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 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 worldwide1. 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 year1. With regard to public spending on health, approximately R$ 250 million were spent on the treatment of acute myocardial infarction (MI) alone in 20112.

In MI, the ultimate therapeutic goal is to restore blood flow to the ischemic region as quickly as possible1 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 death3–4. 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 patients5. 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 myocardium6–9. Notably, a single exercise session prior to IR injury is sufficient to promote an increase in cardiac output10 and improve myocardial contractile function during IR injury11–15. 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 flow16–18. However, the specific mechanism involved in exercise-induced cardiac preconditioning is still under debate19–21. 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.
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. 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. 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. To compensate for the low pH, intracellular water accumulates, thus causing cellular edema. 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 (Ca2+) exchanger occurs, resulting in the efflux of Na+ in exchange for the influx of Ca2+ into the cell.

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 Ca2+ released in the cytosol back into the sarcoplasmic reticulum after muscle contraction. Therefore, the inactivity of these pumps results in additional Na+ and Ca2+ overload, which prevents cell repolarization and culminates in contractile dysfunction. Furthermore, high levels of Ca2+ 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.

During reperfusion, the damage caused by ischemia is exacerbated because the restoration of the oxygen flow promotes the release of ROS in the mitochondria. The production of ROS and Ca2+ overload are the main factors contributing to IR-induced cell damage because they culminate in mitochondrial permeability transition pore (MPTP) opening. 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. 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.

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. /2013 | Running wheel 4 weeks | Exe: exercise NO<sub>2</sub>; supplemented nitrite Exe + NO<sub>2</sub> Sed: sedentary | Infarcted area and troponin-1: Sed > Exe = Exe + NO<sub>2</sub> Ejection fraction: Sed < Exe = Exe + NO<sub>2</sub> Myoglobin and NFAT: Sed = NO<sub>2</sub> > Exe = Exe + NO<sub>2</sub> | Exercise decreases cardiac myoglobin levels by inhibiting the calcium<sub>2+</sub>-NFAT pathway. Moderate exercise-induced cardioprotection is due to diminished ability to reduce nitrite to NO. |
| Akita et al. /2007 | 7 consecutive days 60 min/session 60%–70% VO<sub>2max</sub> | Phenol: sympathetic ablation Antioxidant supplemented eNOS<sup>(−/−)</sup>; eNOS knockout 1400W: iNOS blockade iNOS<sup>(−/−)</sup>; iNOS knockout | Infarcted area: Exe < other groups eNOS and NO activity: Exe > other groups Oxidative stress: > in Exe and eNOS<sup>(−/−)</sup> | Exercise stimulates cardiac sympathetic innervation, causing eNOS activation with consequent iNOS elevation, which acts as a mediator in late cardioprotection. |
| Hajnal et al. /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 | Arhythmias: 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. /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 aminoxyquinidine and iNOS activity increases 24 h after exercise. |
| Farah et al. /2013 | 5 weeks 5 days/week 45 min/session 70% VO<sub>2max</sub> 25 m/min | CT: control Exe: exercise Exe + L-NAME eNOS blockade Exe + L-NIO: more specific eNOS blockade Exe + BH<sub>4</sub>; NO donor | Nitrite and GMPc levels: similar between groups eNOS function: Exe and BH<sub>4</sub> > 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<sub>2</sub> and the consequent formation of peroxynitrite (highly cytotoxic). |
| Frasier et al. /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 BCNU + Exe: inhibited glutathione reductase Vias2870 + 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. /2012 | 5 consecutive days 60 min/session 70% VO<sub>2max</sub> 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 pathogenesis. | Cardioprotection is partially mediated by beneficial adaptations in mitochondrial phenotype, increasing their resistance to the oxidative damage due to IR injury. |
| Kavazis et al. /2008 | 5 consecutive days 60 min/session 30 m/min | Sed: sedentary Exir: trained | Antioxidant enzymes and mitochondrial function: Exir > Sed Expression of proapoptotic proteins: Sed > Exir | Exercise induces mitochondrial adaptations that contribute to cardioprotection. |
| French et al. /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 (MnSOD) that promote cardioprotection by attenuating necrosis/apoptosis |
| Lennon et al. /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

| Study                  | Study details                                      | Control                                                                 | Experimental conditions                                                                                   |
|------------------------|---------------------------------------------------|------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|
| Hamilton et al. 23/2003| 5 consecutive days 60 min/session 30 m/min         | Untrained control (1)                                                  | Infarcted area: 1 > 2 = 3 > 4                                                                          |
|                        |                                                   | Untrained and antioxidant diet (2)                                      | Antioxidant activity: 1 > 3 > 2 = 4                                                                          |
|                        |                                                   | Trained and antioxidant diet (3)                                        | HSP72/73: 3 > 1 = 2 > 4                                                                                   |
|                        |                                                   | Trained and antioxidant diet (4)                                        | Intraventricular pressure: 4 > 3 > 2 > 1                                                                      |
|                        |                                                   |                                                                       | Exercise and use of antioxidants can promote cardioprotection independently and the combination of these strategies does not interfere in the response. |
| Hamilton et al. 23/2001| 3–5 days 60 min/session 70% VO₂max 30 m/min        | C: control                                                            | Infarcted area: C = 3 h = 24 h = 72 h                                                                      |
|                        |                                                   | E-cold: exercised at 4°C                                                | MnSOD: E-cold = E-warm > C                                                                                |
|                        |                                                   | E-warm: exercised at 25°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. |
|                        |                                                   |                                                                       | Intraventricular pressure: 4 > 3 > 2 > 1                                                                      |
|                        |                                                   |                                                                       | 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. |
| Yamashita et al. 15/1999| 1 session 25–30 min 27–30 m/min                   | C: control                                                            | Cardiac function: High > Mod > C                                                                           |
|                        |                                                   | Ex: exercised (0.5 h, 3 h, 24 h, 36 h, 48 h, 60h, and 72 h after exercise) | MnSOD: High > Mod > C                                                                                     |
|                        |                                                   |                                                                       | The cardioprotective benefits of exercise are proportional to its intensity and occur via HSPs and antioxidant defense. |
|                        |                                                   |                                                                       | The cardioprotective effect of short-duration exercise is not associated with the regulation of stress proteins such as HSPs. |
| Esposito et al. 23/2011| 10 weeks 3 days/week 60% VO₂max                  | UNT: untrained                                                         | Infarcted area: High < Low = High-det < UNT                                                                |
|                        |                                                   | Low: low-intensity exercise                                             | HSP70 and MnSOD: High > Low > High-det > UNT                                                              |
|                        |                                                   | High: high-intensity exercise                                           | The cardioprotective benefits of exercise are proportional to its intensity and occur via HSPs and antioxidant defense. |
|                        |                                                   | High-det: untrained after high-intensity exercise                       | The cardioprotective effect of short-duration exercise is not associated with the regulation of stress proteins such as HSPs. |
| Lennon et al. 23/2004| 3 consecutive days 60 min/session 55% or 75% VO₂max| C: sedentary control                                                   | Cardiac function: High > Mod > C                                                                           |
|                        |                                                   | Mod: exercise at 55% VO₂max                                             | MnSOD: High > Mod > C                                                                                     |
|                        |                                                   | High: exercise at 75% VO₂max                                            | HSP72: Tr > Sed                                                                                           |
|                        |                                                   |                                                                       | Oxidative stress and adenosine: Tr = Sed                                                                  |
|                        |                                                   |                                                                       | MnSOD and GR: Tr < Sed                                                                                   |
|                        |                                                   |                                                                       | The cardioprotective effect of short-duration exercise is not associated with the regulation of stress proteins such as HSPs. |
| Murlasits et al. 23/2007| 5 consecutive days 60 min/session 70% VO₂max      | C: sedentary control                                                   | Infarcted area: C > Trained                                                                               |
|                        |                                                   | Trained                                                                | HSP72: Trained > C                                                                                        |
|                        |                                                   |                                                                       | The exercise-induced increase in HSP72 levels is not essential for protection against infarction and apoptosis. |
| Quindry et al. 23/2007| 3 consecutive days 60 min/session 30 m/min        | Sed: sedentary                                                         | Exercise at 55%–60% of VO₂max induces an increase in HSP70 levels, but this increase is below the threshold for inducing cardioprotection. |
|                        |                                                   | W: Ex: exercised at 22°C                                               | Exercise-induced cardioprotection occurs via increase in HSP70 levels and not through the increase of antioxidant or adenosine levels. |
|                        |                                                   | C: Ex: exercised at 8°C                                                | Cardioprotective benefits of exercise are proportional to its intensity and occur via HSPs and antioxidant defense. |
|                        |                                                   |                                                                       | Cardioprotective benefits of exercise are proportional to its intensity and occur via HSPs and antioxidant defense. |
| Starnes et al. 23/2005| 16 weeks 5 days/week 60% VO₂max                   | Sed: sedentary                                                         | Intraventricular pressure: 4 > 3 > 2 > 1                                                                      |
|                        |                                                   | RUN: exercised                                                         | The cardioprotective benefits of exercise are proportional to its intensity and occur via HSPs and antioxidant defense. |
|                        |                                                   |                                                                       | The cardioprotective benefits of exercise are proportional to its intensity and occur via HSPs and antioxidant defense. |
| Moran et al. 23/2005| 24 weeks 5 days/week 45 min/session 25 m/min      | Sed: sedentary                                                         | Cardiac function: Sed = RUN                                                                               |
|                        |                                                   | Tr: trained                                                            | HSP70: RUN > Sed                                                                                           |
|                        |                                                   |                                                                       | Exercise-induced cardioprotection occurs via increase in HSP70 levels and not through the increase of antioxidant or adenosine levels. |
|                        |                                                   |                                                                       | Cardioprotective benefits of exercise are proportional to its intensity and occur via HSPs and antioxidant defense. |
| Lennon et al. 23/2004| 3 consecutive days 60 min/session 70% VO₂max      | CT: control                                                            | Cardiac function: 1 day = 9 days > 18 days > other groups                                                 |
|                        |                                                   | 1, 3, 9, and 18 days after exercise                                    | Cardioprotective benefits of exercise are proportional to its intensity and occur via HSPs and antioxidant defense. |
|                        |                                                   |                                                                       | The cardioprotective benefits of exercise are proportional to its intensity and occur via HSPs and antioxidant defense. |
| Harris et al. 23/2001| 3, 6, and 9 weeks 5 days/week 60 min/session 23°C or 8°C | Sed: sedentary                                                         | Cardiac function: 1 day = 9 days > 18 days > other groups                                                 |
|                        |                                                   | 3WK, 6WK, and 9WK: exercised for 3/6/9 weeks at 23°C                   | Cardioprotective benefits of exercise are proportional to its intensity and occur via HSPs and antioxidant defense. |
|                        |                                                   | 3WKC, 6WKC, and 9WKC: exercised for 3/6/9 weeks at 8°C                 | Cardioprotective benefits of exercise are proportional to its intensity and occur via HSPs and antioxidant defense. |
|                        |                                                   |                                                                       | Exercise-induced cardioprotection occurs via increase in HSP70 levels and not through the increase of antioxidant or adenosine levels. |
| Taylor et al. 15/1999| 1 or 3 days, 100 min/ day, 20 min/m at 23°C        | CTRL: sedentary control                                                | Infarcted area: Exe = Ex5HD < ExHMR = Sed                                                                |
|                        |                                                   | HS: sedentary heated to 42°C                                            | MnSOD: Sed < other groups                                                                                 |
|                        |                                                   | 1DR and 3DR: exercised for 1/3 days at 23°C                           |   | |
|                        |                                                   | 1CR: exercised for 1 day at 8°C                                        |   | |
|                        |                                                   | Sed: sedentary control                                                |   | |
|                        |                                                   | Ex: exercised                                                          |   | |
|                        |                                                   | ExSHD: exercised with mitochondrial KATP blockade                      |   | |
|                        |                                                   | ExHMR: exercised with sarcolemmal KATP blockade                        |   | |
| Quindry et al. 23/2012| 3 days 60 min/session 70% VO₂max                  |                                                                           |   | |
|                        |                                                   | 30 m/min                                                               |   | |
|                        |                                                   |                                                                           |   | |
Quindry et al. 35/2010
3 consecutive days
60 min/session
30 m/min
Sed: sedentary control
Exe: exercised
ExSHD: exercised with mitochondrial KATP blockade
ExHMR1098: 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.

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

Arrhythmias: Ex = ExHMR1098 < Ex5HD = Sed
MnSOD: Sed < other groups

Mitochondrial KATP channels promote antiarrhythmic protection as part of exercise-induced cardioprotection.

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% VO2max
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. 39/2008
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, VO2max: maximum oxygen uptake, eNOS: endothelial nitric oxide synthase, iNOS: induced nitric oxide synthase, IR: ischemia and reperfusion, NO: nitric oxide, NOS: nitric oxide synthase, CGMP: cyclic guanosine monophosphate, HSP: heat shock protein, TNF-α: tumor necrosis factor alpha, IL: interleukin, Grp: glucose regulated protein, CHOP: CCAAT-enhancer-binding protein homologous protein, GR: glutathione reductase, KATP: ATP-dependent potassium channel
has gained relevance in the biomedical sector. In this sense, Murry et al. 41 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 7,17,42; (2) exercise-induced preconditioning, characterized by inducing a cardiac phenotype that is resistant to myocardial injury after exercise 8,11,12,19,20; (3) pharmacological preconditioning, which advocates the use of drug agents (such as adenosine and cyclosporin A) 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 injury46,47.

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 indubitably 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 31,12,19,20; increased cardiac antioxidant activity associated with decreased ROS production in myocardial mitochondria 13-15,21,22,25; improved sarcoplasmic 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 31,50. In response to these factors, the body synthesizes HSPs, which help maintain homeostasis 4.

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 31. Several HSP families, including HSP10, HSP60, and HSP90, are associated with cardioprotective effects; however, the HSP70 family deserves greater attention 6. 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 31, particularly IR injury.

The reviewed studies indicate that acute aerobic exercise can increase myocardial HSP70 levels 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 Quinndry 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 6, 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. 7, 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 42 and...
glutathione peroxidase, whereas others have found no differences in these levels. There is a greater consensus regarding the role of exercise in promoting increased SOD activity, specifically the mitochondrial isomorph manganese superoxide dismutase (MnSOD)\(^\text{3,15,18}\). 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.\(^\text{15}\) 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.\(^\text{16}\) 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\(^\text{3,34}\). These channels are believed to act as sensors to identify the cellular ionic and bioenergetic balance to preserve cardiac homeostasis during metabolic stress situations. 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\(^\text{3,53,55}\). 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. This limits the entrance of Ca\(^2+\) through type-L channels and prevents intracellular Ca\(^2+\) accumulation. Collectively, there is a decrease in cardiac metabolic demand, decreasing the electron transport chain activity, thereby preventing ROS production\(^\text{46}\).

The beneficial cardioprotective role of sarcolemmal KATP channels in ischemia and pharmacological preconditioning\(^\text{57}\) has also been observed in exercise preconditioning\(^\text{36,33,35}\). Brown et al.\(^\text{36}\) 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, Quinndry et al.\(^\text{35}\) 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 alkalization, decreased ROS production, decreased mitochondrial Ca\(^2+\) accumulation, and improved energy production in the mitochondria\(^\text{9}\). Nevertheless, Brown et al.\(^\text{36}\) used a mitochondrial KATP channel inhibitor and concluded that these channels were not essential mediators of exercise-induced cardioprotection against IR injury. In contrast, Quinndry et al.\(^\text{35}\) 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. Because the opioid system is influenced by exercise\(^\text{9}\), 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.\(^\text{60}\) 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\(^\text{15}\). Endogenous opioid peptide levels increase during stressful situations, such as during ischemia\(^\text{61,62}\). Previous studies have demonstrated that the levels of beta-endorphins (an endogenous opioid agent) increase in patients with myocardial ischemia\(^\text{61}\) and after coronary angioplasty\(^\text{62}\). 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\(^\text{39}\).

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\(^\text{64}\). 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\(^\text{84}\).

Exercise and other stress conditions can increase the levels of opioid agents\(^\text{65}\). Howlett et al.\(^\text{66}\) 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\(^\text{87}\). Furthermore, during an incremental exercise test in humans, the blockade of opioid receptors by naloxone decreased test time, VO\(_{\text{max}}\), and maximal heart rate observed in response to exercise\(^\text{90}\); 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.
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. Furthermore, Michelsen et al. and Galvão et al. 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. 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. 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.

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. showed that exercise-induced ST-segment depression was markedly attenuated in high-risk patients during consecutive exercise sessions. Similarly, Lambiase et al. 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, involvement of the NO pathway, increase in the cardiac antioxidant capacity, increase in KATP channel function, and activation of the opioid system.

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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