Mechanisms Involved in Exercise-Induced Cardioprotection: A Systematic Review

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Abstract

Background: Acute myocardial infarction is the leading cause of morbidity and mortality worldwide. Furthermore, research has shown that exercise, in addition to reducing cardiovascular risk factors, can also protect the heart against injury due to ischemia and reperfusion through a direct effect on the myocardium. However, the specific mechanism involved in exercise-induced cardiac preconditioning is still under debate.

Objective: To perform a systematic review of the studies that have addressed the mechanisms by which aerobic exercise promotes direct cardioprotection against ischemia and reperfusion injury.

Methods: A search was conducted using MEDLINE, Literatura Latino-Americana e do Caribe de Informação em Ciências da Saúde, and Scientific Electronic Library Online databases. Data were extracted in a standardized manner by two independent researchers, who were responsible for assessing the methodological quality of the studies.

Results: The search retrieved 78 studies; after evaluating the abstracts, 30 studies were excluded. The manuscripts of the remaining 48 studies were completely read and, of these, 20 were excluded. Finally, 28 studies were included in this systematic review.

Conclusion: On the basis of the selected studies, the following are potentially involved in the cardioprotective response to exercise: increased heat shock protein production, nitric oxide pathway involvement, increased cardiac antioxidant capacity, improvement in ATP-dependent potassium channel function, and opioid system activation. Despite all the previous investigations, further research is still necessary to obtain more consistent conclusions. (Arq Bras Cardiol. 2015; 105(1):71-81)

Keywords: Myocardial Infarction; Exercise/physiology; Myocardial Reperfusion Injury/etiology; Myocardial Ischemia; Heat-Shock Proteins.

Introduction

Despite numerous therapeutic advances during the past decade, coronary artery disease (CAD) is still a leading cause of mortality worldwide[1]. In Brazil, according to the data from the Brazilian Ministry of Health, cardiovascular diseases account for approximately 20% of all deaths in individuals over 30 years of age, which corresponds to an average of 195,000 deaths per year[1]. With regard to public spending on health, approximately R$ 250 million were spent on the treatment of acute myocardial infarction (MI) alone in 2011[2].

In MI, the ultimate therapeutic goal is to restore blood flow to the ischemic region as quickly as possible[3] because the reversibility of the process and extent of tissue damage are directly related to the duration of ischemia. Nonetheless, the biochemical changes caused by the reperfusion process can result in numerous damages, including cell death[3,4]. Due to the severity of the clinical outcomes of MI, it is necessary to investigate preventive measures for and/or treatment of the injury. In this sense, epidemiological evidence indicates a strong correlation between physically active individuals and MI survival patients[5]. Several studies have demonstrated that exercise not only reduces cardiovascular risk factors but also promotes cardioprotection against ischemia and reperfusion (IR) injury through a direct effect on the myocardium[6-9]. Notably, a single exercise session prior to IR injury is sufficient to promote an increase in cardiac output[10] and improve myocardial contractile function during IR injury[11-15]. This experimental evidence supports the idea that the cardiac phenotype acquired after exercise is due to specific biochemical adaptations of the myocardium, which are independent of muscle disorders and blood flow[6,7,16,17]. However, the specific mechanism involved in exercise-induced cardiac preconditioning is still under debate[7,8,18]. Therefore, this study aimed to review the pathophysiology of myocardial IR injury and assess the mechanisms by which exercise promotes direct cardioprotection against IR injury.

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Methods

Bibliographical search

The electronic databases MEDLINE (accessed via PubMed), Literatura Latino-Americana e do Caribe de Informação em Ciências da Saúde (LILACS), and Scientific Electronic Library Online (SciELO) were searched without date restriction. Studies published in English, Portuguese, or Spanish whose primary target of the investigation was aerobic exercise were included. Data were extracted in a standardized manner by two independent researchers, who were responsible for assessing the methodological quality of the studies. Articles in duplicate, reviews articles, editorials, and articles evaluating the influence of gender and/or age in cardioprotection were excluded. The literature search was conducted in September, 2014, and the search strategy included the following terms: "cardioprotection"; "ischemia reperfusion injury" or "ischemia reperfusion"; and "exercise," "physical exercise," "exercise training," or "exercise preconditioning".

Article selection was conducted in two stages. First, the abstracts were evaluated, and those that did not fulfill the inclusion criteria of the study were excluded. Second, the remaining articles were completely read and those that did not fulfill the inclusion criteria were excluded.

Results

The bibliographical search retrieved 78 studies, of which 30 were excluded after evaluation of the abstracts: 23 were review articles, five investigated the effect of age and/or gender in exercise-induced cardioprotection, one was an editorial, and one addressed the cardioprotective effect of strength exercise. Of the 48 remaining articles, 20 were excluded: 12 for not incorporating exercise as a primary research target and eight because the complete article was not available. Therefore, a total of 28 studies were included in this systematic review.

For a better presentation of the results, the selected articles were methodologically classified, as shown in Table 1, by grouping these studies according to the main cardioprotective mechanism evaluated. Therefore, of the 28 studies selected, 11 investigated the myocardial antioxidant capacity, seven focused on cardiac heat shock proteins (HSPs), four addressed the influence of the nitric oxide (NO) pathway, three studied the increase in ATP-dependent potassium channels (KATP), and three evaluated the involvement of the opioid system in exercise-induced cardioprotection.

Notably, when using the aforementioned descriptors in the main scientific search portals, none of the 78 originally retrieved studies were clinical studies, i.e., all intervention studies were performed in animals. Furthermore, in all studies, the outcomes were analyzed after IR injury.

Discussion

Pathophysiology of ischemia-reperfusion myocardial injury

In recent decades, several efforts have been made to elucidate the pathogenesis of IR injury. The ischemia-related events are highly complex, characterized by numerous rapid metabolic and biochemical changes in the myocardium that result in myocardial damage[39,40] (Figure 1). During ischemia, oxygen supply to the mitochondria is blocked, interrupting the Krebs cycle and leaving almost no energy available from oxidative phosphorylation. Therefore, to fulfill the myocardial energy demand, cellular ATP is generated via glycolysis[41]. However, this change in cellular metabolism is accompanied by an increase in cytosolic lactate levels and a reduction in intracellular pH. This occurs in such a way that, 30 min after ischemia, the hydrogen ion (H^+) concentration in the cytosol markedly increases and the cellular pH may reach up to 5.5–6.0[42]. To compensate for the low pH, intracellular water accumulates, thus causing cellular edema[3]. Taken together, these changes activate an ATP-independent membrane ion transporter, known as the Na^+/H^+ exchanger, which regulates intracellular pH and volume while promoting an efflux of H^+ and an influx of sodium ions (Na^+) into the cell. Owing to the increase in cytosol Na^+ concentration, the reverse activation of the Na^+/calcium ion (Ca^{2+}) exchanger occurs, resulting in the efflux of Na^+ in exchange for the influx of Ca^{2+} into the cell[4,41].

In parallel, decreased ATP levels disrupt the activity of active pumps that are important in homeostasis, such as Na^+/K^+ ATPase and SERCA. The function of Na^+/K^+ ATPase is to remove Na^+ and to allow the entry of K^+ to maintain the resting electric potential of the cell, whereas SERCA is responsible for "recapturing" the Ca^{2+} released in the cytosol back into the sarcoplasmic reticulum after muscle contraction. Therefore, the inactivity of these pumps results in additional Na^+ and Ca^{2+} overload, which prevents cell repolarization and culminates in contractile dysfunction[41]. Furthermore, high levels of Ca^{2+} in the cytosol activate certain enzymes, such as phospholipases, proteases (particularly calpain), endonucleases, and ATPases, which are associated with lipid peroxidation, reactive oxygen species (ROS) production, contractile protein impairment, cell function loss, and ultimately, cell death[4,8].

During reperfusion, the damage caused by ischemia is exacerbated because the restoration of the oxygen flow promotes the release of ROS in the mitochondria[8]. The production of ROS and Ca^{2+} overload are the main factors contributing to IR-induced cell damage because they culminate in mitochondrial permeability transition pore (MPTP) opening[4]. The opening of this pore results in the loss of the action potential in the mitochondrial membrane and uncoupling of oxidative phosphorylation, triggering ATP depletion and cell death. Moreover, MPTP opening acts as a chemical attractor for neutrophils, resulting in dysfunctions in the sarcoplasmic reticulum (in the ryanodine receptors) associated with the increase and/or maintenance of the calcium overload and exacerbation of the deleterious effects caused by calcium-activated enzymes[4,8]. The acidic condition ($pH < 7.0$), characteristic of ischemia, prevents MPTP opening and cardiomyocyte hypercontraction. However, during reperfusion, the lactate washout and restoration of physiological pH results in MPTP opening. Although required for the reversal of ischemia, the restoration of blood flow can ultimately be more harmful than the ischemia process itself[18].

In light of this major clinical problem, the development of therapeutic approaches aimed at the treatment of IR injury

Table 1 - Methodological classification of the selected studies

Author/year	Exercise	Groups	Main outcomes	Conclusion
Nicholson et al. [19]/2013	Running wheel 4 weeks	Exe: exercise NO_2: supplemented nitrite Exe + NO_2 Sed: sedentary	Infarcted area and troponin-1: Sed > Exe = Exe + NO_2 > NO_2 Ejection fraction: Sed < Exe = Exe + NO_2 < NO_2 Myoglobin and NFAT: Sed = NO_2 > Exe = Exe + NO_2	Exercise decreases cardiac myoglobin levels by inhibiting the calcineurin-NFAT pathway. Moderate exercise-induced cardioprotection is due to diminished ability to reduce nitrite to NO.
Akita et al. [20]/2007	7 consecutive days 60 min/session 60%–70% VO_{2max}	Exe: exercise Wild: control Phenol: sympathetic ablation Antioxidant supplemented $eNOS^{(-/-)}$: eNOS knockout 1400W: iNOS blockade $iNOS^{(-/-)}$: iNOS knockout	Infarcted area: Exe < other groups eNOS and iNOS activity: Exe > other groups Oxidative stress: > in Exe and $eNOS^{(-/-)}$	Exercise stimulates cardiac sympathetic innervation, causing eNOS activation with consequent iNOS elevation, which acts as a mediator in late cardioprotection.
Hajnal et al. [11]/2005	1 session 21 min Remaining not reported	CT: control L-NAME CT: eNOS blockade AEST CT: iNOS blockade Exe: exercise Exe + L-NAME Exe + AEST	Arrhythmias: less frequent only in Exe Infarcted area: similar among all groups	NO acts as a catalyst and mediator in exercise-induced late protection against IR injury.
Babai et al. [12]/2012	1 session 21 min	CT: control without exercise CT + Phe: phenylephrine CT + AG: iNOS blockade Exe 24 h: 24 h after exercise Exe 48 h: 48 h after exercise Exe 24 h + AG: iNOS blockade 24 h after exercise	Baroreflex sensitivity: Exe 24 h and 48 h > other groups Survival: Exe 24 h: 70% and CT: 9%	Exercise-induced cardioprotection is mediated by NO because this effect is abolished by aminoguanidine and iNOS activity increases 24 h after exercise.
Farah et al. [21]/2013	5 weeks 5 days/week 45 min/session 70% VO_{2max} 25 m/min	CT: control Exe: exercise Exe + L-NAME: eNOS blockade Exe + L-NIO: more specific eNOS blockade Exe + BH_4: NO donor	Nitrite and GMPc levels: similar between groups eNOS function: Exe and BH_4 > other groups Infarcted area: Exe < other groups Oxidative stress: Exe > other groups	Exercise results in increased antioxidant capacity, which prevents an excessive synthesis of NO, limiting its binding to O_2 and the consequent formation of peroxynitrite (highly cytotoxic).
Frasier et al. [22]/2013	10 consecutive days 60 min/session 15' at 15 m/min, 30' at 30 m/min, and 15' at 15 m/min	Sed: sedentary Exe: exercise BCNU + Exe: inhibited glutathione reductase Vas2870 + Exe: inhibited NADPH oxidase	Infarcted area and arrhythmias: Exe < other groups Antioxidant activity: Exe > other groups	Adaptive signaling of exercise-induced cardioprotection is triggered by EROS, which increases glutathione reductase activity.
Lee et al. [23]/2012	5 consecutive days 60 min/session 70% VO_{2max} 30 m/min	CP: sedentary without IR CIR: sedentary with IR EP: exercise without IR EIR: exercise with IR	Proapoptotic proteins and EROS: CIR > other groups Functional parameters: CP = EP > EIR > CIR Respiratory function: EIR = CP = EP	Cardioprotection is partially mediated by beneficial adaptations in mitochondrial phenotype, increasing their resistance to the oxidative damage due to IR injury.
Kavazis et al. [24]/2008	5 consecutive days 60 min/session 30 m/min	Sed: sedentary Extr: trained	Antioxidant enzymes and mitochondrial function: ExTr > Sed Expression of proapoptotic proteins: Sed > ExTr	Exercise induces mitochondrial adaptations that contribute to cardioprotection.
French et al. [13]/2008	3 consecutive days 60 min/session 30 m/min	C: sedentary T: trained T-AS: trained with anti-MnSOD treatment T-M: trained with sham anti-MnSOD treatment	MnSOD: T and T-M > T-AS = C Catalase and GPX: similar among groups Infarcted area and apoptosis: C > T-AS > T = T-M	Exercise increases the activity of antioxidant enzymes (SOD) that promote cardioprotection by attenuating necrosis/apoptosis
Lennon et al. [16]/2004	3 consecutive days 60 min/session 30 m/min	S-C: sedentary control E-C: trained without treatment E-AS: trained with anti-MnSOD treatment E-MM: trained with sham anti-MnSOD treatment	MnSOD: E-C = E-MM > E-AS = S-C Antioxidant activity: catalase > in E groups Double product: S-C < other groups	Prevention of exercise-induced elevation of an antioxidant enzyme (MnSOD) does not abolish cardioprotection.

Continuation

Hamilton et al.[14]/2003	5 consecutive days 60 min/session 30 m/min	Untrained control (1) Untrained and antioxidant diet (2) Trained and antioxidant diet (3) Trained and antioxidant diet (4)	Infarcted area: 1 > 2 = 3 = 4 Antioxidant activity: 1 = 3 < 2 = 4 HSP72/73: 3 > 1 = 2 = 4 Intraventricular pressure: 4 > 3 > 2 > 1	Exercise and use of antioxidants can promote cardioprotection independently and the combination of these two strategies does not interfere in the response.
Hamilton et al.[25]/2001	3–5 days 60 min/session 70% VO_{2max} 30 m/min	C: control E-cold: exercised at 4°C E-warm: exercised at 25°C	Intraventricular pressure and MnSOD: E-cold = E-warm > C HSPs: E-warm > E-cold = C GPx: E-cold > E-warm = C	The protection is not dependent on increased myocardial HSP levels [Remark 1] but rather on increased myocardial antioxidant defense.
Yamashita et al.[15]/1999	1 session 25–30 min 27–30 m/min	C: control Ex: exercised (0.5h, 3 h, 24 h, 36 h, 48 h, 60h, and 72 h after exercise)	Infarcted area: C = 3 h = 24 h = 72 h > 0.5 h = 36 h = 48 h = 60 h MnSOD activity: 0.5 h = 48 h > other groups Expression of MnSOD: 48 h > other groups	The exercise-induced production of EROS, TNF-α, and IL-1β results in MnSOD activation, which plays an important role in biphasic cardioprotection against IR injury.
Esposito et al.[26]/2011	10 weeks 3 days/week 60 min/session 60% or 80% VO_{2max}	UNT: untrained Low: low-intensity exercise High: high-intensity exercise High-det: untrained after high-intensity exercise	Infarcted area: High < Low = High-det < UNT HSP70 and MnSOD: High > Low > High-det > UNT	The cardioprotective benefits of exercise are proportional to its intensity and occur via HSPs and antioxidant defense.
Lennon et al.[27]/2004	3 consecutive days 60 min/session 55% or 75% VO_{2max}	C: sedentary control Mod: exercise at 55% VO_{2max} High: exercise at 75% VO_{2max}	Cardiac function: High = Mod > C MnSOD: High > Mod = C HSP72: High > Mod > C	Moderate- and high-intensity exercise promotes similar protection against IR injury.
Murlasits et al.[28]/2007	5 consecutive days 60 min/session 70% VO_{2max}	C: sedentary control Trained	Infarcted area: C > Trained HSP72: Trained > C Grp78, Grp94, calreticulin, ATF3, CHOP, Caspase 12, Noxa, Puma: Trained = C	The cardioprotective effect of short-duration exercise is not associated with the regulation of stress proteins such as HSPs.
Quindry et al.[29]/2007	3 consecutive days 60 min/session 30 m/min	Sed: sedentary W Ex: exercised at 22°C C Ex: exercised at 8°C	Infarcted area and Tunnel: Sed > W Ex = C Ex HSP72: W Ex > C Ex = Sed	The exercise-induced increase in HSP72 levels is not essential for protection against infarction and apoptosis.
Starnes et al.[30]/2005	16 weeks 5 days/week 40 min/session 55%–60% VO_{2max}	Sed: sedentary RUN: exercised	Cardiac function: Sed = RUN HSP70: RUN > Sed	Exercise at 55%–60% of VO_{2max} induces an increase in HSP70 levels, but this increase is below the threshold for inducing cardioprotection.
Morán et al.[31]/2005	24 weeks 5 days/week 45 min/session 25 m/min	Sed: sedentary Tr: trained	HSP72: Tr > Sed Oxidative stress and adenosine: Tr = Sed MnSOD and GR: Tr < Sed	Exercise-induced cardioprotection occurs via increase in HSP72 levels and not through the increase of antioxidant or adenosine levels.
Lennon et al.[32]/2004	3 consecutive days 60 min/session 70% $VO_{2\ max}$	CT: control 1, 3, 9, and 18 days after exercise	Catalase and HSP72: 1 day = 3 days > other groups Cardiac function: 1 day = 3 days = 9 days > 18 days = CT	Cardioprotection is abolished 18 days after the end of exercise and is not related to HSP72 and catalase.
Harris et al.[33]/2001	3, 6, and 9 weeks 5 days/week 60 min/session 23°C or 8°C	Sed: sedentary 3WK, 6WK, and 9WK: exercised for 3/6/9 weeks at 23°C 3WKC, 6WKC, and 9WKC: exercised for 3/6/9 weeks at 8°C	HSP70: 3WK = 6WK = 9WK > CT = 3WKC = 6WKC = 9WKC Cardiac function: 9WK > SED = 9WKC SOD: 9WK = 9WKC	Exercise-induced cardioprotection appears to be due to the increase in HSP70 levels.
Taylor et al.[10]/1999	1 or 3 days, 100 min/day, 20 m/min at 23°C 1 day, 100 min/day, 20 m/min at 8°C	CTRL: sedentary control HS: sedentary heated to 42°C 1DR and 3DR: exercised for 1/3 days at 23°C 1CR: exercised for 1 day at 8°C	Cardiac function: CTRL < other groups HSP72: 1DR = 3DR = HS > 1CR = CTRL	Acute exercise can produce cardioprotective response without increase in HSP72 levels.
Quindry et al.[34]/2012	3 days 60 min/session 70% VO_{2max} 30 m/min	Sed: sedentary control Exe: exercised Ex5HD: exercised with mitochondrial KATP blockade ExHMR: exercised with sarcolemmal KATP blockade	Infarcted area: Exe = Ex5HD < ExHMR = Sed MnSOD: Sed < other groups	Sarcolemmal KATP channels are more important than mitochondrial KATP channels in the prevention of tissue death after exercise.

Continuation

Quindry et al. [35]/2010	3 consecutive days 60 min/session 30 m/min	Sed: sedentary control Exe: exercised Ex5HD: exercised with mitochondrial KATP blockade ExHMR1098: exercised with sarcolemmal KATP blockade	Arrhythmias: Ex = ExHMR1098 < Ex5HD = Sed MnSOD: Sed < other groups	Mitochondrial KATP channels promote antiarrhythmic protection as part of exercise-induced cardioprotection.
Brown et al. [36]/2005	12 weeks Remaining not informed	Sed: sedentary Tr: trained 5HD: exercised with mitochondrial KATP blockade HMR1098: exercised with sarcolemmal KATP blockade	Infarcted area: HMR1098 > other groups Calcium content: 5HD > other groups Blood pressure: 5HD < other groups	Exercise increases the expression of sarcolemmal KATP channels, which when blocked, annuls the cardioprotective benefits of exercise.
Michelsen et al. [37]/2012	Bicycle 1 session 25 min 4× 2' 400W + 3' 250W	ExPC: exercise-induced preconditioning ExPC + N: exercise-induced preconditioning + opioid blockade rIPC: Remote ischemic preconditioning rIPC + N: Remote ischemic preconditioning + opioid blockade	Infarcted area: rIPC < rIPC + N / ExPC < ExPC + N Blood pressure: rIPC > rIPC + N = ExPC > ExPC + N	Exercise remotely preconditions the heart through the opioid receptor activation-dependent humoral effector.
Galvão et al. [38]/2011	12 weeks 5 days/week 60 min/session 60% VO_{2max}	C: control ET: physical training M: morphine IR: ischemia and reperfusion M + N: opioid blockade ET + M ET + N	Infarcted area: C, M + N, and ET + N > other groups Intraventricular pressure and capillary density: similar between groups	The chronic effect of exercise in reducing the infarcted area is due to the activation of opioid receptors rather than to increased myocardial perfusion.
Dickson et al. [9]/2008	1 session 25 min 25 m/min	Exe: exercise Exe N: exercise with opioid blockade	Intraventricular pressure: equal between groups Infarcted area: Exe < Exe + N	Cardioprotection is mediated by an opioid receptor-dependent mechanism.

NFAT: nuclear factor of activated T-cell, VO_{2max}: maximum oxygen uptake, eNOS: endothelial nitric oxide synthase, iNOS: induced nitric oxide synthase, IR: ischemia and reperfusion, NO: nitric oxide, CGMP: cyclic guanosine monophosphate, O_2: oxygen, NADPH: nicotinamide adenine dinucleotide phosphate (reduced), ROS: reactive oxygen species MnSOD: manganese superoxide dismutase, GPX: glutathione peroxidase, SOD: superoxide dismutase, HSP: heat shock protein, TNF-α: tumor necrosis factor alpha, IL: interleukin, Grp: glucose regulated protein, ATF: activating transcription factor, CHOP: CCAAT-enhancer-binding protein homologous protein, GR: glutathione reductase, KATP: ATP-dependent potassium channel

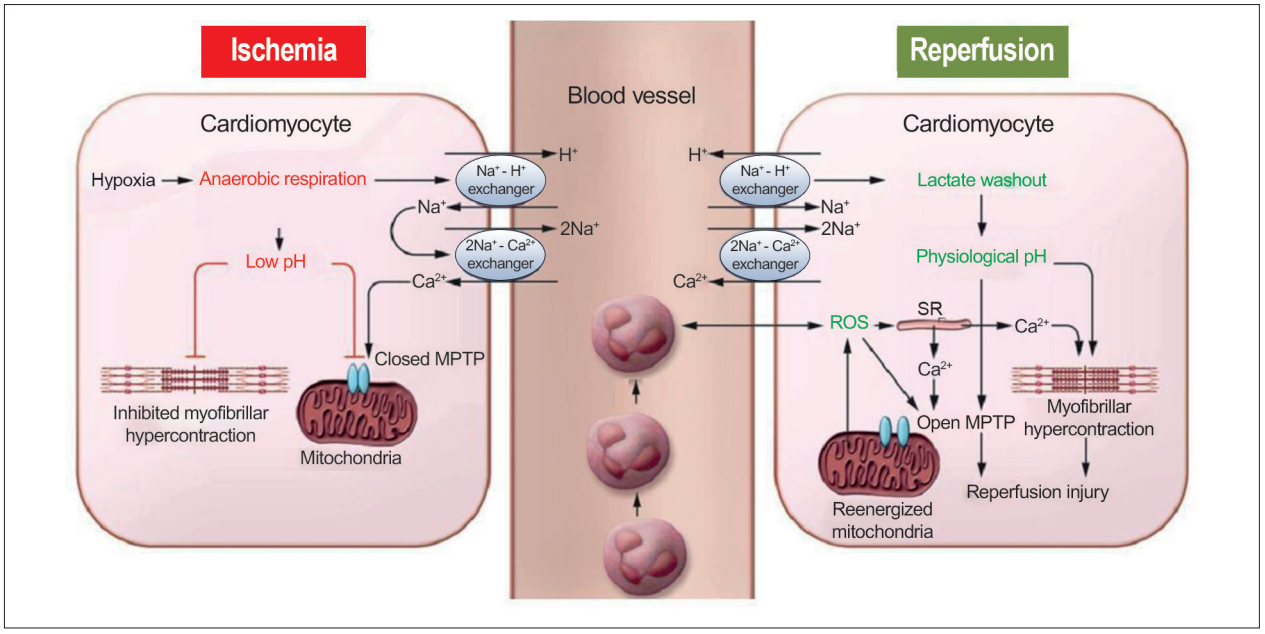

Figure 1 - *Metabolic and biochemical changes in the myocardium in response to ischemia and reperfusion*
Adapted from: Hausenloy et al.[4]
ROS: reactive oxygen species; SR: sarcoplasmic reticulum; MPTP: mitochondrial permeability transition pore.

has gained relevance in the biomedical sector. In this sense, Murry et al.[43] observed that four 5-min periods of myocardial ischemia interspersed with 5 min of reperfusion protected the myocardium against subsequent longer periods of ischemia. From this observation, which became known as "infarction preconditioning," numerous studies have investigated other possible cardioprotective strategies, including (1) myocardial ischemic post-conditioning, in which short periods of ischemia during the initial minutes of reperfusion after prolonged arterial occlusion could reduce the infarcted area[7,17,44]; (2) exercise-induced preconditioning, characterized by inducing a cardiac phenotype that is resistant to myocardial injury after exercise[6,7,9,16,17,41,45]; and (3) pharmacological preconditioning, which advocates the use of drug agents (such as adenosine and cyclosporin A)[4] as intracellular pathway modulators to reduce the myocardial necrosis area due to IR injury.

Exercise-induced preconditioning, the object of the present review, is the only treatment strategy that can be performed regularly to protect the heart against IR injury[46,47].

Mechanisms of exercise-induced myocardial preconditioning

In 1978, McElroy et al.[48] demonstrated that regular physical activity could provide cardioprotection. In that study, mice were subjected to physical training that consisted of swimming (1 h/session, 5 days/week, 5 weeks). After irreversible occlusion of the left coronary artery, a 30% reduction of the infarcted area was observed in trained mice when compared with the sedentary control. A similar result was found by Brown et al.[49], whose protocol comprised 20 weeks of training on the treadmill and resulted in a 25% reduction in the infarcted area among the trained animals after 1 h of ischemia and 2 h of reperfusion. In addition, the authors also reported an improvement in cardiac function after physical training due to better maintenance of intraventricular pressure during IR.

Aerobic exercise undeniably protects the heart against IR injury, both by attenuating tissue death and by promoting greater maintenance of cardiac function. However, the mechanism by which this occurs is still unclear[6,8,18]. According to the retrieved studies, the main proposed mechanisms include increased production of heat shock proteins (HSPs)[31,33], involvement of the NO pathway[11,12,19,20], increased cardiac antioxidant activity associated with decreased ROS production in myocardial mitochondria[13-15,21,22,25], improved sarcolemmal and mitochondrial KATP channel function[34-36], and activation of the opioid system[9,37,38].

HSPs

Several proteins have been demonstrated to play an important role in maintaining homeostasis at least at the cellular level. When the body is exposed to stress (for example, during hypoxia, hyperthermia, ischemia, and acidosis), the synthesis of these proteins may be compromised[8,50]. In response to these factors, the body synthesizes HSPs, which help maintain homeostasis[6].

HSPs are classified into several groups according to their molecular weight: 8–32 kDa, 40–60 kDa, 70 kDa, 90 kDa, and 100–110 kDa[51]. Several HSP families, including HSP10, HSP60, and HSP90, are associated with cardioprotective effects; however, the HSP70 family deserves greater attention[8]. The most prominent members of the HSP70 family are HSP73 and HSP72. HSP73 is constitutively synthesized in all cells, and its levels slightly increase slightly after a stressful event. Conversely, HSP72 can only be detected after a stressful event[51], particularly IR injury.

The reviewed studies indicate that acute aerobic exercise can increase myocardial HSP70 levels[10,26,29,31,33]. However, although the mechanism by which this occurs remains unclear, studies have associated this increase with thermal stress, hypoxia, reduction in intracellular pH, oxidative stress, depletion of glucose, and/or increase in cytosolic calcium levels. Previous studies have suggested that the increase in exercise-induced HSP72 expression is associated with protection against IR injury[26,31,33], and this could explain the exercise-induced preconditioning. Nonetheless, in the studies by Taylor et al.[10] and Quindry et al.[29], animals were trained in a cold environment and at room temperature to prevent an increase in HSP72 levels. Both studies found that, regardless of the level of HSP72, the two animal groups showed cardioprotection against IR injury. Although the increase in HSP72 expression can promote a cardioprotective response, this increase is not a prerequisite for exercise-induced cardioprotection.

Involvement of the NO pathway

After ischemia, the heart immediately uses endothelial NO synthase (eNOS) for the immediate release of NO, along with an increase in the induced NO synthase (iNOS) levels, to convert a more defensive cell phenotype through an increment in NO biosynthesis[8]. Considering that exercise promotes the release of eNOS through shear stress, NO may play an important role in exercise-induced cardioprotection against IR injury[8], acting as a trigger and mediator of late stage preconditioning (after 24 h)[11]. In this sense, Babai et al.[12] demonstrated that even a single exercise session conferred cardioprotection against NO-mediated IR. Further evidence for this was contributed by Farah et al.[21], who observed that exercise-induced cardioprotection was abolished when using an eNOS blocker. In this context, Nicholson et al.[19] demonstrated that exercise could moderately increase the heart's ability to reduce nitrite to NO, which probably contributed to exercise-induced cardioprotection.

Increased antioxidant capacity

The human body has a highly complex antioxidant system, composed primarily of enzymatic agents that function synergistically to protect cells and organ systems from damage caused by oxidative stress. Among them, superoxide dismutase (SOD), catalase, and glutathione peroxidase should be highlighted[8].

Although widely studied, there is still no consensus on the effect of exercise on the activity of myocardial antioxidant agents[8]. The transient release of ROS during exercise has been demonstrated to trigger a specific adaptation that increases the antioxidant capacity[22]. Some authors have shown that exercise can increase the levels of catalase[32] and

glutathione peroxidase[22], whereas others have found no differences in these levels[9,52]. There is a greater consensus regarding the role of exercise in promoting increased SOD activity, specifically the mitochondrial isoform manganese superoxide dismutase (MnSOD)[8,15,18]. However, because exercise-induced cardioprotection is a multifactorial process, it may be associated with factors other than the increased antioxidant capacity. To address this question, Yamashita et al.[15] used a technique with antisense oligonucleotides for MnSOD gene silencing and demonstrated that the inhibition of the increased cardiac MnSOD expression induced by exercise resulted in the loss of cardioprotection. In contrast, Lennon et al.[16] used the same silencing technique and observed that cardioprotection was still present even after preventing the increase in MnSOD expression. Therefore, it is clear that further research is necessary to elucidate the role of antioxidant enzymes in exercise-induced cardioprotection.

KATP channels

The KATP channels, highly expressed in the sarcolemma and mitochondria, have been associated with a cardioprotective effect[53,54]. These channels are believed to act as sensors to identify the cellular ionic and bioenergetic balance to preserve cardiac homeostasis during metabolic stress situations[55]. To perform this function, they control the amount of ATP available in the cytosol. The KATP channels are closed in the presence of cytosolic ATP. However, the decreased ATP levels due to metabolic stress (such as ischemia) stimulate their opening[8,53,55]. The opening of the sarcolemmal KATP channels causes an efflux of K^+ from cardiomyocytes, resulting in hyperpolarization of the cardiac cell and a decrease in the number of action potentials[56]. This limits the entrance of Ca^{2+} through type-L channels and prevents intracellular Ca^{2+} accumulation[8]. Collectively, there is a decrease in cardiac metabolic demand, decreasing the electron transport chain activity, thereby preventing ROS production[56].

The beneficial cardioprotective role of sarcolemmal KATP channels in ischemic and pharmacological preconditioning[57] has also been observed in exercise preconditioning[36,53,58]. Brown et al.[36] demonstrated that a 12-week training program increased the expression of these channels in cardiomyocytes, and their pharmacological blockade prevented the cardioprotective effect of exercise against IR injury. Similarly, Quindry et al.[34] stressed the importance of sarcolemmal KATP channels in the prevention of tissue death after exercise.

However, the role of mitochondrial KATP channels in cardioprotection is more controversial. With regard to the cardioprotective mechanisms, evidence indicates that their opening causes mitochondrial matrix alkalinization, decreased ROS production, decreased mitochondrial Ca^{2+} accumulation, and improved energy production in the mitochondria[7]. Nevertheless, Brown et al.[36] used a mitochondrial KATP channel inhibitor and concluded that these channels were not essential mediators of exercise-induced cardioprotection against IR injury. In contrast, Quindry et al.[35], by inhibiting the mitochondrial KATP channels, observed that the protection against arrhythmia in trained mice was lost. Thus, the hypothesis that mitochondrial KATP channels, in addition to the sarcolemmal channels, can promote cardioprotection after exercise cannot be excluded.

Although the association of exercise-induced cardioprotection with the opening of KATP channels is well established, the intracellular signaling cascade triggered in response to exercise and responsible for the opening of these channels remains unclear. In a simplified manner, KATP channels are believed to be associated with opioids and protein kinase C, as shown in Figure 2[9]. Because the opioid system is influenced by exercise[9], this system may be mediating exercise-induced cardioprotection.

Opioid system

Opioid peptides have been used to treat pain for hundreds of years. However, investigations of the cardioprotective properties of opioids began recently, when Schultz et al.[60] demonstrated, in 1995, that myocardial preconditioning, a phenomenon that can help reduce myocardial ischemic injury, includes opioid receptors among its mediators. These studies led to the discovery that morphine (an exogenous opioid agent), in addition to treating the pain associated with MI, may also aid in the reduction of the MI area. Since then, the opioid system and the drugs that act on it have gained importance owing to its effect on the cardiovascular system[59]. Endogenous opioid peptide levels increase during stressful situations, such as during ischemia[59,61]. Previous studies have demonstrated that the levels of beta-endorphins (an endogenous opioid agent) increase in patients with myocardial ischemia[62] and after coronary angioplasty[63]. Therefore, the increase in opioid peptide levels in infarcted ventricular tissue may be due to a compensatory mechanism that counteracts the high levels of catecholamines released during ischemia to minimize the infarcted area[59].

Notably, hibernation of animals, a period characterized by a marked decrease in metabolism, heart rate, and respiratory rate, is induced by a substance of opioid nature, the hibernation-inducing factor[64]. The use of DADLE, a widely used opioid agonist and the hibernation-inducing factor, has been demonstrated to be effective in extending organ survival and tissue preservation before organ transplantation[64].

Exercise and other stress conditions can increase the levels of opioid agents[9,65]. Howlett et al.[66] demonstrated that acute bouts of running exercise markedly increased beta-endorphin levels in young women and that this pattern was not changed 8 weeks after physical training. These authors reported that exercise was the first physiological stimulus capable of increasing the levels of another endogenous opioid agent, enkephalin. With regard to the impact of exercise in the expression of opioid receptors, a transient increase of these receptors in the heart immediately after an acute exercise session has been demonstrated[9]. Furthermore, during an incremental exercise test in humans, the blockade of opioid receptors by naloxone decreased test time, VO_{2max}, and maximal heart rate observed in response to exercise[67]; therefore, healthy individuals, when subjected to maximum test and naloxone injection, ended the maximum stress test primarily because of the perception of fatigue rather than physiological limitation.

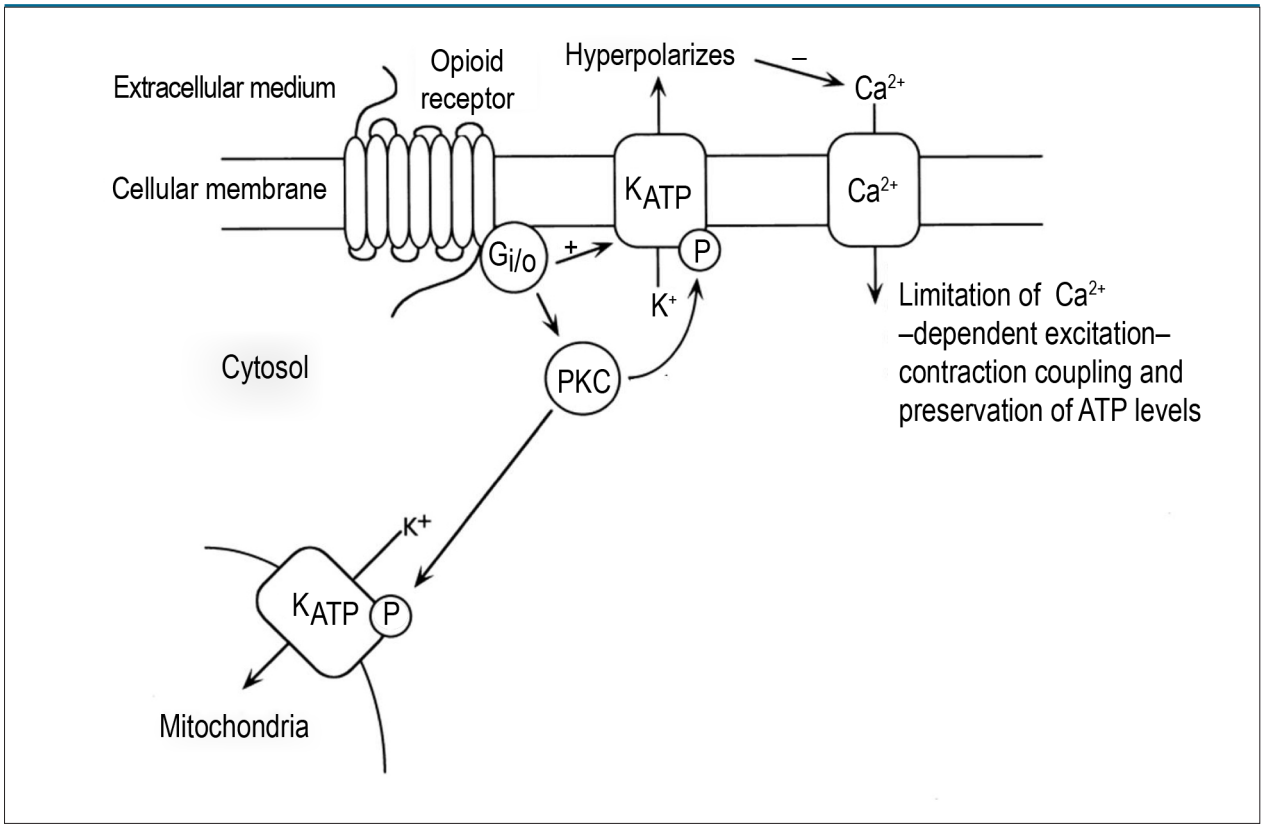

Figure 2 - *Schematic diagram of a possible signaling pathway involved in opioid- and KATP channel-induced cardioprotection*
Adapted from: Schultz et al[69]
PKC: protein kinase C

Despite this analgesic effect of the opioid system, the role of this system in cardiovascular function in response to exercise cannot be excluded. Although the relationship of opioids with the cardioprotective effect of exercise has been little studied, it has been demonstrated that a 50% reduction in the infarcted area, associated with a single exercise session (25 min at 25 m/min), was completely abolished after the pharmacological blockade of opioid receptors[9]. Furthermore, Michelsen et al.[37] and Galvão et al.[38] showed that exercise-induced cardioprotection is mediated by an opioid receptor-dependent mechanism.

From experimental research to clinical practice

Over the years, various therapeutic interventions have been tested to prevent reperfusion injury in patients[68]. Among them, remote myocardial ischemic conditioning is noteworthy for its broader application in clinical practice. This strategy may be reproduced in humans through the use of a sleeve cuff to induce small IR cycles in the arm, a procedure that would protect the heart from IR injury[69,70]. However, these results are still incipient mostly because of the difficulty of translating the cardioprotection obtained from animal studies into clinical practice. In addition, the infarction models adopted in animals do not adequately represent the typical cardiac patient with regard to age, comorbidities, drug therapy, and infarction pathophysiology[68].

With regard to exercise-induced cardioprotection against IR injury and its mechanisms, the intervention is required before the ischemic event, which in case of myocardial infarction is impossible to predict, making clinical research even more difficult. This explains the fact that all the evidence retrieved on this subject was obtained from studies with animal models, primarily mice and dogs. However, to establish associations and clinical implications, Zdrenghea et al.[71] showed that exercise-induced ST-segment depression was markedly attenuated in high-risk patients during consecutive exercise sessions. Similarly, Lambiase et al.[72] trained CAD patients prior to PCI and observed that ST-segment deflation, common in PCI, decreased in these patients. These results indicate the promising use of exercise in the promotion of clinical cardioprotection, making it a future research target.

Conclusion

Despite the considerable research on this subject, the mechanisms responsible for exercise-induced cardioprotection against IR injury have not been completely elucidated. In view of the serious clinical complications due to IR injury and considering that physical exercise is the only known sustained cardiac preconditioning strategy against this type of injury, it is important to better understand the pathways involved in this mechanism. In light of the studies included

in this review, the main mechanisms include increase in the HSP production[31,33], involvement of the NO pathway[11,12,19,20], increase in the cardiac antioxidant capacity[13-15,21,22,25], increase in KATP channel function[34-36], and activation of the opioid system[9,37,38].

Although previous evidence indicates that exercise-induced cardioprotection is more directly associated with antioxidant capacity and the role of KATP channels, along with the opioid system, further research is necessary to obtain more consistent conclusions.

Author contributions

Conception and design of the research: Borges JP, Lessa MA. Acquisition of data: Borges JP, Lessa MA. Analysis and interpretation of the data: Borges JP, Lessa MA. Obtaining financing: Borges JP, Lessa MA. Writing of the manuscript: Borges JP, Lessa MA. Critical revision of the manuscript for intellectual content: Borges JP, Lessa MA.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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