

Mechanisms of Exercise-Induced Cardioprotection

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Myocardial ischemia-reperfusion (IR) injury can cause ventricular cell death and is a major pathological event leading to morbidity and mortality in those with coronary artery disease. Interestingly, as few as five bouts of exercise on consecutive days can rapidly produce a cardiac phenotype that resists IR-induced myocardial injury. This review summarizes the development of exercise-induced cardioprotection and the mechanisms responsible for this important adaptive response.

Coronary artery disease remains a major cause of death worldwide (71). The major pathology associated with coronary artery disease is commonly the direct consequence of an ischemia-reperfusion (IR) insult. In this regard, the resultant myocardial damage due to IR injury is proportional to ischemic duration (98). Given the high incidence of coronary artery disease and the associated IR-induced cardiac injury, developing a countermeasure to protect the heart against IR-induced damage is important. In this regard, it is well established that exercise training provides robust cardioprotection against IR injury. For example, human epidemiological studies reveal that regular exercise reduces the risk of death during a myocardial IR insult (reviewed in Ref. 49). Importantly, numerous animal studies provide direct evidence that endurance exercise protects the heart from IR-induced injury (reviewed in Refs. 28, 56, 92).

This review summarizes the present understanding of the mechanisms responsible for exercise-induced cardioprotection against IR-induced injury. We begin with an introduction to IR-induced myocardial injury followed by a brief summary of the evidence demonstrating that exercise is cardioprotective. The remainder of this report focuses on the putative mechanisms responsible for exercise-induced cardioprotection with an emphasis on the important role that mitochondrial adaptations play in producing the protected phenotype.

Levels of IR-Induced Cardiac Injury

Depending on the duration of ischemia, three different levels of IR-induced cardiac injury ensue (FIGURE 1). The lowest level of IR-induced injury is the occurrence of cardiac arrhythmias. In this scenario, rapid reperfusion occurring after 1–5 min of ischemia often promotes ventricular tachycardia or fibrillation without depressed myocardial contractile performance or cardiac cell death (25). Reperfusion following 5–20 min of ischemia produces a

higher level of myocardial injury termed “myocardial stunning” (25). Myocardial stunning is characterized by impaired ventricular contractility that occurs in the absence of cardiac cell death (9). Typically, IR-induced myocardial stunning results in cardiac contraction deficits that last 24–72 h following the insult (9). The final and most severe level of IR injury occurs when the duration of ischemia exceeds 20 min. During this most severe level of IR injury, cardiac myocytes are irreversibly damaged, and death occurs due to both apoptosis and necrosis (25). Additional evidence indicates that apoptotic tissue death can be either mitigated or potentiated by autophagic processes within ventricular myocytes exposed to ischemia (61, 100).

Cellular Events Leading to IR-Induced Cardiac Injury

Despite the complexity of the events leading to IR-induced cardiac damage, the key factors responsible for IR-induced myocardial cell death are largely understood (for a review, see Refs. 33, 77, 91). During prolonged myocardial ischemia, numerous interrelated events occur resulting in decreased cellular [ATP], small increases in reactive oxygen species (ROS) production, accumulation of hydrogen ions, increased levels of cytosolic-free calcium, and activation of the calcium-activated protease calpain (FIGURE 2). During reperfusion, ROS production is exacerbated, cellular calcium overload continues, calpain remains active, and activation of caspase-3 occurs (50, 77). Furthermore, leukocyte infiltration to the area of cardiac injury occurs following severe IR damage to the heart (i.e., ischemia longer than 20 min) (77). Collectively, these factors promote mitochondrial injury and subsequent cardiac myocyte death (33, 77).

It is established that mitochondrial ROS production is central in IR-induced myocardial injury (124). Indeed, mitochondrial ROS are produced during both ischemia and reperfusion, with the

majority of ROS production occurring during reperfusion (4, 124). The importance of ROS-mediated damage to the heart during an IR event is confirmed by reports demonstrating that delivery of exogenous antioxidants provide protection against IR-induced cardiac injury (2, 18, 44, 59). Although IR-induced ROS production occurs in several cellular locations, mitochondrial production of ROS plays a dominant role in IR-mediated oxidant injury in the heart (2, 59).

Of the various pathological events that lead to IR injury, increased ROS production and increased cytosolic calcium levels play key roles. Furthermore, several lines of evidence indicate that cross talk occurs between ROS and calcium to magnify IR injury. For example, increased cytosolic calcium levels mediate cellular injury by activation of calpain, promotion of mitochondrial damage, and facilitating mitochondrial ROS production (33, 65, 86). Conversely, oxidative stress fosters cellular calcium overload in at least two ways. First, ROS-mediated formation of reactive aldehydes (i.e., 4-hydroxyl-2,3-trans-nonenal) attenuates plasma membrane calcium ATPase activity and diminishes calcium removal from the cell, resulting in increased cellular calcium levels (102). Second, ROS-mediated oxidation of the sarcoplasmic reticulum calcium release channel (i.e., ryanodine receptor) fosters release of calcium into the cytosol (3, 34).

Among the numerous factors that contribute to IR-induced cardiac cellular injury, mitochondria viability is a key arbitrator of cardiac cell life or death. Indeed, the degree to which aerobic ATP production is preserved in mitochondria following an IR insult determines whether the cell will live or die via necrosis or apoptosis (35, 36, 39, 65, 81). The main factor linking mitochondria to IR-induced cell death involves permeabilization of the mitochondrial outer membrane and/or opening of the mitochondrial permeability transition pore, which is linked to both apoptotic and necrotic cell death (81). Specifically, IR-induced apoptosis occurs

during reperfusion by permeabilization of the outer mitochondrial membrane and the release of cytochrome *c* into the cytosol (65). Necrotic cell death can occur during both ischemia and reperfusion and involves the opening of the mitochondrial permeability transition pore followed by mitochondrial swelling and rupture (65).

In summary, numerous factors interact to produce cardiac cell injury during an IR insult. Of the various events responsible for IR injury, mitochondrial damage plays a central role, since these organelles are vital gate keepers of life and death in the cardiac myocyte. It follows that preventing IR-induced mitochondrial injury protects cardiac myocytes during an IR insult. Given the wealth of evidence indicating that exercise training produces robust protection against IR injury, it has been postulated that beneficial mitochondrial adaptations underpin these observations. A brief summary of the evidence that exercise provides protection against IR-induced myocardial injury follows.

Exercise Promotes Cardioprotection

It is well established that repeated bouts of exercise improve myocardial tolerance to IR in animals, which is commonly referred to as exercise-induced cardioprotection (reviewed in Refs. 28, 56, 92). Similar to the ischemic preconditioning (PC) phenomenon produced by a brief period of surgically induced ischemia, exercise-induced cardioprotection is also biphasic. The first phase of cardioprotection is acquired rapidly following an acute exercise bout (i.e., 0.5 h after exercise). However, this initial protection is rapidly lost within 3 h postexercise. The mechanisms responsible for the early phase of preconditioning are not well characterized but likely include allosteric activation of the endogenous antioxidant enzyme superoxide dismutase (SOD2) located within mitochondria of ventricular myocytes (46, 118). Given the similar time course for the early window of protection in both exercise-induced cardioprotection and PC, it is tempting to speculate that common mechanisms could mediate both forms of cardioprotection, although this conclusion requires scientific verification. The second or late phase of exercise-induced cardioprotection is achieved within 24 h after the exercise bout and persists for at least 9 days following a 5-day exercise routine and is far more robust than the aforementioned early protective window (66). This review is focused on the mechanisms responsible for this second window of exercise-induced cardioprotection.

As mentioned earlier, repeated bouts of endurance exercise protect against IR-induced arrhythmias (29, 43, 75), myocardial stunning (11, 22, 23,

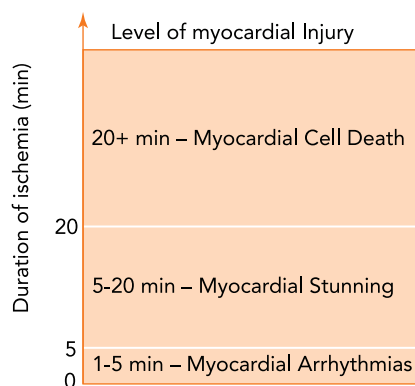


FIGURE 1. Relationship between duration of ischemia and the level of IR-induced cardiac injury

32, 42, 69, 70, 89, 113, 114), and myocardial infarction (29, 31, 95, 118). Interestingly, only 3–5 consecutive days of endurance exercise is required to achieve a significant level of cardioprotection against IR-induced myocardial infarction (FIGURE 3) (23, 31, 44). Complete details about the dose-response impact of aerobic exercise intensity on cardioprotection remain unknown. Findings from several studies provide insight into the influence of exercise intensity on resulting cardioprotection. One investigation into the role of intensity suggested that exercise below 55–60% $\dot{V}O_{2max}$ did not achieve IR injury resistance (107), whereas another study concluded that both moderate- (i.e., 50% $\dot{V}O_{2max}$) and relatively high-intensity (i.e., 70% $\dot{V}O_{2max}$) exercise appear to be equally protective against IR-induced myocardial stunning (67). Regardless, it is feasible that there may be an exercise intensity threshold above which cardioprotection is achieved. Predictably, exercise-induced cardioprotection in rodents is lost rapidly (i.e., within 9–18 days) following the cessation of exercise training (66).

Although it is clear that continuous aerobic exercise (e.g., 60 min) produces a cardioprotective phenotype, growing evidence suggests that high-intensity interval training (1 min of exercise at $\geq \dot{V}O_{2max}$) also produces a cardioprotective phenotype (69, 70). Furthermore, a recent study suggests that 12 wk of resistance exercise training provides protection against IR-induced myocardial infarction in rats (24, 103).

What Are the Mechanisms Responsible for Exercise-Induced Cardioprotection?

The mechanism(s) responsible for exercise-induced myocardial protection against IR injury remains a debated issue as numerous putative mediators have been proposed. In theory, exercise-induced cardioprotection could be achieved by any physiological adaptation that attenuates one or more of the damaging events that occur during ischemia and/or reperfusion. For example, exercise-induced cardioprotection could be acquired by changes in the coronary arteries (i.e., increased collateral circulation) and/or intrinsic changes in the cardiac myocyte. Potential intrinsic changes in the cardiac myocyte that could provide cellular protection against IR injury include increased glycolytic flux, altered nitric oxide (NO) signaling, increased levels of heat shock proteins (HSPs), amplified myocardial cyclooxygenase-2 (COX-2) activity, elevated endoplasmic reticulum (ER) stress proteins, enhanced function of sarcolemmal and/or mitochondrial ATP-sensitive potassium channels, increased cytosolic antioxidant capacity, and/or altered mitochondrial antioxidant capacity (FIGURE 4). An overview of the evidence to support or deny the role for each of these

potential cardioprotective mediators is highlighted in the next segments.

Exercise-Induced Changes in Coronary Circulation Are Not Required for Cardioprotection

Endurance exercise-induced structural changes in the coronary circulation include increased conduit artery diameters and increased arteriolar densities and diameters (reviewed in Ref. 62). Specifically, in both young rats and humans, several months of endurance exercise increases the volume of the coronary vasculature because of increased conduit artery size (45, 68). Furthermore, regular aerobic exercise induces functional adaptations in the coronary circulation, including enhanced endothelium-dependent vasodilation (62). Although both of these training-induced adaptations within the coronary circulation could protect the heart during an IR event, at present, no experimental data exists to directly link these exercise-induced changes to short-term (e.g., 3–5 days) exercise-mediated cardioprotection. Indeed, a few consecutive days of exercise training can provide cardioprotection against IR injury (23, 42, 113), but structural changes in the coronary circulation do not occur within this short period of exercise training (118). Furthermore, evidence indicates that cardiac myocytes isolated from the heart of exercise-trained animals are protected against hypoxia-reoxygenation injury (55). Therefore, these results indicate that exercise can directly modify the cardiac myocyte to achieve a cardioprotective phenotype, and, consequently, a change in the coronary circulation is not required to achieve exercise-induced cardioprotection. Indeed, although exercise training has the potential to increase coronary flow during reperfusion (62), studies of isolated perfused hearts demonstrate that short-term exercise provides cardioprotection independent of improvements in

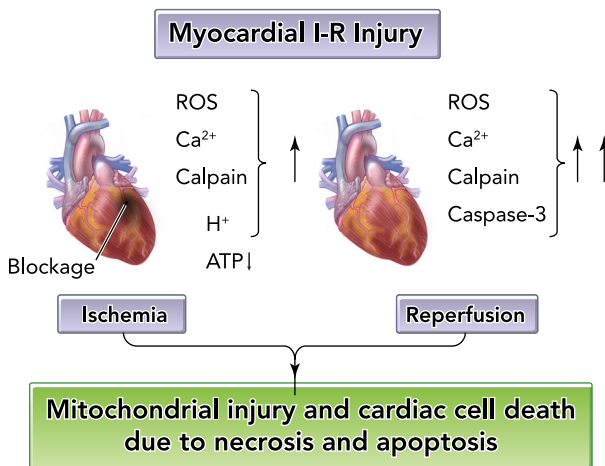


FIGURE 2. Cellular events leading to ischemia-reperfusion injury in the heart
ROS, reactive oxygen species.

coronary flow (11, 66, 93). Thus the remainder of this review will focus on exercise-induced endogenous changes in the cardiac myocyte that can contribute to cardioprotection.

Altered Glycolytic Flux as a Potential Mediator of Exercise-Induced Cardioprotection

Myocardial survival during an IR insult is dependent, at least in part, on cellular energy status. To limit the energy deficit during ischemia and hypoxia, cardiac energy production switches from the preferential use of fatty acids to carbohydrates; this switch in fuel utilization is essential to sustain ATP production via glycolysis (116). However, whether increased glycolytic flux is advantageous or detrimental during ischemia depends on the duration of the ischemic insult. Indeed, increased glycolysis in the ischemic heart is a double-edged sword since the accumulation of glycolytic end-products (e.g., lactate) during prolonged ischemia are detrimental to postischemic recovery (104). Hence, limiting glycolysis during long-duration myocardial ischemia could be a protective strategy to minimize IR injury (116). In this regard, it has been reported that PC results in a decrease in glycolytic flux in the ischemic heart (116). Similarly, evidence indicates that endurance exercise training decreases the rate of glycolysis in the rat heart during ischemia (14). Although the mechanism(s) by which exercise training alters the metabolic phenotype of the heart to produce this response is unknown, it is feasible that a reduction in glycolysis during ischemia could be cardioprotective. Nonetheless, to date, no direct evidence exists to mechanistically connect exercise-induced changes in myocardial glycolytic flux to cardioprotection.

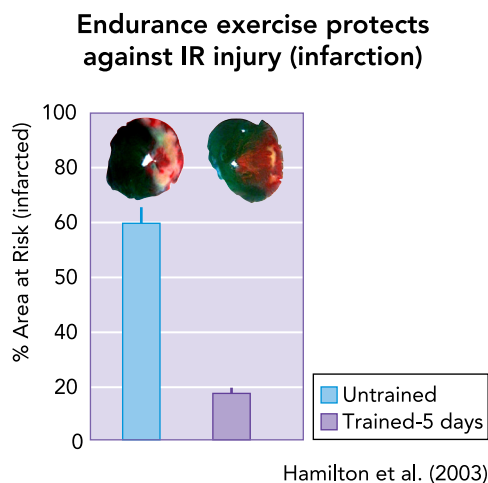


FIGURE 3. As few as 5 consecutive days of exercise can provide significant protection against IR-induced myocardial infarction. Data are from Ref. 44.

Altered NO Signaling as a Facilitator of Exercise-Induced Cardioprotection

NO is produced in tissues from L-arginine, oxygen, and NADPH by NO synthase (NOS) enzymes (90). Numerous studies reveal that endurance exercise training results in increased phosphorylation and activity of endothelial NOS (eNOS) in both humans and animals (19, 38, 41). This exercise-induced rise in eNOS activity is associated with increased production of NO, as evidenced by augmented levels of both nitrite and nitrosothiols in tissue and blood. In this regard, nitrite is produced by the oxidation of NO in aerobic conditions (117), whereas nitrosothiols are formed when cysteine thiols in proteins are modified by NO via a process known as S-nitrosylation (27). It follows that circulating levels of nitrite and nitrosothiols are commonly used as biomarkers of NO availability (15). Importantly, nitrite is a potentially important storage form of NO in both blood and tissues because nitrite can be converted to NO by either acid reduction or nitrite reductases during ischemia (64).

A recent review summarized the possible role that NO metabolites play in the cardioprotective effects of exercise (15). In regard to the mechanisms linking NO to cardioprotection, increased NO levels can increase the S-nitrosylation of cardiac proteins during myocardial IR, which can attenuate injurious processes such as apoptosis (15). Indeed, caspase-3 activity can be inhibited via protein S-nitrosylation, and NO production of nitrosothiols can modify complex I of the mitochondrial electron transport chain, resulting in reduced mitochondrial ROS production during an IR event (15). Together, these changes could protect cardiac myocytes against IR-induced damage (117), and a recent study employing eNOS knockout mice concluded that increased production of NO is essential to achieve the cardioprotection associated with endurance exercise. However, it is also possible that some of the exercise-induced adaptations in the heart are not achieved in the eNOS knockout mouse. Indeed, a recent study reveals that several exercise-induced adaptations within the heart require eNOS expression (20). Therefore, additional experiments are required to confirm or deny whether NO or other NO metabolites are a requirement to achieve exercise-induced cardioprotection.

Elevated Myocardial Heat Shock Proteins Are Not Essential for Exercise-Induced Cardioprotection

It is clear that transgenic overexpression of heat shock protein 72 (HSP72) protects the heart against IR-induced injury (48, 51, 111). Furthermore, repeated bouts of endurance exercise result in a three- to fivefold increase in cardiac HSP72 levels

(21, 44, 89, 113). In theory, elevated cellular levels of HSP72 can protect the myocardium against IR injury by augmenting myocardial antioxidant capacity, protecting mitochondria against IR injury, and preventing apoptosis (51, 109, 111).

Although exercise promotes the accumulation of HSP72 in the heart and HSP72 is cardioprotective, three independent studies demonstrate that an exercise-induced increase in HSP72 is not essential for exercise-induced cardioprotection (42, 95, 113). Therefore, although overexpression of HSP72 is sufficient to promote cardioprotection, increases in myocardial HSP72 are not a requirement to achieve exercise-induced cardioprotection.

Increased Myocardial Cyclooxygenase-2 is not Responsible for Exercise-Induced Cardioprotection

Cyclooxygenase-2 (COX-2) is the rate-limiting enzyme in prostaglandin biosynthesis catalyzing the conversion of arachidonic acid to prostaglandins. Over the past decade, COX-2 has emerged as an obligatory mediator of the late phase of ischemic preconditioning-induced cardioprotection, and it follows that COX-2 could also be a candidate molecule to explain exercise-induced cardioprotection (8, 10, 101).

The PC cardioprotective actions of COX-2 appear to spring from PGE₂ and/or PGI₂ production, since this elevated prostanoid production improves vasodilation within the coronary vasculature (1, 47). Nonetheless, several lines of evidence suggest that COX-2 is not required for exercise-induced cardioprotection. For example, exercise does not elevate COX-2 levels in the rat heart (94). Similarly, pharmacological inhibition of COX-2 does not prevent exercise-induced protection against IR-induced cardiac arrhythmias (78). Together, these findings suggest that an increase in COX-2 is not essential to achieve exercise-induced cardioprotection against IR injury.

Elevated Endoplasmic Reticulum Stress Proteins do not Contribute to Exercise-Mediated Cardioprotection

Endoplasmic reticulum (ER) stress proteins represent a group of cardioprotective proteins that could contribute to exercise-induced cardioprotection, since recent evidence indicates that ER stress contributes to IR-induced myocardial injury (80). Indeed, IR-induced ER dysfunction can promote both mitochondrial-dependent and -independent cell death resulting from a disturbance in calcium homeostasis and/or impaired protein folding (115).

Two proteins that could protect against ER stress are the glucose-regulated proteins Grp78 and Grp94. Both Grp78 and Grp94 function in ER protein folding and also exhibit calcium-binding

properties, and overexpression of Grp94 and Grp78 can protect cardiomyocytes against both calcium overload and oxidative damage (115, 120, 121). Moreover, increased Grp78 and Grp94 expression is linked to a reduction in IR-induced necrosis and apoptosis in the heart (74). Nevertheless, exercise does not elevate Grp78 and Grp94 (76). Therefore, the existing evidence indicates that increased ER stress proteins are not a requirement for exercise-induced cardioprotection against IR injury.

Sarcolemmal and/or Mitochondrial ATP-Sensitive Potassium Channels May Participate in Exercise Trained-Mediated Cardioprotection

ATP-sensitive potassium channels located in the sarcolemma (sarcoK_{ATP}) and mitochondria (mitoK_{ATP}) of cardiac myocytes are important regulators of cardiac myocyte function (79, 84). Both sarcoK_{ATP} and mitoK_{ATP} channels are composed of two protein complexes, an inwardly rectifying potassium channel pore and an associated sulfonylurea receptor subunit that governs channel opening. These energy-sensing channels are named according to a classical understanding of their function, in which potassium ion currents are inhibited by an abundance of cellular ATP. Channel activation also occurs in response to other activating factors such as acute ischemia, increases in adenosine and MgADP, along with activation of protein kinase C-ε (PKC-ε) (99). Independent of the activating stimulus, opening either sarcoK_{ATP} or mitoK_{ATP} channels before an IR insult confers cardioprotection (40, 60). The means by which K_{ATP} channels confer cardioprotection are not fully understood. Nonetheless, it is clear that K_{ATP} channel opening prevents IR injury through multiple

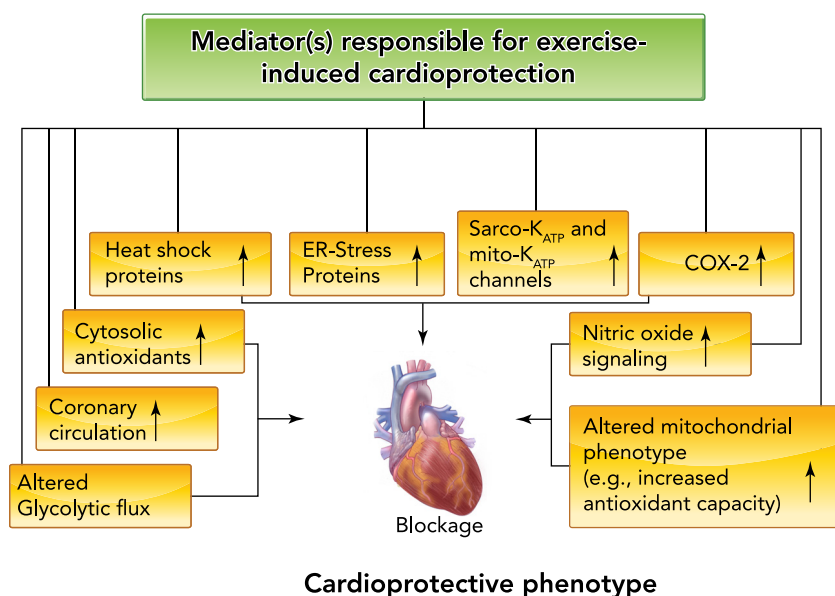


FIGURE 4. A list of proposed mediators of exercise-induced cardioprotection
See text for details.

biochemical alterations within the ventricular myocyte. For instance, opening of sarcoK_{ATP} channels is predicted to protect against IR injury by shortening the cardiac action potential duration via the acceleration of phase 3 repolarization (40). Shortening the cardiac action potential could inhibit Ca²⁺ entry into the cell via L-type Ca²⁺ channels and avert Ca²⁺ overload (40). Furthermore, the slowing of depolarization could also reduce Ca²⁺ entry by avoiding the reversal of the Na⁺/Ca²⁺ exchanger. Together, these actions could protect the cardiac myocyte during IR by reducing the cytosolic Ca²⁺ overload. Another role that sarcoK_{ATP} channels play is triggering the opening of the mitoK_{ATP} channels (84). The specific details of how mitoK_{ATP} channels protect the heart against IR injury remains controversial, but it is believed that opening the mitoK_{ATP} channels protects mitochondria against IR-induced Ca²⁺ overload and damage (5, 40).

The role that sarcoK_{ATP} channels play in exercise-induced protection against IR injury has received limited investigative attention, but two studies suggest that endurance exercise training increases the expression of sarcoK_{ATP} channels in the cardiac myocyte (12, 122). Furthermore, other reports reveal that pharmacological blockage of the sarcoK_{ATP} channels impairs the exercise-induced protective benefits against IR-induced myocardial necrosis (12, 96). Nonetheless, because of concerns associated with the pharmacological inhibitors used in these studies, it is difficult to form a firm conclusion regarding the mechanistic role that sarcoK_{ATP} channels play in exercise-induced cardioprotection.

Finally, using pharmacological inhibitors of the MitoK_{ATP} channel, it appears that MitoK_{ATP} channel activation protects the heart against IR-induced ventricular arrhythmias (97) but does not protect against IR-induced infarction (12). Nonetheless, the inability to detect the molecular identity of the MitoK_{ATP} channel and concerns associated with the specificity of the channel blocker used in these experiments does not permit firm conclusions regarding the role that the MitoK_{ATP} channel plays in exercise-induced cardioprotection.

Increased Cytosolic Antioxidant Capacity May Play a Required Role in Exercise-Induced Cardioprotection

Although the etiology of cardiac IR injury involves numerous factors and varies as a function of the duration of ischemia, mitochondrial-produced ROS are significant contributors to the necrotic and apoptotic cell death following an IR insult (reviewed in Refs. 77, 91). There is abundant evidence that endurance exercise training increases several components of the antioxidant buffering system in the heart. In particular, many studies agree that endurance exercise increases SOD2, which is the

SOD isoform located in the mitochondrial matrix (reviewed in Refs. 28, 56, 92). Another SOD isoform (SOD1) is located in both the cytosol and the mitochondrial intermembrane space (90). Emerging evidence indicates that exercise training also elevates SOD1 in the mitochondria (63). In contrast, the impact of exercise on the cytosolic isoform of SOD1 in the heart remains equivocal.

SOD is a first line of defense against superoxide in cells, and SOD-mediated dismutation of superoxide results in formation of the nonradical ROS hydrogen peroxide (H₂O₂). Cardiac myocytes are equipped to eliminate H₂O₂ via several routes, including the enzymatic removal by catalase, thioredoxins, and glutathione peroxidase (90). However, most studies report that the activities of catalase, thioredoxins, and glutathione peroxidase are not increased in the heart following exercise (28, 30, 31, 53, 97, 105, 112). Nonetheless, growing evidence suggests that exercise training increases the activity of glutathione reductase in the heart via post-translational modifications (29, 30). An increase in glutathione reductase activity would amplify the heart's ability to replenish cardiac levels of glutathione that is required for glutathione peroxidase to remove H₂O₂. In this regard, a recent report concludes that increases in glutathione reductase activity play an essential role in exercise-induced cardioprotection (29). However, it is currently unclear whether this exercise-induced increase in glutathione reductase activity in cardiac myocytes is confined to the cytosolic compartment alone or whether glutathione reductase activity also increases in other cellular compartments such as the mitochondrion. Regardless of the cellular location of this enzyme, it appears likely that increases in myocardial glutathione reductase activity contribute to exercise-induced cardioprotection.

Exercise-Induced Alterations in Mitochondrial Proteins and Phenotype Are Central to Exercise-Induced Cardioprotection

Growing evidence reveals that endurance exercise training results in alterations in mitochondrial phenotype, and this adaptation is required to achieve exercise-induced cardioprotection. In the following segments, we discuss exercise-induced changes in mitochondrial phenotype and evaluate evidence that exercise-induced increases in key mitochondrial proteins are required to achieve cardioprotection against an IR insult.

Exercise alters mitochondrial phenotype in the heart. Emerging evidence reveals that exercise induces a mitochondrial phenotype that resists apoptotic stimuli and IR-induced mitochondrial damage (6, 57, 63, 73, 105). Indeed, both subsarcolemmal (SS) and intermyofibrillar (IMF) mitochondria undergo biochemical adaptations in response to endurance

exercise that lead to decreased apoptotic susceptibility (57, 58). For example, *in vitro* experiments using isolated cardiac mitochondria reveal that exercise training results in a mitochondrial phenotype that resists cytochrome *c* release from both SS and IMF mitochondria exposed to ROS and/or calcium challenges (58). The concept that exercise training results in a mitochondrial phenotype that resists IR-mediated damage is also supported by experiments using isolated cardiac mitochondria exposed to anoxia followed by reoxygenation. These studies reveal that, following anoxia-reoxygenation, state 3 respiration is better preserved in mitochondria isolated from the hearts of exercised rats (6). This finding was associated with attenuated oxidative damage to mitochondrial proteins and is in contrast to the severe metabolic dysfunction and oxidative damage observed in cardiac mitochondria isolated from sedentary animals.

Furthermore, a recent *in vivo* study employing a physiologically relevant experimental model provides more evidence that endurance exercise protects cardiac mitochondria from IR-induced damage (63). This work confirms that exercise training protects both SS and IMF mitochondria against IR-induced functional impairment and corroborates the concept that exercise training attenuates IR-induced increases in ROS release from both SS and IMF mitochondria. Moreover, this investigation establishes that exercise training also retards the IR-induced release of proapoptotic proteins from cardiac mitochondria (63). This observation may explain previous findings that exercise training is associated with attenuated cardiac apoptosis following IR insults *in vivo* (31, 95, 96). The next segment discusses the potential molecular mechanisms responsible for exercise-induced protection of cardiac mitochondria.

Exercise-induced alterations in mitochondrial proteins. To understand the mechanisms by which exercise training alters mitochondrial phenotype to resist pro-apoptotic stimuli and stress-induced by hypoxia-reoxygenation, studies have investigated exercise-induced changes in mitochondrial protein expression (13, 57, 82, 88, 110). Collectively, these reports identify both increased and decreased expression of 21 different mitochondrial proteins in hearts from exercise-trained animals. These exercise-induced changes in mitochondrial proteins can be classified into five functional categories: 1) fatty acid metabolism; 2) amino acid metabolism; 3) mitochondrial respiratory chain/TCA cycle; 4) creatine kinase phospho-transfer circuit; and 5) redox balance. Increased expression of mitochondrial proteins involved in both fatty acid and amino acid metabolism could result in improved outcomes following IR (54, 72). Moreover, it is feasible that exercise-induced expression of mitochondrial respiratory chain/TCA cycle proteins

can assist cardiac myocytes in maintaining energy homeostasis during IR insults (11, 106, 108). It is also possible that exercise-induced increases in mitochondrial creatine kinase could benefit the heart during an IR insult (26). In this regard, one study suggests short-term endurance training in humans increases the activity of mitochondrial creatine kinase in the heart (123). Nonetheless, at present, there is no direct evidence demonstrating cause and effect between cardioprotection and exercise-induced increases in proteins involved in fatty acid metabolism, amino acid metabolism, respiratory chain/TCA cycle function, and/or changes in mitochondrial creatine kinase activity.

Again, ROS play a major role in IR-induced cardiac injury, and much of the IR-induced ROS production in the heart is due to superoxide production in the mitochondria (91). Most of this IR-induced increase in superoxide is dismutated enzymatically by SOD1 and SOD2 located in the mitochondrial intermembrane space and matrix, respectively (90). However, dismutation of superoxide results in H_2O_2 that is converted to H_2O and O_2 by other antioxidant enzymes to avoid oxidative damage in cells (90). Importantly, studies report that exercise increases the protein abundance of several mitochondrial antioxidant enzymes including SOD1 and SOD2, along with the H_2O_2 removing enzymes glutathione peroxidase-1 and catalase (reviewed in Ref. 92). Hence, this exercise-induced fortification of these antioxidant enzymes has the potential to increase the removal of ROS produced in cardiac mitochondria.

Furthermore, exercise training decreases the expression of the mitochondrial enzyme monoamine oxidase A (MAO-A) in both SS and IMF mitochondria in the rat heart (57). This observation is important because MAO-A catalyzes the oxidative deamination of several monoamines, resulting in increased ROS production (85). Importantly, ROS production by MAO-A is a contributor to myocardial apoptosis during postischemia reperfusion (7, 85). Therefore, decreased mitochondrial levels of MAO-A in exercised hearts could be a beneficial adaptation that contributes to cardioprotection. Nonetheless, additional experiments are required to provide direct evidence for this postulate.

To summarize, endurance exercise training benefits the mitochondrion through increased expression of beneficial antioxidant proteins and decreased expression of proteins with potentially deleterious functions. Collectively, these changes improve mitochondrial capacity to produce ATP, eliminate ROS, and maintain a healthy mitochondrial redox balance. By extension, these exercise-induced changes result in a mitochondrial phenotype that is resistant to IR-induced damage. Direct

evidence to support this prediction is highlighted in the next section.

Experimental evidence linking mitochondrial proteins to cardioprotection. Again, endurance exercise training alters the expression of numerous mitochondrial genes, resulting in a resilient mitochondrial phenotype that is less sensitive to apoptotic stimuli and more resistant to IR-induced mitochondrial damage. The key question becomes: Which of these differentially expressed mitochondrial proteins are required contributors to exercise-induced cardioprotection? Although a complete answer to this question is not currently available, experimental evidence reveals that increased expression of mitochondrial SOD2 plays a major role in exercise-induced cardioprotection. For example, transgenic overexpression of SOD2 alone is sufficient to provide protection against IR-induced cardiac injury in mice (17). Furthermore, two independent studies have shown that exercise-induced expression of SOD2 in the heart plays a vital role in exercise-induced protection against IR-mediated myocardial infarction (118). Explicitly, using antisense oligonucleotides to prevent exercise-induced increased SOD2 expression in the heart, Yamashita et al. first demonstrated that preventing the exercise-induced increase in SOD2 expression abolished the cardioprotection normally associated with exercise (118). These findings were confirmed in a study demonstrating that prevention of exercise-induced increases in SOD2 greatly diminished the exercise-induced protection against IR-induced cardiac cell death due to both necrosis and apoptosis (31). Furthermore, although it is established that the primary regulation of SOD2 is through gene expression, deacetylation of SOD2 via Sirt3 increases SOD2 activity (16). The finding that exercise increases the protein levels of Sirt3 in the heart suggests that increases in Sirt3 may also play a role in exercise-induced cardioprotection via regulation of SOD2 activity (83).

After confirming that exercise-induced increases in myocardial SOD2 are important for exercise-induced cardioprotection, French et al. then investigated the downstream mechanisms by which increased myocardial SOD2 activity contributes to cardioprotection (31). This work reveals that exercise-induced increases in mitochondrial SOD2 protects against IR-induced myocardial infarction in part by attenuating IR-induced oxidative damage of calcium handling proteins and prevention of calpain activation (31). Indeed, an IR insult results in calpain activation in the cardiac myocyte, which promotes the degradation of four vital calcium handling proteins, including the sarcoplasmic reticulum calcium ATPase (SERCA2a), phospholamban, L-type calcium channels, and Na⁺/Ca²⁺ exchanger. Exercise training, performed before the

IR insult, successfully protected the heart against IR-induced calpain activation and breakdown of these calcium handling proteins (32). Importantly, exercise-induced protection was lost when the exercise-stimulated increase in cardiac SOD2 was blocked using antisense oligonucleotides. Together, these studies demonstrate that an exercise-induced increase in SOD2 plays a key role in protection against IR-induced cardiac injury.

In synopsis, exercise training promotes the differential expression of numerous mitochondrial proteins, resulting in a mitochondrial phenotype that resists IR-induced injury (FIGURE 5). Present evidence suggests that an exercise-induced increase in SOD2 in cardiac mitochondria is essential to achieve the full benefit of exercise-induced cardioprotection. Furthermore, other exercise-induced mitochondrial proteins may also play a role in cardioprotection, but additional research is required to demonstrate cause and effect.

Conclusions and New Frontiers

Myocardial IR injury is a major cause of morbidity and mortality around the world, and, therefore, protecting the heart against IR injury is important. Currently, the only pragmatic method of providing sustainable cardioprotection against IR-induced myocardial injury is endurance exercise training,

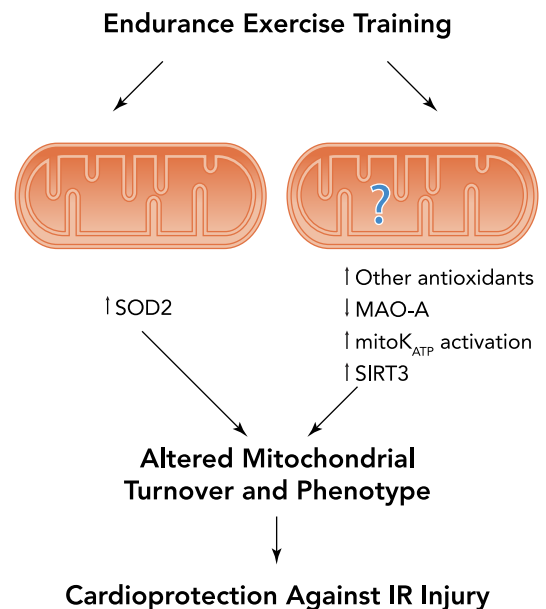


FIGURE 5. Illustration of several exercise-induced mitochondrial alterations that promote cardioprotection against IR injury Exercise increases mitochondrial levels of the important antioxidant enzyme superoxide dismutase 2 (SOD2). Exercise training could also increase the expression of mitochondrial ATP-sensitive potassium channels along with other mitochondrial proteins that could contribute to cardioprotection. MAO-A, monoamine oxidase; SIRT3, sirtuin 3; MitoK_{ATP}, mitochondrial potassium ATP-sensitive channel.

and regular participation in endurance exercise protects the heart against all levels of IR-induced injury.

The precise cellular adaptations responsible for exercise-induced cardioprotection remain a topic of debate. Nonetheless, there is evolving evidence that exercise training promotes alterations in mitochondrial protein expression and a change in mitochondrial phenotype that resists IR-induced damage. Furthermore, convincing evidence indicates that elevated myocardial SOD2 plays a major role in exercise-induced cardioprotection by protecting mitochondria and the cardiac myocyte against IR-induced oxidative damage. Moreover, it is feasible that other cardioprotective proteins exist in mitochondria that contribute to exercise training-induced cardioprotection. Moreover, there is reason to believe that both physiological and biochemical mechanisms exert synergistic protection in the exercised heart. Relative to the anti-arrhythmic protection afforded by elevated SOD and MitoKATP content of exercised ventricles, there is motive to suspect that autonomic changes associated with exercise may also contribute to the observed cardioprotection. This matter of synergistic protection against IR injury could help to explain clinical observations that exercise appears to be more protective than can be accounted for statistically using traditional risk factor analyses (52).

Although much has been learned about exercise-induced cardioprotection, many unanswered questions remain. For example, the role that posttranslational modifications play in exercise-induced cardioprotection against IR-injury is unknown. Indeed, posttranslational modifications to proteins such as phosphorylation, methylation, and acetylation are essential regulators of cell signaling pathways, and their contributions to cardioprotection are poorly understood (87).

Finally, another unanswered question regarding the mechanisms of exercise-induced cardioprotection relates to the impact of exercise training on mitochondrial turnover in cardiac myocytes. This type of mitochondrial quality control depends on the dynamic balance between mitophagy and mitochondrial biogenesis along with fission and fusion processes (37, 119). Theoretically, increased mitophagy of damaged mitochondria that produce high levels of ROS would benefit the exercised heart by leaving behind only the healthiest mitochondria. Therefore, these remaining mitochondria should have a higher threshold for opening of the mitochondrial permeability transition pore (37). This raises the question of "What role does mitochondrial quality in the cardiac myocyte play in cardioprotection?" Clearly, there is much more to be learned about the mechanisms responsible

for exercise-induced cardioprotection against IR injury. ■

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