed. Biotechnol. 2011 (2011) 569628, https://doi.org/10.1155/2011/569628.
[118] W.G. Pyle, J.R. Solaro, At the crossroads of myocardial signaling. The role z-discs in intracellular signaling and cardiac function, Circ. Res. 94 (2004) 296–305.
[119] C.W. Tong, R.D. Gaffin, D.C. Zawieja, M. Muthuchamy, Roles of phosphorylation of myosin binding protein-C and troponin I in mouse cardiac muscle twitch dynamics, J. Physiol. 558 (2004) 927–941.
[120] R. Zhang, J. Zhao, A. Mandveno, J.D. Potter, Cardiac troponin I phosphorylation increases the rate of cardiac muscle relaxation, Circ. Res. 76 (1995) 1028–1035.
[121] S. Vahebi, A. Ota, M. Li, C.M. Warren, P.P. de Tombe, Y. Wang, R.J. Solaro, p38-MAPK induced dephosphorylation of alpha-tropomyosin is associated with depression of myocardial sarcomeric tension and ATPase activity, Circ. Res. 100 (2007) 408–415.
[122] V.S. Rao, L.R. La Bonte, Y. Xu, Z. Yang, B.A. French, W.H. Guilford, Alterations to myofibrillar protein function in nonischemic regions of the heart early after myocardial infarction, Am. J. Physiol. Heart Circ. Physiol. 293 (2007) H654–H659.
[123] N. Gedik, M. Krüger, M. Thielmann, E. Kottenberg, A. Skyschally, U.H. Frey, E. Cario, J. Peters, H. Jakob, G. Heusch, P. Kleinbongard, Proteomics/phosphoproteomics of left ventricular biopsies from patients with surgical coronary revascularization and pigs with coronary occlusion/reperfusion: remote ischemic preconditioning, Sci. Rep. 7 (2017) 7629.
[124] R. Schulz, M. Kelm, G. Heusch, Nitric oxide in myocardial ischemia/reperfusion injury, Cardiovasc. Res. 61 (2004) 402–413.
[125] R. Schreckenberg, P. Weber, H.A. Cabrera-Fuentes, I. Steinert, K.T. Preissner, P. Bencsik, M. Sarkozy, C. Csonka, P. Ferdinandy, R. Schulz, K.D. Schluter, Mechanism and consequences of the shift in cardiac arginine metabolism following ischaemia and reperfusion in rats, Thromb. Haemost. 113 (2015) 482–493.
[126] G. Heusch, K. Boengler, R. Schulz, Cardioprotection: nitric oxide, protein kinases, and mitochondria, Circulation 118 (2008) 1915–1919.
[127] T. Rassaf, M. Totzeck, U.B. Hendgen-Cotta, S. Shiva, G. Heusch, M. Kelm, Circulating nitrite contributes to cardioprotection by remote ischemic preconditioning, Circ. Res. 114 (2014) 1601–1610.
[128] D.T. Hess, A. Matsumoto, S.O. Kim, H.E. Marshall, J.S. Stamler, Protein S-nitrosylation: purview and parameters, Nat. Rev. Mol. Cell Biol. 6 (2005) 150–166.
[129] N. Hogg, K.A. Broniowska, J. Novalija, N.J. Kettenhofen, E. Novalija, Role of S-nitrosothiol transport in the cardioprotective effects of S-nitrosocysteine in rat hearts, Free Radic. Biol. Med. 43 (2007) 1086–1094.
[130] C. Figueiredo-Freitas, R.A. Dulce, M.W. Foster, J. Liang, A.M. Yamashita, F.L. Lima-Rosa, J.W. Thompson, M.A. Moseley, J.M. Hare, L. Nogueira, M.M. Sorenson, J.R. Pinto, S-nitrosylation of sarcomeric proteins depresses

myofilament (Ca^{2+}) sensitivity in intact cardiomyocytes, Antioxid. Redox Signal. 23 (2015) 1017–1034.
[131] G.W. Hart, C. Slawson, G. Ramirez-Correa, O. Lagerlof, Cross talk between O-GlcNAcylation and phosphorylation: roles in signaling, transcription, and chronic disease, Annu. Rev. Biochem. 80 (2011) 825–858.
[132] R.V. Jensen, N.E. Zachara, P.H. Nielsen, H.H. Kimose, S.B. Kristiansen, H.E. Bøtker, Impact of O-GlcNAc on cardioprotection by remote ischaemic preconditioning in non-diabetic and diabetic patients, Cardiovasc. Res. 97 (2013) 369–378.
[133] G.A. Ramirez-Correa, W. Jin, Z. Wang, X. Zhong, W.D. Gao, W.B. Dias, C. Vecoli, G.W. Hart, A.M. Murphy, O-linked GlcNAc modification of cardiac myofilament proteins: a novel regulator of myocardial contractile function, Circ. Res. 103 (2008) 1354–1358.
[134] P. Ding, J. Huang, P.K. Battiprolu, J.A. Hill, K.E. Kamm, J.T. Stull, Cardiac Myosin light chain kinase is necessary for Myosin regulatory light chain phosphorylation and cardiac performance in vivo, J. Biol. Chem. 285 (2010) 40819–40829.
[135] O. Seguchi, S. Takashima, S. Yamazaki, M. Asakura, Y. Asano, Y. Shintani, M. Wakeno, T. Minamino, H. Kondo, H. Furukawa, K. Nakamaru, A. Naito, T. Takahashi, T. Ohtsuka, K. Kawakami, T. Isomura, S. Kitamura, H. Tomoike, N. Mochizuki, M. Kitakaze, A cardiac myosin light chain kinase regulates sarcomere assembly in the vertebrate heart, J. Clin. Invest. 117 (2007) 2812–2824.
[136] S. Kötter, M. Kazmierowska, C. Andresen, K. Bottermann, M. Grandoch, S. Gorressen, A. Heinen, J.M. Moll, J. Scheller, A. Gödecke, J.W. Fischer, J.P. Schmitt, M. Krüger, Titin-based cardiac myocyte stiffening contributes to early adaptive ventricular remodeling after myocardial infarction, Circ. Res. 119 (2016) 1017–1029.
[137] A. Divald, S. Kivity, P. Wang, E. Hochhauser, B. Roberts, S. Teichberg, A.V. Gomes, S.R. Powell, Myocardial ischemic preconditioning preserves postischemic function of the 26S proteasome through diminished oxidative damage to 19S regulatory particle subunits, Circ. Res. 106 (2010) 1829–1838.