



## Review

# Exercise-induced cardioprotection: From endogenous to exogenous mechanisms

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## ABSTRACT

**Background:** Acute myocardial infarction (AMI) remains the leading form of cardiovascular morbidity and mortality, while exercise is a preventative and therapeutic countermeasure. The collective benefits of exercise on the heart are called cardioprotection. Exercise-induced cardioprotection encompasses four broad areas: 1) cardiovascular disease (CVD) risk factor improvement, 2) anatomical remodeling of the heart, 3) improved cardiac physiologic function, and 4) mechanisms of exercise preconditioning.

**Discussion:** With respect to the latter area of cardioprotection, research indicates that a few days of moderate intensity aerobic exercise preconditions the heart against cardiac dysrhythmias, ventricular pump dysfunction, and tissue death. The short duration protective timeframe, hours to days after exercise, indicates that the mechanisms are biochemical in nature. Protective mechanisms within exercised hearts include endogenous antioxidant enzymes, better regulation of cytosolic  $Ca^{2+}$ , and more efficient bioenergetics. However, a formative body of work conducted over the last decade indicates that additional exogenous mechanisms may be receptor mediated, presumably providing cardioprotection via circulating factors. Preliminary findings indicate that tissue-to-tissue cross talk involves cardioprotective paracrine factors derived from muscle or autocrine factors originating from the heart itself. This protection is termed exogenous (or remote) cardiac preconditioning, and appears to include  $\delta$ -opioid receptors, IL-6 receptors, and perhaps other surface receptors on exercised cardiac tissue.

**Conclusion:** The current review outlines existing knowledge on exercise and factors of cardiac preconditioning, and highlights the avenues for next-step scientific advances to understanding treatments against AMI.

## 1. Introduction

Cardiovascular diseases (CVD) remain the leading cause of morbidity and mortality in wealthy countries, manifesting as ischemic heart diseases such as acute myocardial infarction (AMI).<sup>1</sup> Importantly, the pathological aspects of AMI have been described over the last few decades, and include phases of cardiac injury. The first phase occurs during the lack of blood flow (ischemia) to the heart. The second injury phase happens during the restoration of blood flow (reperfusion). Collectively, this injury is described as ischemia reperfusion injury (reviewed in detail elsewhere).<sup>2</sup> The persistence of AMI as a public health problem contrasts successes in pharmacologic therapies (e.g., statins, antihypertensives, adrenergic blockers, etc.), improved interventional strategies (e.g., refinement in revascularization techniques), and other medical approaches used to treat cardiovascular diseases. Collectively, this understanding highlights the medical need for additional scientific advances to

combat AMI frequency and severity. Given that AMI remains a public health problem, there is a critical need for sustainable approaches to restore cardiac health in people at risk for ischemic heart disease.

Public health data are unequivocal in supporting the notion that sedentary behavior is among the most modifiable risk factors for AMI.<sup>3</sup> Indeed, when the population is stratified for rates of physical activity, the highest rates of CVD are observed in the most inactive individuals.<sup>4</sup> In contrast, the elevation of cardiorespiratory fitness in sedentary and low-fit individuals is accompanied by dramatic reductions in all-cause and CVD morbidity and mortality.<sup>5</sup> Thus, it is now undeniable that engaging in regular exercise can serve both as a primary and secondary preventative strategy against AMI. Moreover, in persons diagnosed with AMI, exercise is a potent therapeutic rehabilitation modality for the prevention of disease recurrence and progression. To this end, the term *exercise-induced cardioprotection* includes a series of clinical and scientific observations that contribute to the prevention of infarcts, in addition to bolstering the resilience of hearts subjected to an AMI.<sup>3,6–9</sup>

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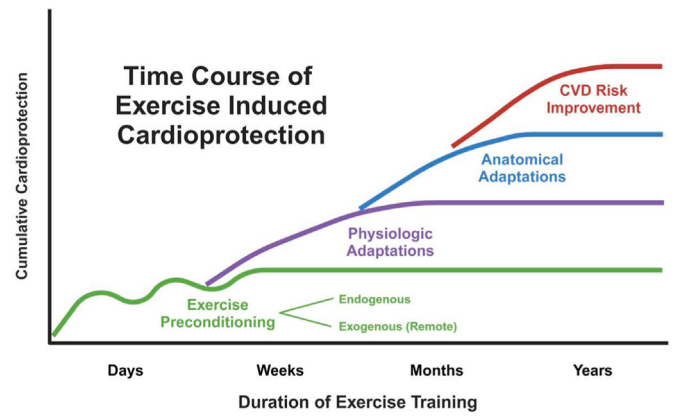
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List of abbreviations	
AMI	Acute myocardial infarction
CVD	Cardiovascular disease
ECG	Electrocardiogram
FIIT	Frequency, Intensity, Time, Type of exercise
GR	Glutathione reductase
HSP	Heat shock protein
IL-6	Interleukin-6
MitoK <sub>ATP</sub>	Mitochondrial ATP sensitive potassium channel
SarcK <sub>ATP</sub>	Sarcolemmal ATP sensitive potassium channel
SOD-2	Superoxide dismutase-2
VO <sub>2peak</sub>	Peak oxygen consumption observed during a graded exercise test

The purpose of the current review is to summarize the collective understanding of exercise-induced cardioprotection. Because much of this work is based on animal research findings, we provide an overview of the clinical relevance of the exercise and myocardial infarction models. With respect to improved outcomes in animals exposed to experimental AMI, special emphasis is focused on the endogenous mechanisms of myocardial cardioprotection against ischemia reperfusion injury. Preliminary evidence for exogenous mechanisms (also called remote cardiac preconditioning) of exercise preconditioning is also provided. Exogenous protection comes in the form of circulating factors that bind with receptors found on myocardial cells. Finally, findings from exercise studies indicate that the exercise stimulus is fundamentally different from several common non-exercise avenues of preconditioning research. The scientific and clinical implications of these differences between exercise and non-exercise approaches to cardioprotection research are discussed.

## 2. The four facets of exercise-induced cardioprotection

Exercise-induced cardioprotection is characterized by four broad facets of improved heart health: 1) beneficial modification of CVD risk factors, 2) anatomical remodeling of cardiac structures and coronary blood supply, 3) physiologic adaptations to cardiovascular control and function, and 4) exercise preconditioning. Exercise preconditioning collectively describes a series of biochemical changes which protect the heart when fortified in cardiac tissue.<sup>10</sup> The four aspects of exercise induced cardioprotection are highlighted in Fig. 1. Notably, cardioprotective facets 1–3 appear to require regular exercise training (Fig. 2), adhering to physical activity guidelines put forth by the Office of Disease Prevention and Health Promotion, and training recommendations provided by the American College of Sports Medicine.<sup>11,12</sup> In the average adult, for example, exercise

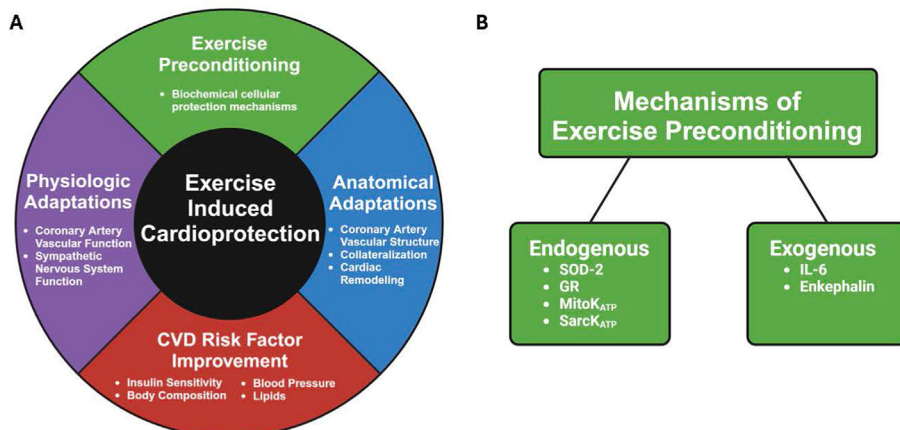


**Fig. 2. The time course of exercise induced cardioprotection.** Exercise induced cardioprotection includes 1) cardiovascular disease (CVD) risk factor improvement (e.g., sustained effect on resting blood pressure, waist/hip ratios, etc), 2) Anatomic remodeling of the heart and coronary blood supply, 3) physiologic adaptations (e.g., improved stroke volume, etc.), and 4) biochemical factors of exercise preconditioning. Exercise preconditioning occurs within 1–3 days after the first bout of regimented physical activity. Exercise preconditioning, which includes endogenous and exogenous biochemical mechanisms, is the first level of cardioprotection to benefit heart health, with the other factors accumulating over weeks to years of continued exercise training.

provides consistent, even if sometimes modest, improvements in modifiable risk factors for CVD (e.g., dyslipidemia, hypertension, hyperglycemia, etc).<sup>13,14</sup> Anatomical adaptations to exercise training include the development of collateral coronary circulation and ventricular remodeling.<sup>15</sup> These anatomical changes in the exercised heart serve to mitigate morbidity and mortality in the face of an ischemic insult.<sup>16</sup> Similarly, physiologic adaptations to several months of exercise training evoke significant improvements in cardiac function, an observation that is linked to improved outcomes following AMI.<sup>17–20</sup> Collectively, these three clinical areas of cardioprotection significantly improve heart health outcomes for those who participate in regular exercise. For individuals that engage in regular exercise, it generally takes weeks-to-months, if not years, for the benefits of cardioprotective exercise to accrue. Accordingly, these aspects of cardioprotective exercise reflect the positive influence of an exercise lifestyle.

## 3. Exercise preconditioning: the clinical relevance of animal models of myocardial infarction and exercise

In contrast to the lifestyle-based heart benefits described above, cardiac preconditioning can occur after just a few days (e.g., 3–5 consecutive days) of moderate-intensity aerobic exercise sessions (Fig. 2).<sup>21,22</sup>



**Fig. 1. The four facets of exercise induced cardioprotection.** The exercised heart is resistant to acute myocardial infarction (AMI) because of four types of cardioprotection. **A)** The four facets include (1) cardiovascular disease (CVD) risk factor improvement, (2) physiologic adaptations, (3) anatomical adaptations, and (4) exercise preconditioning. **B)** The mechanisms of exercise preconditioning include endogenous and exogenous factors. Notably, the cardioprotective areas 1–3 are evoked by prolonged exercise training, while exercise preconditioning occurs after 1–3 bouts of exercise.

Because the clinical incidence of myocardial infarction can be nearly impossible to predict, even in persons with numerous CVD risk factors, research studies of exercise and cardiac preconditioning are usually performed using laboratory rat and mouse models. Fortunately, it is possible to prescribe exercise to rats and mice (e.g., treadmill running, swimming, etc.). Because the exercise is sustained (e.g., 30–60 min of continuous exercise), it mimics human exercise prescriptions in important ways. Even though some animals are more anatomically akin to human forms of cardiovascular disease (e.g., pigs), rodent models have been the most commonly used for exercise and cardiac preconditioning research.

Accordingly, scientists using rodent models of exercise and myocardial infarction have demonstrated that in previously sedentary animals, 3–5 days of moderate-intensity aerobic exercise will elicit consistent cardiac preconditioning against an experimental MI.<sup>21,22</sup> In fact, an early study of exercise preconditioning found that the protected phenotype could be induced within 30 min of the first bout of exercise.<sup>23</sup> As such, in these types of experiments, “sedentary” (e.g., previously untrained) animals receive a 1–3 day exercise regimen (outlined subsequently) followed by an experimental MI on the day immediately following the last bout of exercise (e.g., day 4 following a 3-day exercise program).

Given this almost immediate time frame for the induction of cardioprotection after the onset of exercise, the protected phenotype must be due to biochemical mediators. The animal models of myocardial infarction generally utilize one of two experimental techniques: 1) the surgical ligation of the left anterior descending coronary artery in anesthetized animals, or 2) through isolated perfusion heart models. In the latter model, isolated hearts can be subjected to coronary artery ligation or be deprived of oxygen by shutting off buffer perfusion to the entire heart. Studies that use either the surgical approach or the isolated perfused heart technique typically conclude with the quantification of biochemical and histological outcome measures. Many of these studies also include clinically relevant variables such as ECG ventricular abnormalities,<sup>24–27</sup> myocardial pump dysfunction,<sup>19–21,28–31</sup> and circulating biomarkers for cardiac tissue death.<sup>9,32</sup>

Rodent (rat and mouse) exercise models also warrant discussion for their relevance to clinical applications in humans. The experimental models effectively scale the animal exercise to an approximation of the human exercise prescription, including considerations for Frequency, Intensity, Time, and Type (FITT) to the respective animal model. First, the exercise regimen is usually concluded within 1 week, most often employing between 3 and 5 exercise sessions, inferring that if the program were continued, the animals would engage in the recommended 3+ days/week in a standard exercise prescription. Next, the exercise modalities include repetitive activities involving large muscle groups, such as treadmill running and swimming.<sup>33</sup> In addition, the exercise intensity is typically set between 50% and 75% of the peak aerobic capacity of the animal species being examined.<sup>32,34,35</sup> The exercise duration typically varies between 30 and 60 min,<sup>23,24</sup> matching human recommendations.<sup>12</sup> Finally, while the forced exercise aspect of rodent exercise (e.g., a rodent treadmill with an electrified shocker, etc.) could be viewed with scientific skepticism, when animals are provided with free access to a running wheel, they accumulate daily distance totals that are comparable to the regimented exercise imposed on them in exercise preconditioning studies.<sup>25,36</sup> By extension, free-wheel physical activity has been observed to induce an ischemic-resistant phenotype.<sup>37</sup> In total, the animal models used to evoke an exercise preconditioned phenotype are generally believed to be sufficiently comparable to human physical activity and exercise recommendations intended to improve cardiovascular health.<sup>3,6,33</sup>

#### 4. Exercise preconditioning: sex, age, and dose considerations

With respect to the potential clinical applications of this research line, there are several aspects of exercise preconditioning phenomenology that should be discussed. First, CVD incidence disproportionately impacts

middle-aged and older adults.<sup>1</sup> This clinical reality could be problematic given that nearly all exercise preconditioning studies are conducted in young adult rats and mice. Moreover, across the many rat and mouse strains used for exercise preconditioning research, the average life expectancy is quite variable, making animal-to-human comparisons somewhat tenuous. Nonetheless, multiple research groups have conducted exercise preconditioning research in aged animals. For example, two foundational studies utilized 2-year-old Fisher 344 rats, a timepoint that coincides with the average lifespan of that rat strain. In both of these studies, consistent evidence of cardiac preconditioning was observed against the experimental heart attacks,<sup>30,38</sup> indicating that short-term exercise preconditioning is possible in senescent hearts. In addition to age, it is important to highlight the unfortunate fact that most of the findings from exercise preconditioning studies have been performed in male animals. Nonetheless, several studies have been performed in both sexes, demonstrating that exercise can precondition the female heart.<sup>39–41</sup>

In addition to animal age and sex, scientists were curious about the dose of exercise needed to elicit an exercise preconditioned phenotype. Two important studies examined whether there was a dose-response relationship between the exercise performed and the amount of cardioprotection observed. The first study examined exercise preconditioning in response to exercise of various intensities relative to  $\dot{V}O_{2peak}$  (the peak oxygen consumption observed during an incremental graded exercise test). A classic study randomly assigned rats to either sedentary control, 3 consecutive days of treadmill running at 55%  $\dot{V}O_{2peak}$  (“moderate intensity”), or an identical 3 days of treadmill exercise performed at 75%  $\dot{V}O_{2peak}$  (“high intensity”). At the conclusion of the exercise regimen, hearts received global ischemia in an isolated perfused heart apparatus. Cardiac work in the post-ischemic period was quantified. Notably, animals from both the moderate intensity exercise and high intensity exercise groups exhibited similar levels of cardioprotection against the ischemic insult. That both 55%  $\dot{V}O_{2peak}$  and 75%  $\dot{V}O_{2peak}$  achieved similar levels of cardioprotection lends support to the idea that exercise preconditioning is a threshold-dependent phenomenon.<sup>32</sup> Separately, other studies have not observed cardioprotection when rats performed exercise at 55%–60% of  $\dot{V}O_{2peak}$ .<sup>42</sup> While these discordant findings between comparable study approaches cannot be resolved currently, it is worth noting that the determination of  $\dot{V}O_{2peak}$  can be inexact in rodent models, where metabolic estimates must be determined from animals exercising in large mixing chambers (e.g., as compared to human testing with mouthpieces and nose clips). This uncertainty has led some authors to suggest that exercise preconditioning is a threshold-dependent phenomenon.<sup>32</sup> The proposed threshold may occur around 55%–60% of  $\dot{V}O_{2peak}$ , where these discordant findings overlap. However, the presence of this threshold, or the exact intensity above which preconditioning is evoked, remains unknown. Moreover, whether the threshold is expressed consistently across age and sex also remains a matter of speculation.

Another compelling question raised about exercise preconditioning is the duration of protection provided by a short-term exercise regimen. If 1–3 days of exercise evokes robust cardioprotection, how long does that protection last? To answer this question, the established model of 3 consecutive days of exercise and a myocardial infarction surgery on the 4<sup>th</sup> experimental day was re-examined with a slight twist. In a landmark investigation, Lennon and colleagues assigned rats to one of 5 treatment groups, either a sedentary control treatment or 4 separate groups of identical exercise (3 consecutive days at moderate-high intensity treadmill exercise). Across the exercise groups, the ischemia-reperfusion challenge occurred at different times after the conclusion of the 3-day exercise regimen: 1 day after, 3 days after, 9 days after, or 18 days after. Measures of post-ischemia contractile dysfunction were examined in isolated hearts exposed to ischemia-reperfusion. Findings revealed that the hearts were preconditioned for 1–9 days after the last bout of treadmill exercise. However, the exercise group that received the

ischemia reperfusion injury 18 days after the final treadmill session was not protected.<sup>43</sup> This observation suggests that exercise preconditioning provides a remarkable return on investment, where the protection afforded by three consecutive days of exercise can persist for up to nine days after the last exercise bout.

##### 5. Endogenous cellular mechanisms of exercise preconditioning: protection from within

What cellular mechanisms are responsible for the exercise preconditioned phenotype? The implications of this question extend beyond exercise and have been the focus of ongoing research from the non-exercise preconditioning field. Indeed, since conducting the first observation of cardiac preconditioning using a non-exercise ischemic stimulus in 1986,<sup>44</sup> there have been numerous attempts to resolve the biochemical mechanisms responsible for infarct-resilient hearts in hopes of reverse engineering a pharmacologic solution to AMI.<sup>45,46</sup> Accordingly, in the late 1990s-early 2000s, exercise emerged as one of the important sub-fields of cardiac preconditioning research, a scientific venue through which the translatable mechanisms might be discovered.

The first step in understanding cardiac preconditioning from any stimulus (e.g., exercise, ischemia, etc.) is to search for potential mechanisms that counter the pathological mechanisms of ischemia reperfusion injury. First, it is important to note that both the induction of ischemia, and paradoxically the necessary act of coronary artery reperfusion, induce cardiac injury. From a cellular perspective, the key mechanisms of injury include acute oxidative damage (i.e., inflammation, free radical production), calcium overload in the cytosol and mitochondrial matrix, widespread protease activation, and membrane disruption (e.g., sarcolemma, cellular organelles, mitochondrial inner and outer membrane, etc.). Notably, as reviewed in detail elsewhere, all of these pathological cellular mechanisms are interrelated, and of course, undeniably linked to the bioenergetic challenges imposed by AMI.<sup>2,45–47</sup>

Importantly, the exercise preconditioned phenotype is characterized by the cellular fortification of biochemical factors that parallel the facets of ischemia-reperfusion pathology. For example, where oxidative stress is foundational to an ischemic insult, exercise increases the cellular levels and enzymatic activity of several endogenous antioxidant enzymes, including superoxide dismutase-2 (SOD-2).<sup>23,41,48</sup> SOD-2 is an important endogenous antioxidant because the enzyme is essential in a two-step biochemical reduction of superoxide anions to stable end products. To test the role of this enzyme in exercised hearts, antisense oligonucleotide technology was used to block the overexpression of SOD-2 in exercised hearts. As such, where exercise would normally increase the cellular protein content of SOD-2, under the condition of antisense oligonucleotides directed against SOD-2, the basal levels of the enzyme are maintained even after exercise. As such, without the exercise induced increase in SOD-2, the cardioprotection effect was partially lost.<sup>23,41,48</sup> Collectively, these findings demonstrate an essential role of SOD-2 for exercise-induced protection.

Furthermore, the role of SOD-2 in exercised hearts was compared to several important post-MI outcomes such as ventricular arrhythmias. To undertake these observations, ECG tracings were recorded during and after surgical MI, and the presence of ventricular arrhythmias was quantified for paraventricular contractions, ventricular tachycardia, and ventricular fibrillation using standardized techniques.<sup>49–51</sup> Findings revealed that exercised hearts that received the antisense oligonucleotides against SOD-2 experienced ventricular arrhythmias at a rate that was similar to hearts from the sedentary group, indicating that SOD-2 is essential for mitigating ECG dysrhythmias during an MI.<sup>24</sup> Additionally, in these studies, histological markers of tissue death were used to examine the role of SOD-2 against post-infarct tissue death. Findings indicated that exercised hearts that received the antisense oligonucleotides against SOD-2 lost protection against post-infarction tissue death.<sup>23</sup> Thus, exercise results in the overexpression of SOD-2 in heart muscle cells, and having fortified levels of this endogenous antioxidant is

protective against ventricular dysrhythmias and tissue death following an MI.

The neutralization of superoxide requires two biochemical steps. The first step is an enzymatic reduction of superoxide by the enzyme SOD-2. This reaction results in the production of hydrogen peroxide, which requires further reduction to unreactive end products. There are several antioxidant enzymes that can reduce hydrogen peroxide. Peroxiredoxin III, found in mitochondria, is among the possible candidate molecules. In a classic investigation, Kavazis et al.<sup>52</sup> observed that peroxiredoxin III protein expression was upregulated in hearts taken from Sprague-Dawley rats that completed a 5-day treadmill running protocol. The peroxiredoxin III overexpression was observed in the subsarcolemmal mitochondria. Furthermore, when rat hearts were exposed to ischemia reperfusion injury using an isolated perfused heart apparatus, the exercised hearts were cardioprotected. Specifically, in the post-ischemic measurements of cardiac performance, the exercised hearts had preserved levels of cardiac output, cardiac work (e.g. systolic blood pressure  $\times$  cardiac output), coronary flow, and rate pressure product (e.g., systolic blood pressure  $\times$  heart rate), while sedentary hearts exhibited significant declines in all of these performance outcomes.<sup>52</sup> The proximity of peroxiredoxin III to SOD-2, both being found in the mitochondria, suggests that these two antioxidant enzymes work in tandem to quench the reactive oxygen species responsible for oxidative stress during ischemia reperfusion injury in the heart.

Other antioxidant enzymes that may aid in quenching hydrogen peroxide are found in the cytosol of heart muscle cells. For example, cardiac levels of glutathione reductase, a key antioxidant enzyme of the cellular glutathione network, are also fortified by exercise training.<sup>53</sup> Separately, the antioxidant enzyme catalase is also capable of neutralizing hydrogen peroxide. Examination of cardiac levels of catalase, in addition to measures of catalase' enzymatic activity, have been equivocal.<sup>30,32</sup> Accordingly, the extent to which catalase and components of the glutathione system contribute to the quenching of reactive oxygen species during ischemia reperfusion remains unknown. Nonetheless, the possibility exists that these and other factors could act as redundant mechanisms of exercise preconditioning.

In addition to protection against oxidative stress, hearts from exercised animals exhibit better calcium control during and following experimental models of AMI.<sup>28,29</sup> Finally, at the conclusion of an experimental ischemia-reperfusion challenge, exercised hearts exhibit evidence of a significantly improved bioenergetic state.<sup>54</sup> While there are likely to be multiple mechanisms responsible for the improved metabolic tolerance in exercised hearts exposed to experimental ischemia-reperfusion, ATP-sensitive potassium channels ( $K_{ATP}$ ) are among the mechanisms that may contribute to the improved metabolic state in exercised hearts.

As a brief review,  $K_{ATP}$  channels are located on both the sarcolemma ( $SarcK_{ATP}$ ) and on the inner mitochondrial membrane ( $MitoK_{ATP}$ ). In the unstressed state, these ion channels are closed in the presence of ample cellular levels of ATP. As the bioenergetic state of the cell changes, however, the ion channels open as ATP levels decline. Once opened,  $K_{ATP}$  channels appear to confer protection through a number of possible ways, although the exact mechanisms remain a topic of open debate.<sup>55</sup> Nonetheless, both the  $SarcK_{ATP}$  and the  $MitoK_{ATP}$  channels appear to be involved in exercise-induced cardiac preconditioning.<sup>27,39,40,56,57</sup>

To test the potential role of  $K_{ATP}$  channels in exercised hearts, pharmacologic inhibitors to the respective  $SarcK_{ATP}$  and  $MitoK_{ATP}$  have been used. Pharmacologic inhibitors to the  $K_{ATP}$  channels are necessary, as opposed to gene knockout or other reductionist methodological approaches, because the ion channels are essential to animal viability (e.g., knockout of  $K_{ATP}$  channels results in a lethal mutation). While the use of pharmacologic inhibitors invariably comes with the potential for non-specific effects of the blockers, which could confound study outcomes, the approach remains a negotiable scientific limitation that is arguably inherent to these types of whole animal research experiments.

With respect to the evolving pathological profile of ischemia

reperfusion injury, the MitoK<sub>ATP</sub> was found essential to protection against ventricular dysrhythmias following a limited-duration infarction, while the SarcK<sub>ATP</sub> channel was not essential for protection.<sup>27</sup> When a similar study of anti-arrhythmic resilience was conducted using a longer duration infarction, however, neither the SarcK<sub>ATP</sub> nor MitoK<sub>ATP</sub> channels were found to be essential for exercise preconditioning against ventricular dysrhythmias.<sup>56</sup> This finding is important because it suggests that the protective mechanisms of exercise afford the heart with a finite duration of protection but do not eliminate the critical need for immediate reperfusion. By extension, both K<sub>ATP</sub> channels were examined as potential mediators of exercise-induced cardioprotection against tissue infarction. Findings from several studies confirm that the SarcK<sub>ATP</sub> channels are protective against post-infarction tissue death,<sup>39,40,56</sup> while the MitoK<sub>ATP</sub> channels do not appear to be essential for protection in exercised hearts.<sup>56</sup>

As described above, it appears that individual mediators may protect against specific forms of cardiac injury. Moreover, from a cellular perspective, ischemia reperfusion injury includes damage to sarcolemmal membranes, sub-cellular organelles, the mitochondria, and both functional proteins (e.g., enzymes) and structural proteins.<sup>45,58,59</sup> Accordingly, the mechanisms responsible for exercise-induced preconditioning appear to prevent or delay the damage imposed on many cellular components during an ischemia reperfusion event. Nonetheless, exercise does not fully prevent the damage imposed by ischemia and reperfusion. Rather, the activation of the mechanisms of exercise preconditioning appears to slow the time-dependent pathology of ischemia reperfusion injury. In the end, long-term survival is invariably dependent upon reperfusion. Accordingly, the multi-level protective mechanisms produced by short-term exercise training provide the ischemic-resistant myocardium the potential of additional critical life-saving minutes during life-threatening cardiac events.

## 6. Exogenous exercise preconditioning: protection from beyond

In recent years, the field of exercise and cardiac preconditioning has taken a significant step forward, with multiple research groups exploring the idea that cellular mechanisms of preconditioning may be (at least partially) controlled by circulating factors that interact with surface receptors on heart muscle cells. Dickson and colleagues were the first to work on this topic, examining the potential role of  $\delta$ -opioid receptors on preconditioning following a short-term exercise regimen (days).<sup>60</sup> Importantly, the chemical nature of the endogenous compounds examined by Dickson et al. is likely derived from the opioid sub-family called enkephalins.<sup>61</sup> Thus, these endogenous opiates are fundamentally different from the more commonly discussed endorphins, a separate opioid sub-class of analgesic compounds that are generally found to be elevated in circulation only after extended duration exercise bouts (e.g., more than 90 min of continuous exercise), and only in a portion of well-trained individuals (e.g., months – years of structured long duration exercise training).<sup>62</sup>

Dickson et al. were the first to investigate enkephalins as a possible mediator of exercise preconditioning. In their study, Dickson et al. injected Naltrexone, a non-selective opioid receptor blocker, prior to the initiation of exercise. From an experimental perspective, pre-exercise injections of the opioid blocker were used to prevent interactions between circulating opioid factors and their conjugate receptors. This approach presumably also attenuated the potential for activation of downstream cellular mediators of cardioprotection. Animals in this study were assigned to several days of treadmill exercise followed by an ischemia reperfusion procedure using an isolated perfused heart model.<sup>60</sup> The key dependent measure was post-mortem histological measures of tissue death (e.g., triphenyl tetrazolium chloride staining). Findings revealed that exercise training prevented roughly 50% of the tissue death caused by ischemia reperfusion. In exercised hearts pre-treated with the opioid blocker Naltrexone, however, elevated levels of cardiac tissue necrosis were statistically similar to sedentary control

animals that had not received cardioprotective exercise. This important finding suggested that exercise-induced release of enkephalin compounds into circulation appears to confer a cardioprotective phenotype that is resistant to an AMI-like challenge. As a secondary aim of the study, unstressed hearts from exercised animals were also examined. Animals were sacrificed, and unstressed hearts were probed for mRNA expression of opioid precursors and opioid receptors. Findings indicated that in the hours following an acute bout of exercise, proenkephalin compounds and  $\delta$ -opioid receptor expression were increased in exercised hearts.<sup>60</sup> Collectively, these foundational experiments demonstrated for the first time that exercise results in the release of cardioprotective enkephalin into circulation. Furthermore, the preconditioned phenotype is dependent upon circulating enkephalin binding with opioid receptors on the heart. Unknown, however, was whether the circulating proenkephalin was derived from the heart and/or skeletal muscle. In addition, it was uncertain whether the  $\delta$ -opioid receptor (versus other opioid receptor subtypes) was specifically responsible for the observed preconditioning against ischemic insults.

In response, Miller et al.<sup>63</sup> conducted a similar short-term training experiment designed to confirm the potential role of the  $\delta$ -opioid receptor as an agent for exercise preconditioning of the heart. Moreover, they also examined both heart and skeletal muscle for increases in proenkephalin gene expression following exercise. Lastly, their study utilized pre-exercise infusions of Naltrindole, a selective pharmacologic agent used to block  $\delta$ -opioid receptors. Separately, the model of ischemia-reperfusion involved surgical ligation of the left anterior descending artery in anesthetized and ventilated animals.<sup>63</sup> This approach was viewed as complementary to the isolated perfused heart experiments described previously. For example, the *in vivo* approach provided additional clinical translatability because of the contribution of blood factors, an intact immune system, and the ability to quantify tissue infarct relative to the ischemic area of the heart (versus the total heart cross-section). Findings from Miller et al.<sup>63</sup> indicated that in unstressed rat hearts, the levels of proenkephalin gene transcripts were elevated, while skeletal muscle gene transcript levels of proenkephalin were not elevated. This finding suggests that the source of circulating enkephalin comes from the heart and acts to precondition in a paracrine/autocrine fashion.<sup>63</sup>

Interestingly, in the study by Miller et al., cardiac gene expression for the  $\delta$ -opioid receptor was not elevated by the exercise intervention. This finding suggests that any potential role of  $\delta$ -opioid receptors on preconditioning would be linked to constitutive levels of the receptors in exercise hearts. Accordingly, in animals exposed to the surgical ischemia-reperfusion insult, exercise prevented approximately 60% of the tissue death, while pharmacologic blockade of the  $\delta$ -opioid receptor eliminated about ½ of that protection. Moreover, post-mortem tissue analyses included histological measures of apoptosis.<sup>63</sup> While numerical trends followed the protective relationships observed with the tissue necrosis measure, findings were not statistically significant, indicating that in exercised hearts, the  $\delta$ -opioid receptor does prevent post-infarct necrosis but does not appear to be responsible for the anti-apoptotic protection observed previously.<sup>25,30,47,63</sup> In total, these findings provide mounting evidence that exercise-induced cardiac preconditioning includes proenkephalin circulating factors that interact with  $\delta$ -opioid receptors on the surface of myocardial tissue. Moreover, secondary level experimental data from both the Dickson et al. and the Miller et al. studies suggest that the source of the proenkephalin appears to be derived from the heart,<sup>60,63</sup> presumably acting in either paracrine or autocrine fashion. However, this preliminary conclusion requires scientific confirmation.

Another compelling question regarding cardiac preconditioning is whether any circulating factors of cardioprotection originate from exercised skeletal muscle. That is, do circulating factors derived from contracting skeletal muscle, termed *myokines*, induce cytoprotection in other tissues, including the heart? To answer this question, a growing body of research has examined numerous tissue-to-tissue interactions. While many candidate factors exist, interleukin-6 (IL-6) is among those that

have been most studied to date. Moreover, while elevated circulating levels of IL-6 are sometimes linked to pathological inflammatory responses (e.g., elevations concomitant to a spike in circulating TNF- $\alpha$ ), skeletal muscle-derived increases in this compound are generally found to evoke beneficial adaptations.<sup>64</sup>

To this end, McGinnis et al. conducted a short-term exercise study in mice to examine the potential role of IL-6 as a possible mediator of exogenous cardiac preconditioning. The exercise protocol was undertaken in C57BL6 mice and corresponding IL-6 knockout mice (e.g. genetically altered so that they do not produce IL-6). In unstressed (no ischemia) C57BL6 mice, they confirmed that an acute bout of exercise produced a transient spike in circulating IL-6, with values peaking 30 min after exercise and returning to baseline levels by 60 min in the post-exercise recovery. Interestingly, soluble IL-6 receptors were observed to be elevated in the post-exercise circulation. Moreover, after acute exercise, IL-6 receptors were elevated in both skeletal muscle and cardiac tissue. Notably, in subsets of animals exposed to the surgical ischemia reperfusion challenge, exercise was protective against ventricular dysrhythmia on ECG. Meanwhile, exercised IL-6 knockout animals were not protected against ventricular dysrhythmias. This conclusion was based on ECG tracings that were examined for ventricular ectopy using the aforementioned scoring system.<sup>49–51</sup> In this regard, the post-infarct ECG scores from exercised IL-6 knockout mice were statistically comparable to the post-infarction scores from sedentary animals. Similarly, post-infarction tissue staining indicated that short-term exercise prevented ~60% of the tissue death as compared to sedentary control mice. Exercised IL-6 knockout mice, however, had myocardial infarction levels that were similar to the sedentary control group, indicating that IL-6 also protects against post-infarction tissue death.<sup>25</sup>

Thus, it appears that exogenous exercise preconditioning involves at least two types of circulating factors and their conjugate receptors. Specifically, enkephalin interacts with the  $\delta$ -opioid receptor to promote exercise preconditioning, while IL-6 interacts with its corresponding soluble and/or cardiac tissue receptors (Fig. 3).<sup>25,60,63</sup> To date, however, there is no existing evidence to link cardiac tissue receptors to the known intracellular mediators of exercise preconditioning. One scientific limitation to this understanding is that standard practice in exercise preconditioning research frequently relies on end-point experimental models. This approach is limiting because the verification of cell signaling pathways in intact animal studies typically involves numerous time points to track the chemical cascade process. Nonetheless, secondary experiments have been undertaken to explore the potential role of

intermediary signaling molecules and pathways. In the Miller et al. study, for example, blood and tissue were examined for calcitonin gene-related peptide,<sup>63</sup> an intermediary molecule thought to be related to opioid receptor activation.<sup>65</sup> Findings, from Miller et al.,<sup>63</sup> however, found no associative relationships between calcitonin gene related peptide and other study outcomes.<sup>63</sup> Similarly, McGinnis et al. performed secondary experiments in tissue and blood samples to examine possible cell signaling pathways linked to IL-6-mediated preconditioning. Specifically, tissues were examined for alterations in STAT3 signaling, a pathway sometimes linked to non-exercise preconditioning.<sup>66</sup> Findings from McGinnis et al. did not indicate STAT3 involvement, but preliminary evidence suggested that MAPK signaling was consistently elevated in the exercise preconditioned hearts.<sup>25</sup> While these findings are compelling, they are not conclusive and require extensive follow-up studies to confirm or refute.

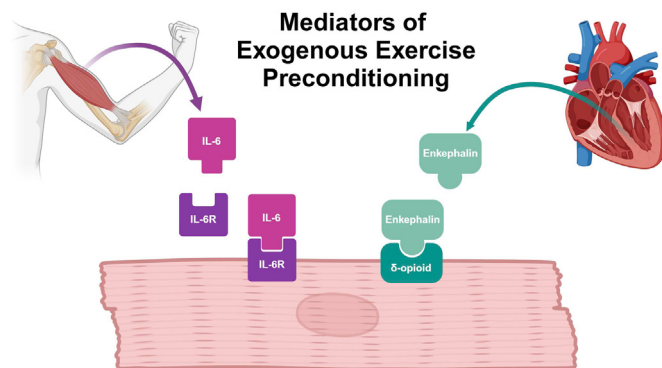
Finally, it is worth noting that the scientific gaps between receptor-based exercise preconditioning and known cellular mediators of protection may be related to the faceted nature of exercise preconditioning. That is, exercise evokes numerous complementary cellular and extracellular adaptive responses against a host of chronic diseases, including AMI. As reviewed elsewhere, the adaptive response to exercise includes intracellular and myokine signals.<sup>67</sup> In many instances, these responses are irreducible due to the redundant nature of many protective cellular mechanisms linked to exercise training. Moreover, the sustainable nature of the exercise stimulus contrasts many non-exercise approaches to stimulate cytoprotection (e.g., non-lethal ischemia, pharmacologic preconditioning, etc.).<sup>45,68</sup> These interesting contrasts between exercise and non-exercise stimuli are discussed further in the next section of this review.

## 7. Exercise preconditioning, a unique cardioprotective stimulus

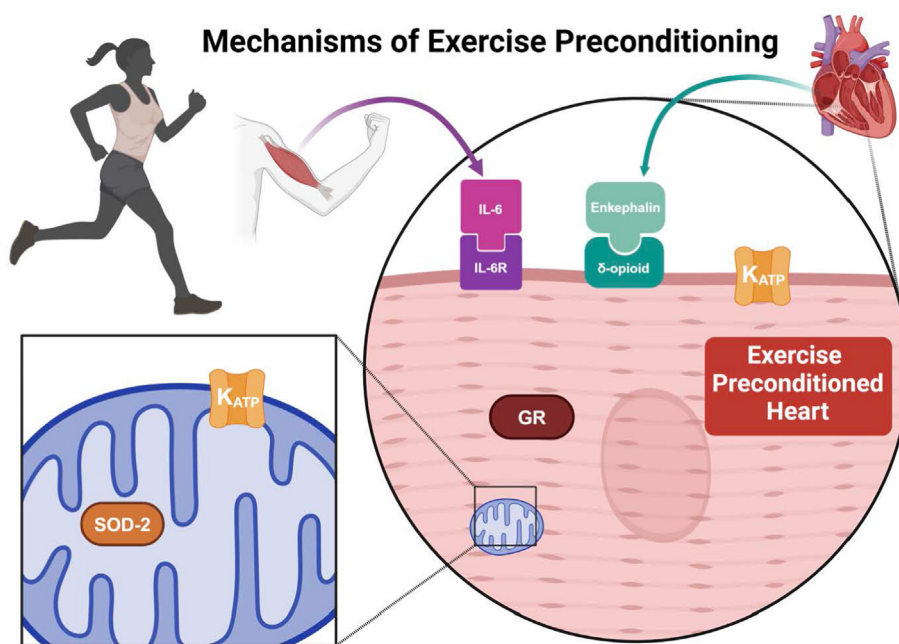
In summary, exercise appears to cardioprotect against ischemia-reperfusion injury via multiple exogenous preconditioning factors, including proenkephalin and IL-6.<sup>25,60,61,63</sup> How these factors are linked to cellular mechanisms of cardiac preconditioning is currently unknown. Moreover, it seems plausible that these, and possibly other yet-to-be identified circulating factors, may mediate cardioprotection via unidentified cellular factors. While exercise and cardiac preconditioning research has been undertaken for more than 30 years, the sub-field remains in its infancy relative to other non-exercise fields of cardiac preconditioning.

The value of exercise as a comparative field, however, should not be undervalued. For example, the preconditioning stimulus of exercise is sustainable, cost-effective, and pragmatic in addition to offering a host of other benefits. In contrast, non-exercise stimuli for cardiac preconditioning (e.g., pharmacologic activation, ischemic preconditioning, etc.) result in cellular habituation within a matter of days to weeks.<sup>46,68</sup> Indeed, the transient nature of non-exercise preconditioning approaches resulted in call to action published by preeminent physicians and scientists in the field where it was concluded: “hundreds of experimental interventions protect the ischemic myocardium in experimental animals; however, with the exception of early reperfusion, none has been translated into clinical practice.”<sup>69</sup> Given that the goal of this work is to find both robust cellular mechanisms of cardioprotection, in addition to druggable cellular targets, logic would hold that success is critically dependent upon the nature of the adaptive stimulus.

There is already a wealth of evidence to suggest that exercise adaptations are different from the non-exercise approaches to exercise. For example, the key mediators linked to ischemic preconditioning (e.g., repetitive bouts of short duration coronary vessel ligation) evoke a number of cytoprotective mediators including several heat shock proteins (e.g., HSP-32, HSP-70 family proteins, etc.), inducible nitric oxide synthase, cyclooxygenase-2, and other inflammatory mediators (reviewed by Bolli).<sup>46</sup> However, these mediators have been investigated in multiple exercise preconditioning studies and found to be either



**Fig. 3. Suspected mediators of exercise and exogenous cardiac preconditioning.** Strong evidence indicates that exercise preconditioning includes tissue-to-tissue cross talk via exogenous (remote) factors. Specifically, these circulating factors include the myokine interleukin-6 (IL-6) released from exercise skeletal muscle appears to interact with IL-6 receptors, and an endogenous  $\delta$  opioid (e.g., pro-enkephalin/enkephalin) is produced by the heart and interacts with  $\delta$ -opioid receptors in paracrine fashion. As proposed, IL-6 is acting as a myokine. How these ligand-receptor interactions interface with cellular mediators of exercise preconditioning is currently unknown.



**Fig. 4. A summary of the endogenous and exogenous mechanisms of exercise preconditioning.** Exercise preconditioning includes several endogenous mechanisms of cellular protection. Endogenous factors include the up-regulation of the antioxidant enzymes superoxide dismutase-2 (SOD-2), glutathione reductase (GR), in addition to ATP-sensitive potassium channels (KATP) located on the outer membrane of the mitochondria and sarcolemma. Exogenous (remote) factors of exercise preconditioning include interleukin-6 (IL-6) and enkephalin. To date, it is unknown how exogenous factors of exercise preconditioning may activate and/or support endogenous mechanisms of exercise preconditioning.

non-essential or completely unrelated to the protected phenotype.<sup>26,30,41</sup> Similarly, pharmacologic preconditioning with exogenous opiates is initially cardioprotective when first administered, but the phenotype reverts to an unprotected state within 9 days and, thereafter, is unresponsive to repeated doses of ischemic or pharmacologic stimuli.<sup>70,71</sup> As indicated earlier, this response is in stark contrast to the nature of exercise preconditioning,<sup>49,60</sup> which is sustainably cardioprotective when included as part of a healthy lifestyle. Moreover, whereas exercise is cardioprotective in aged hearts,<sup>30,38</sup> the non-exercise stimuli are ineffective for inducing a cardioprotective phenotype in aged hearts.<sup>72</sup> Collectively, these observations suggest that the exercise stimulus of preconditioning is fundamentally different from ischemic and pharmacologic forms of preconditioning. Indeed, the mechanisms central to ischemic preconditioning rely heavily on inflammatory pathways, where sustained exposure is maladaptive and not biologically intended for supporting long-term survival.<sup>73</sup>

## 8. Conclusion

In total, exercise provides the heart with a faceted and comprehensive amount of cardioprotection. Classically, the cardioprotection provided by exercise includes cardiac anatomical remodeling and a mitigation of CVD risk factors. More recent studies indicate that exercise-induced cardioprotection includes biochemical factors that precondition the heart against ischemia-reperfusion injury. Exercise preconditioning appears to include preservation of the bioenergetic state during and following ischemia-reperfusion injury. In addition, cellular calcium control mechanisms appear to be better preserved in exercised hearts exposed to ischemia reperfusion. As presented in Fig. 4, Exercise preconditioning also includes the fortification of at least two endogenous antioxidant enzymes, SOD-2 and glutathione reductase. In addition to these intracellular factors, more recent evidence suggests that circulating factors derived from the heart and skeletal muscle likely contribute to the cardiac preconditioning of exercised hearts. Preliminary evidence suggests that IL-6, presumably released from exercised skeletal muscle, may precondition the heart by interacting with IL-6 receptors expressed on cardiac tissue. Moreover, proenkephalin produced by exercised hearts and its corresponding  $\delta$ -opioid receptors may also contribute to exercise preconditioning.

Future research efforts are needed to better understand how circulating factors promote cardioprotection. For example, additional work is needed to fully understand how IL-6 and proenkephalin mediate cardioprotection in exercised hearts. Central to this future understanding will be the discovery of the cell-signaling events that link receptor binding to mediators of cardiac preconditioning. Given the number of exerkine and myokine factors that have been identified in recent years, it seems plausible that currently unknown circulating factors of exercise preconditioning may exist. Finally, novel experimental designs should be used to clarify the extent to which rodent-based exercise preconditioning studies are clinically relevant to humans suffering from AMI.

## CRediT authorship contribution statement

**John C. Quindry:** Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **Ronald E. Michalak:** Writing – review & editing, Writing – original draft.

## Declaration of competing interest

John C. Quindry is an editorial board member for *Sports Medicine and Health Science* and was not in the editorial review or the decision to publish this article. Otherwise the authors have no other conflicts of interest to report.

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