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336 *Current Cardiology Reviews,* **2014,** *10,* **336-348**

Cardioprotection Acquired Through Exercise: The Role of Ischemic Preconditioning

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Abstract: A great bulk of evidence supports the concept that regular exercise training can reduce the incidence of coronary events and increase survival chances after myocardial infarction. These exercise-induced beneficial effects on the myocardium are reached by means of the reduction of several risk factors relating to cardiovascular disease, such as high cholesterol, hypertension, obesity etc. Furthermore, it has been demonstrated that exercise can reproduce the "ischemic preconditioning" (IP), which refers to the capacity of short periods of ischemia to render the myocardium more resistant to subsequent ischemic insult and to limit infarct size during prolonged ischemia. However, IP is a complex phenomenon which, along with infarct size reduction, can also provide protection against arrhythmia and myocardial stunning due to ischemia-reperfusion. Several clues demonstrate that preconditioning may be directly induced by exercise, thus inducing a protective phenotype at the heart level without the necessity of causing ischemia. Exercise appears to act as a physiological stress that induces beneficial myocardial adaptive responses at cellular level. The purpose of the present paper is to review the latest data on the role played by exercise in triggering myocardial preconditioning.

Keywords: Cardioprotection, cardiovascular disease, heart, ischemia, remote preconditioning, warm-up.

INTRODUCTION

The notion that regular exercise is cardioprotective is supported by numerous human epidemiological studies. According to the World Health Organization, cardiovascular diseases will become the major cause of death in the world as a whole by the year 2020 and will exact human and economic costs that will be unparalleled by any other single disease [1]. The fact that physical activity is cardioprotective and that physical inactivity is a risk factor for these pathologies is supported by a great quantity of scientific research. Furthermore, there is overwhelming proof that an individual's exercise capacity is a strong predictor of increased risk of death from any cause in both healthy subjects and in those with cardiovascular diseases [2].

The concept that regular exercise confers protection against coronary disease can be traced to the seminal work of Morris and co-workers [3] and it has been extensively investigated since in a number of studies which have demonstrated that regular exercise is beneficial for the cardiovascular apparatus since it decreases the incidence of myocardial infarction and increases the chances of survival after coronary events [4-16]. Regular physical activity, assessed over a mean of 2.4 years, was associated with a 27% reduction in total mortality and a 31% reduction in cardiac mortality [17]. Moreover, it is a well-established and useful tool in rehabilitation for stable coronary insufficiency and after infarction [18-20], and the evidence supporting the beneficial effect of physical training in patients with coronary disease is good [21]. Yet, the relative risk of coronary artery disease has been estimated to be circa 2 fold higher for inactive subjects compared with physically active individuals [22].

However, regular exercise protects against chronic and acute coronary disease through mechanisms which have not yet been completely elucidated. It is believed that exercise operates by the reduction of several risk factors related to cardiovascular pathologies, including high blood pressure, dis-lipidemia, obesity, insulin resistance, and autonomic disregulation [18, 23, 24].

Moreover, physical training can also reduce vascular resistance and induce structural adaptations in the coronary tree (i.e. increased number of capillaries and number and size of arteries and arterioles), thereby enhancing the blood transport capacity at this level [25]. Much attention has also been paid to the capacity of exercise to improve functions of the vascular endothelium. Indeed, the vasculature is the largest organ in the body and endothelial cells are important in regulating some key functions in homeostasis such as platelet aggregation, immune responses, and vascular permeability. Moreover, endothelium maintains vascular tone, thereby determining blood flow distribution to each tissue [26], and produces numerous substances, including nitric oxide (NO), which is important in regulating vasomotor function and in maintaining the health of the vascular wall [27]. It is now established that exercise can improve endothelial functions and endothelial-dependent vasodilatation as well as increase gene expression for endothelial NO synthase (eNOS) [28-31]. Finally, it has recently been found both in humans and in animals that physical exercise can also mimic the "Ischemic Preconditioning" (IP) phenomenon, which refers to the capacity of short episodes of ischemia to render the myocardium more resistant to subsequent more prolonged ischemic events [32-35]. These recent findings further

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strengthen the potential benefit of exercise on chronic and acute coronary disease.

The purpose of this article is to review the latest data on the potential role of exercise in inducing the preconditioning phenomenon. The general features of ischemic preconditioning are briefly reviewed in the first part, whilst the second deals with the potential role of exercise in triggering the cardioprotection afforded by preconditioning.

ISCHEMIC PRECONDITIONING: GENERAL CONCEPTS

The heart is able to change its phenotype in response to various kinds of stress in order to become more resistant to subsequent injury. This is well testified by phenomena such as stunning, hibernation, and preconditioning [36]. This latter condition identifies the capacity of various kinds of stimuli such as ischemia, rapid pacing, heat stress, exercise, and drugs to put the heart in a defensive status which is more resistant to ischemia.

The classical form of preconditioning is ischemic preconditioning (IP), which refers to the capacity of brief sublethal episodes of myocardial ischemia to protect against cellular damage due to more sustained periods of ischemia able to induce infarction. IP was first reported by Murry and co-workers [37] in dogs, where it was found that IP could greatly reduce infarction size. This necrosis-sparing effect was remarkable and reduced necrosis from an expected 30% to only 7% of the risk area. Moreover, it is to be emphasized that protection was unrelated to the development of collateral flows. Importantly, in the years that followed this original observation in dogs, the infarct-sparing effect of IP was reproduced in several mammalian species, including the rat, the rabbit, the swine, and the goat [38-42]. Since then the phenomenon has been recognized as "the strongest form of *in vivo* protection against myocardial ischemic injury other than early reperfusion" [36].

Along with the necrosis-sparing effect, IP has been demonstrated to protect the heart against damage caused by ischemia-reperfusion such as ventricular arrhythmias and myocardial stunning [43-46]. Moreover, IP can affect also coronary reactivity, as after preconditioning maneuvers the coronary vascular bed was found to respond to brief coronary occlusions with a reduced reactive hyperemic flow and a more rapid vasodilatation. The latter phenomenon was likely achieved through an increase in endothelial release of NO. Therefore, along with myocardial cell protection, IP can also exert beneficial effects on vascular endothelium responsiveness of the coronary tree [41].

Since experimentally-induced infarction in humans is not a feasible option, *in vivo* research to test directly whether or not IP can effectively reduce infarct size in humans is not possible. However, *in vitro* studies in isolated cultured human atrial myocytes have demonstrated that contractility recovery time after sustained ischemia was shorter if ischemia was preceded by a preconditioning maneuver [47, 48]. Furthermore, studies conducted in the course of coronary angioplasty have reported that the electrocardiographic (ST segment elevation) and clinical (chest pain) signs of myocardial ischemia were attenuated after the first balloon inflation, which can be considered as a preconditioning maneuver [49-51]. Yet, it is well established that if myocardial infarction is preceded by angina, which can be considered a form of ischemic preconditioning, then patients have smaller infarct size and better outcomes after thrombolytic therapy than patients without pre-infarction angina [52-56]. Therefore, these findings taken together support the concept that, like in animals, even human cardiomyocytes can be preconditioned. This fact suggests that IP can be induced in humans.

Finally, the "warm-up" phenomenon (i.e improved exercise performance and reduced severity of angina exhibited by some patients with coronary artery disease a few minutes after a previous effort) has also been considered a sort of ischemic preconditioning, mainly because the warm-up time course is consistent with that of IP. However, it should be taken into account that the opening of collateral flows together with some metabolic adaptations may in part be responsible for the phenomenon [57-62]. Hence, it is unclear at this point whether other mechanisms apart from preconditioning are involved in the warm-up.

As concerns the temporal characteristics, it is now established that IP is a biphasic phenomenon with an early phase of protection, commonly known as the first window of protection (FWOP), and a second phase, termed the second window of protection (SWOP). While FWOP is active immediately after preconditioning ischemia and lasts for about 2-3 hours, SWOP starts 12-24 hours after the initial ischemia and lasts 72-90 hours and confers a delayed protection [63, 64]. This late phase of protection is particularly attractive in a clinical perspective because of its sustained duration. Apart from the duration, other differences between FWOP and SWOP exist. Indeed, SWOP is more effective than FWOP in attenuating myocardial stunning, whereas FWOP exerts a more pronounced effect in reducing infarct size [65, 66].

ISCHEMIC PRECONDITIONING: RECEPTORS, MEDIATORS AND EFFECTORS

The chain of cellular events that confers resistance to ischemia is not completely understood. It has been reported that the accumulation of intracellular adenosine, produced from ATP degradation during the brief ischemic periods of preconditioning preceding the sustained ischemia, interacts with adenosine receptors A_1 and A_2, thus activating a complex metabolic cascade that eventually induces myocardial protection [38, 67]. The fact that adenosine agonist administration confers protection, whilst A_1 receptor blockers eliminate the preconditioning effects bears witness with the concept that adenosine plays a role in the cardioprotection of IP [38, 68]. The role of adenosine has also been confirmed *in vivo* preconditioning of the human myocardium. Indeed, it was found that adenosine infusion could attenuate the ischemic signs due to balloon inflation during angioplasty maneuvers, thus strengthening the role played by this metabolite in IP [69, 70].

In addition to adenosine, the ischemic heart also releases other end-products and metabolites, such as reactive oxygen species (ROS), reactive nitrogen species (RNS), bradykinin, and opioids, which are all considered to be capable of inducing preconditioning [71-74]. All these substances seem to work in parallel by means of interaction with their specific

receptors which activate the protein kinase C (PKC) [67]. Probably, a threshold for IP exists and the accumulation of each single metabolite alone is insufficient to trigger the threshold required for protection, i.e. their effects can sum to exceed the threshold to initiate IP and if any of the metabolites is eliminated by an antagonist, the hypothesized threshold can no longer be reached [67].

A particular role is played by ROS and RNS. Both ROS and RNS are involved in normal cell regulation in which oxidants and redox status are important in signal transduction [75-78]. Several studies report that ROS/RNS are among the principle responsible for the reversible protein kinase activation observed after ischemia, as their production may induce either reversible or irreversible activation of proteins/enzymes [79-81]. Conversely, the ROS/RNS scavengers block preconditioning cardioprotection [78, 82-84]. Although ROS/RNS production (redox stress) may be detrimental, from these studies it appears that their production may be also beneficial (redox signalling) [78, 85]. In fact, excessive ROS and RNS formation during reperfusion that follows infarcting ischemia may enhance cell death, while low concentrations of ROS/RNS modulate cell signalling processes during reperfusion and they are essential for the cardioprotection induced by IP.

Hence, PKC activation appears to be the common path along which all preconditioning stimuli converge. It is noteworthy that oxygen-free radicals can directly activate PKC, without the need to interact with a specific receptor [42]. PKC eventually phosphorylates some unknown effectors responsible for ischemic resistance. The metabolic events following PCK activation are only partially understood. Other kinases besides PKC have been identified as being potentially involved in the IP metabolic chain. Among others, tyrosine kinase seems to be downstream of PCK in this cascade [86]. Moreover, several mytogen activated protein kinases (MAPK) seem to exist downstream of PKC and play a role in cardioprotection. However, this topic is a much debated chapter in the history of IP [42] and exhaustive attention to it was not foreseen in this review.

Notwithstanding the debate over the metabolic cascade following PCK activation, there is general consensus that, at least for FWOP, the final effectors of the cardioprotection induced by IP are the ATP-sensitive potassium (K_{ATP}) channels, although it should be considered that it remains still unknown the exact mechanism which leads to cellular resistance to ischemia. These channels are normally closed in the non-ischemic heart, i.e. when the cellular level of ATP is normal. However, when ischemia occurs the fall in intracellular ATP level open these channels. Moreover, they are also modulated by ADP, NO, pH, fatty acid, G-proteins and various ligands such as adenosine, acetylcholine etc. [87, 88].

The fact that the opening of K_{ATP} channels was involved in the IP phenomenon was first proposed by Gross and coworkers [89]. Since then, it has also been demonstrated that K_{ATP} blockers can abolish IP-induced protection [90, 91] and that channel openers such as cromakalin, bimakalin, or pinacidil can mimic IP protection [92, 93]. Moreover, the importance of K_{ATP} channels also results from the clinical observation that diabetic patients in treatment with glibencamide and other sulfonylureas, which block these channels, exhibit increased mortality from cardiovascular causes and have poorer outcomes at the time of myocardial infarction [94, 95]. Actually, the opening of K_{ATP} channels may trigger several actions against cellular damage. Among others, it may induce depolarization of the cellular membrane and shortening of the action potential, thus preventing Ca^{2+} entry and overload, a phenomenon that may lead to cell death. Furthermore, the shortening of action potential spares energy. All these effects are potentially beneficial and may in part explain the protective action of K_{ATP} channels opening during ischemia.

Recent findings showing that cardiac cells contain at least two different types of K_{ATP} channels, sarcolemmal (sK_{ATP}) and mitochondrial (mK_{ATP}), have contributed to a greater understanding of IP. It has recently been proposed that the mK_{ATP} channels are the true final effectors of IP. In fact, several findings argue against a role of sK_{ATP} channels: for example, bimakalim was found to reduce infarct size at a dose that did not affect action potential duration [96], and this action clearly excludes the sK_{ATP} channels' involvement in the phenomenon; in simulated ischemia models of cultured cardiomyocytes, which were quiescent and did not generate any action potential, it was still possible to induce protection through the administration of K_{ATP} channel openers [97], and this fact means that action potential shortening is not mandatory for cardioprotection; finally, and perhaps most importantly, the use of diazoxide, a K_{ATP} channel opener 1000 to 2000 times more potent in opening mK_{ATP} than sK_{ATP} channels [98], has been demonstrated able to induce cardioprotection in the micromolar range without shortening the action potential, thereby suggesting that the opening of sK_{ATP} channels is not mandatory; yet the specific blocker of mK_{ATP} 5-hydroxidecaonate blocks the cardioprotection induced by mK_{ATP} openers [98, 99]. All these facts strongly suggest that mK_{ATP} channels are the real effectors of the cellular metabolic cascade that confers preconditioning and make it necessary to reconsider the role of sK_{ATP} channels as effectors of preconditioning. However, it is still unclear why opening mK_{ATP} channels is cardioprotective and several not mutually exclusive hypotheses have been developed [100-107].

It is to be noted that the early moments of reperfusion after sustained ischemia are crucial since significant reversible and irreversible organ damage is triggered. This period can lead to reperfusion injury such as arrhythmias, transient mechanical dysfunction, microvascular injury, and inflammatory response [85, 108]. Recently, regulators of mitochondrial membrane permeability have been reported to play a critical role in the IP phenomenon [78, 85, 109]. In detail, mitochondrial permeability transition pores (mPTP) have been found involved in the reperfusion injury following the ischemic insult. These pores open in the reperfusion period, when cellular pH increases after its reduction occurring in the ischemic phase. The consequence of long-lasting mPTP opening is the collapse of mitochondrial membrane potential, matrix swelling, rupture of mitochondrial membrane, and ultimately apoptosis. It has found that IP can delay the post-ischemic recovery of intracellular pH, thereby preventing mPTP opening directly and indirectly [85, 110]. Hence, it is conceivable to hypothesize

that IP, by counteracting mPTP opening, can protect the heart from damage due to the ischemia/reperfusion period.

The putative mechanisms of IP cascade during the FWOP period are schematically illustrated by (Fig. **1**).

If the molecular cascade of FWOP is complex and only partially understood, the cellular events leading to delayed protection, i.e. the SWOP period, are even more complex and less understood. As previously stated, SWOP shows a particular time course, as it starts several hours after FWOP has ceased, i.e. about 12-24 hours after the preconditioning stimuli, and lasts 72-90 hours. This fact is generally interpreted by taking into account that gene expression and new protein synthesis are mandatory for the protection afforded by SWOP [65, 111]. Similarly to what has been described for FWOP, there are also putative triggers, mediators, and final effectors for SWOP. However, exhaustive attention to this particular topic was not foreseen in the present manuscript and readers should refer to other papers for a detailed review of this issue [42, 65, 66]. Here, we believe that elucidating the general features of SWOP should suffice to understand the phenomenon.

As for FWOP, adenosine [112-114], bradykinin [115, 116], oxygen free radicals [117-119], and Opioids [120, 121] are all believed to initiate the cascade which leads to SWOP. Thus, the same stimuli that initiate FWOP can also induce SWOP. It is widely accepted that the cellular kinases that open mK_{ATP} channels in early preconditioning can also activate the transcription of genes that codify some cardioprotective proteins. These probably include inducible NO synthase (iNOS) [122-125], Cyclooxygenase-2 [126, 127], Aldose Reductase [128], Super Oxide Dismutases [129, 130], and Heat Shock Proteins (HSPs) [131-134].

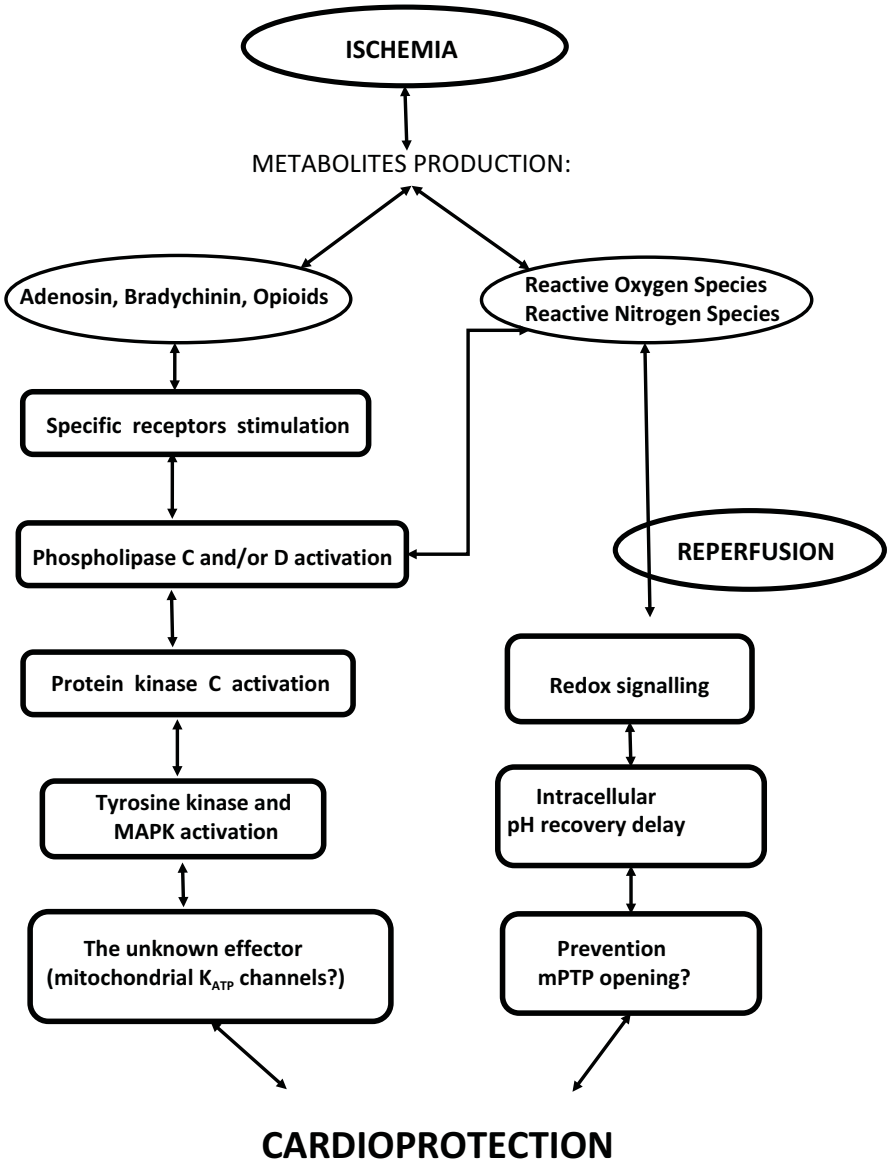

Fig. (1). Putative mechanisms of IP cascade during the first window of ischemic protection. Ischemia causes the production of some metabolites that, in turn, activate Protein Kinase C. This leads to activation of several cell kinases, such as Tyrosine kinase and MAPK. An unknown effector (possibly mitochondrial K_{ATP} channels) is downstream of this cascade. Redox signalling due to reactive oxygen and nitrogen species production also contributes by preventing mPTP opening during reperfusion. See text for more details.

The final effectors of delayed preconditioning are still unknown. Delayed preconditioning appears to be of a polygenic nature and that the heart shifts to a defensive phenotype requiring a complex coordinated activation of multiple genes [65]. Hence, there is probably no single end-effector of protection, but probably the contemporary expression of the same proteins may induce the protective status through several different actions.

Some evidence suggests that mK_{ATP} channel opening may also be a component of delayed preconditioning. In animal model of IP (rats and rabbits) it has been found that diazoxide applied 24 hours before ischemia is able to induce protection against infarction [135] and that this protection can be eliminated by treatment with the mK_{ATP} channels selective blockers 5-hydroxydecanoate [136]. Recently, it has also been reported that delayed preconditioning in humans requires the opening of mK_{ATP} channels [137]. Thus, it appears that mK_{ATP} channels are a component of SWOP, even though the specific importance of their involvement requires further clarification.

A particular role in the SWOP phenomenon is played by NO as it participates in the signaling pathway from FWOP to SWOP as well as acting as a trigger for SWOP. In detail, during the preconditioning maneuvers preceding FWOP, the reduction in pH that occurs in the coronary vessel activates a kininogenase which provokes the production of bradykinin which, in turn, through endothelial B_2 receptors interaction, stimulates NO production by eNOS [66, 124]. The NO generated by eNOS activates PKC and, in turn, the kinases responsible for the transcription of genes which codify the cardioprotective proteins of SWOP, including iNOS [138], which appears to be an essential mediator for delayed preconditioning [65, 122, 125, 139]. As mentioned above, NO also appears to act as a trigger for SWOP since it can directly open mK_{ATP} channels, thus rendering the cell more resistant to ischemia [140]. Bolli and co-workers [141] proposed a scenario where the NO generated early by eNOS initiates the preconditioning cascade that confers immediate protection and activates the iNOS that produces the NO involved in delayed protection. It is noteworthy that both in animals and in humans pre-treatment with NO donor under normoxic or ischemic conditions can mimic the effects of SWOP [35, 142-144]. In our opinion, it is useful to emphasize that delayed preconditioning is probably a universal mechanism whereby the heart responds to general stress, and that it can be activated by several situations such as heat shock, hibernation, pacing, and physical exercise [36, 65, 145].

EVIDENCE THAT EXERCISE CAN INDUCE PRECONDITIONING

Much evidence shows that exercise can induce preconditioning and protect the heart from ischemic insults without the necessity of a previous ischemia. Several studies conducted both in the animal and in the human setting strongly suggest that exercise provides myocardial protection against damage due to ischemia.

ANIMAL STUDIES

Increasing myocardial O_2 consumption with tachycardia may confer cardioprotection in animal models of IP [32, 146, 147]. This result was achieved without ischemia, thus suggesting that tachycardia alone may directly induce preconditioning. This finding leads to speculation that exercise-induced tachycardia might exert the same effect. However, the infarct-sparing effect due to exercise in conscious dogs was greater than that observed due to tachycardia alone in anesthetized dogs, thus suggesting that during exercise, stimuli other than increased myocardial O_2 consumption may activate the cascade that induces preconditioning [32, 147]. Moreover, in isolated hearts from exercise-trained rats it was found that the recovery of cardiac function after global ischemia was greater compared with hearts obtained from sedentary animals [148]. These results are in accordance with findings from other investigations in which it was reported that endurance exercise training enhances myocardial performance during ischemic/reperfusion maneuvers [149, 150].

Overall, the mentioned studies have repeatedly shown that both short-term and long-term exercise training decrease ischemia reperfusion-induced cardiac injury.

HUMAN STUDIES

In the human setting, recent observations during repeated exercise tests performed in patients with stable angina suggest that exercise can trigger the preconditioning phenomenon. As previously illustrated in the introduction, the warm-up phenomenon, which refers to the enhanced exercise performance and the reduced severity of angina shown by some patients with coronary disease a few minutes after a previous effort, has been attributed to a preconditioning-like effect. This mainly because the warm-up time course is consistent with that of FWOP. Furthermore, it has been demonstrated that, if the patient had performed previous exercise, the degree of myocardial stunning following exercise-induced ischemia may be attenuated [151] and that myocardial oxygen consumption was reduced during the warm-up compared to the first effort [152], thereby indicating that the warm-up might improve myocardial performance and metabolism during subsequent efforts. Nevertheless, the opening of collateral flows may potentially play a role in the warm up, thus confounding the interpretation of data. Indeed, warm-up angina has been related to coronary vasodilation despite the fact that arterial vasodilators have little effect on exercise tolerance [153]. Moreover, it has never been explained why enhanced myocardial performance afforded by the second effort appears after a rest period of at least 2-5 min following the first effort, but no more than 30-60 min [154], which is consistent with the FWOP time course. Lambiase and co-workers [33] have demonstrated that the protection due to IP is independent of collateral recruitment. Taken together, these facts support the concept that warm-up is an IP mechanism related phenomenon.

It should be underscored that improved exercising capacity and reduced signs of ischemia can still be present 24 to 48 hours after ischemic exercise, i.e. during the SWOP period, when collateral flows should already be closed. In detail, Lambiase and co-workers [33] were the first to demonstrate that 24 hours after exercise-induced ischemia there is enhanced resistance to further ischemia caused by exertion. These findings were then confirmed by Crisafulli and co-

workers in patients with stable angina [35]. These authors showed that, together with the reduced ischemic signs, global hemodynamics was also improved during exercise performed 48 hours after a previous exercise-induced ischemia and this fact provides clues that in the human being, along with FWOP (which is possibly coincidental with the warm-up phenomenon), SWOP can also be induced by efforts leading to ischemia.

Besides, Michaelides and co-workers [34] showed that most patients (37/50) with coronary insufficiency who underwent a sequence of repetitive exercise had improved myocardial performance measured with thallium-scintigraphy during the last of the sequential tests. Authors concluded that repetitive exercise induced cardiac adaptations, which may represent an aspect of ischemic preconditioning.

Moreover, by employing a sequence of repeated treadmill efforts, it has recently been reported that in the FWOP period (i.e. 30 minutes after a previous ischemia-inducing exercise) signs of the presence of cardioprotection (reduced ST segment depression, increased rate pressure product and longer onset of chest pain) were present. However, these signs disappeared 6 hours later, during the non-protecting period between the fading of FWOP and the appearance of SWOP, to return after 24 hours (i.e. during SWOP) [154]. Nevertheless, it should be mentioned that one study failed to demonstrate the possibility of inducing SWOP in humans by exercise-induced ischemia [155].

Therefore, it appears that the human heart may be preconditioned by exercise, although direct proof (the necrosis-sparing effect) that exercise is cardioprotective is still lacking. Furthermore, it should be highlighted that all the evidence demonstrating the possibility of preconditioning the human heart with exercise derives from studies on patients with coronary disease, in whom the preconditioning effect is triggered by exercise-induced myocardial ischemia. On the contrary, the attempt to enhance cardiac performance in healthy subjects by means of a sequence of maximal tests on a cycle-ergometer and by pharmacological-induced SWOP failed, thereby suggesting that ischemia must occur to detect any beneficial cardiac effect [156]. Thus, to the best of our knowledge, the capability of exercise to recruit the preconditioning phenomenon without the occurrence of ischemia is still to be demonstrated in humans, even though the aforementioned studies carried out on animals provide evidence supporting this possibility.

It should also be considered that the quoted investigation in humans focused on the preconditioning effects due to exercise-induced ischemia while, as yet, no studies have dealt with the effects of regular exercise training programs. This kind of exercise are normally conducted at sub-ischemic threshold intensity. As a consequence, no information is available on thresholds for exercise duration and severity to reach the beneficial effects of preconditioning induced by exercise. In this light, there is a compelling need for studies which investigate on the possibility of preconditioning the human myocardium with exercise and further establish what kind and at which intensity effort should be performed to trigger the preconditioning phenomenon.

EXERCISE AND PRECONDITIONING: POSSIBLE MECHANISMS

Although the mechanisms responsible for exercise-induced cardioprotection remain elusive, some exercise-induced cellular adaptations are likely to be involved in the phenomenon. Probably, exercise can activate the multiple downstream kinase cascade responsible for cardioprotection. Actually, studies performed on animals have demonstrated a necrosis-sparing effect and improved myocardial function after ischemia following exercise training [157-160]. However, at this stage, the precise mechanism through which exercise operates is only speculative and several possible mechanisms have been postulated.

As previously described, exercise-induced tachycardia alone may trigger preconditioning. Furthermore, several metabolites such as adenosine, bradykinin, and opioids are released during exercise. These metabolites are known to induce classic preconditioning, and this fact alone may explain the capability of exercise to recruit the preconditioning phenomenon. Moreover, the possibility that exercise may induce preconditioning by directly causing myocardial hypoxia cannot be ruled out. However, it has been found that a single episode of moderate sub-maximal physical exercise unlikely to induce hypoxia could confer cardioprotection on the rat heart. This protection has been explained through a PKC-mediated mechanism, since pharmacological inhibition of this enzyme during exercise resulted in the abrogation of protection [161]. These results suggest that, for the activation of the preconditioning cascade, hypoxia is not a prerequisite and that the PCK activity may be directly modulated by exercise.

Acute exercise generates ROS and does so in an intensity and duration-dependent manner [162]. The old view of exercise is a potential source of harmful oxidative damage has been changed. In fact, muscle derived ROS produced during prolonged inactivity contribute to muscle atrophy whereas the same stimulus coming from working fibers is essential for adaptations induced by training. This apparent paradox may be explained by the hormesis theory: chemical and toxic substances that are deleterious at high doses can have at low dose beneficial effects. Thus, increases in ROS due to exercise could induce beneficial adaptations [162]. It is noteworthy that antioxidant supplementation can reverse beneficial exercise adaptations [163, 164] The formation of ROS during exercise may be another potential exercise-related mechanism underlying cardioprotection [165, 166] since ROS production can directly activate PKC, without the need to interact with a specific receptor. Furthermore, physical training could up-regulate myocardial anti-oxidant capacity in order to overcome the oxidative stress caused by exercise. Indeed, myocardial Manganese Super Oxide Dismutase and extracellular Superoxide Dismutase levels have been reported to be increased in several studies [34, 160, 167]. The up-regulation of Super Oxide Dismutases is believed to be involved in SWOP, thus providing another mechanism through which exercise can act to protect the heart from ischemic insults.

A key role in cardioprotection due to physical exercise is probably played by NO production. It is well known that exercise increases shear stress within vessels. Shear stress is

considered one of the most important mechanical stimuli leading to NO production and, actually, exercise enhances NO production [25, 29]. As stated in the previous paragraph, NO acts both as a trigger and mediator of SWOP. In fact, exposure to NO donors can reproduce the effects of delayed preconditioning in the absence of ischemia [35, 144]. Furthermore, NO is also believed to participate in FWOP protection, although conflicting results exist on whether NO action during FWOP exerts a beneficial effect or simply triggers SWOP [124]. Thus, it is possible to speculate that exercise-induced increase NO production is a further mechanism by which physical training acts in order to induce cardioprotection. It is also to be considered that skeletal muscle also generates RNS including NO or nitrite ion [162, 168], which at high doses may cause nitrosative stress and tissue damage, but at low doses exerts beneficial effects in signalling transduction, as previously described.

Exercise can act also by increasing the expression of cardiac stress proteins, which are likely to be involved in myocardial protection [169, 170]. In this regard, HSPs have been extensively studied and it has been found that the myocardial level of these proteins can increase up to threefold in response to exercise [169]. It is believed that HSPs can protect cells from a variety of stresses including ischemia-reperfusion injury [138]. However, their contribution to the cardioprotection afforded by exercise remains controversial since the cardioprotection lasts longer than HSP half time [171].

Finally, the phenomenon known as "remote preconditioning", which refers to the possibility of preconditioning the heart by causing ischemia in a remote organ, should also be taken into account. This particular kind of preconditioning has been demonstrated by inducing transient ischemia in several organ and tissue such as the small intestine, kidney, and skeletal muscle [172-175] and it has been shown to induce both early and delayed cardioprotection [176-178]. Probably, remote preconditioning is initiated by humoral factors released into the blood and transferred to the heart where they trigger protection [179]. In the genesis of the remote preconditioning key molecules appear to be adenosine and bradykinin, although the exact mechanisms involved are still unknown [175, 180].

It must also be considered that amount of products deriving from the anaerobic metabolism (such as lactate, ADP, adenosine etc.) is released into the blood during exercise, even in non-ischemic conditions [15, 181, 182], i.e. without the flow reduction setting used to cause remote preconditioning in the aforementioned experiments. It is therefore conceivable to hypothesize that these substances produced during exercise may trigger preconditioning, but to the best of our knowledge this hypothesis has never been verified.

It has been recently demonstrated that dialysated plasma from humans undergoing high-intensity exercise reduced infarct size in isolated rabbit hearts after ischemia-reperfusion injury. This phenomenon was also present with plasma from humans exposed to remote ischemic preconditioning [183]. Authors of the quoted study concluded that exercise-induced cardioprotection was at least partially mediated by systemic release of one or more humoral factors. A similar outcome was also obtained in the mice heart perfused with dialysated plasma from highly trained humans (swimmers) undergoing a protocol of ischemia-reperfusion to trigger the remote preconditioning phenomenon. In this study, along with the infarct reduction effect in the mice heart, the remote ischemic preconditioning maneuvers were also able to enhance the athletic performance during swimming [184]. The fact that remote preconditioning is able to improve exercise performance has been also demonstrated by a series of studies reporting that IP can improve oxygen uptake, maximal power output, blood lactate accumulation, and time to exhaustion in tests performed in the laboratory setting [185-193]. Furthermore, remote ischemic preconditioning has been found able to improve endothelium-dependent function after strenuous exercise [188]. The effect of remote ischemic preconditioning on the endothelium has been attributed to humoral mechanisms that lead to increased activation of ATP-sensitive potassium channels and increased concentration of NO [189-191]. Yet, a recent investigation reported that the ischemic preconditioning induced by intermittent upper-arm ischemia done before primary percutaneous coronary intervention could attenuate reperfusion injury in patients with evolving myocardial infarction, thereby resulting in increased myocardial salvage [192]. Collectively, these data strongly suggest that remote ischemic preconditioning can act as a mechanism whereby the heart can be preconditioned by exercise.

The major putative mechanisms responsible for the exercise-induced preconditioning discussed in the present paragraph are shown by (Fig. **2**).

CONCLUSIONS

Although the exact mechanisms underlying exercise-induced cardioprotection against ischemia still need to be elucidated, there are continuing advances in the understanding of the phenomenon. From the cited studies it appears that exercise acts as a physiological stress that, similar to that due to sub-lethal ischemia, heat, caloric restriction, and hypoxia can enhance cellular defense [193] and induce a defensive phenotype in the heart, which probably depends on the contemporary production of several molecules and proteins. These changes at cellular level render the heart more tolerant to ischemic insult and damage. However, the mechanisms responsible for the phenomenon are only partially understood and require further investigation. In humans, the direct demonstration of the possibility of preconditioning the heart of healthy individuals are still lacking, notwithstanding a preconditioning effect due to ischemia has been observed in patients suffering from coronary disease (i.e. the "warm-up"). Numerous unanswered questions still remain such as. In particular, describing the specific pathways and mechanisms that are involved in exercise-induced cardioprotection is crucial for therapeutic intervention. Moreover, the characterization of exercise intensity and duration required to trigger protection due to ischemic preconditioning is another important topic that needs to be investigated. This is particularly important for patients suffering from coronary heart disease, as it is generally accepted that exercise training should be conducted below the threshold for myocardial ischemia. However, patients may be deprived of the potential benefits of more intense exercise by the use of such a protocol. In controlled conditions, ischemic exercise training does

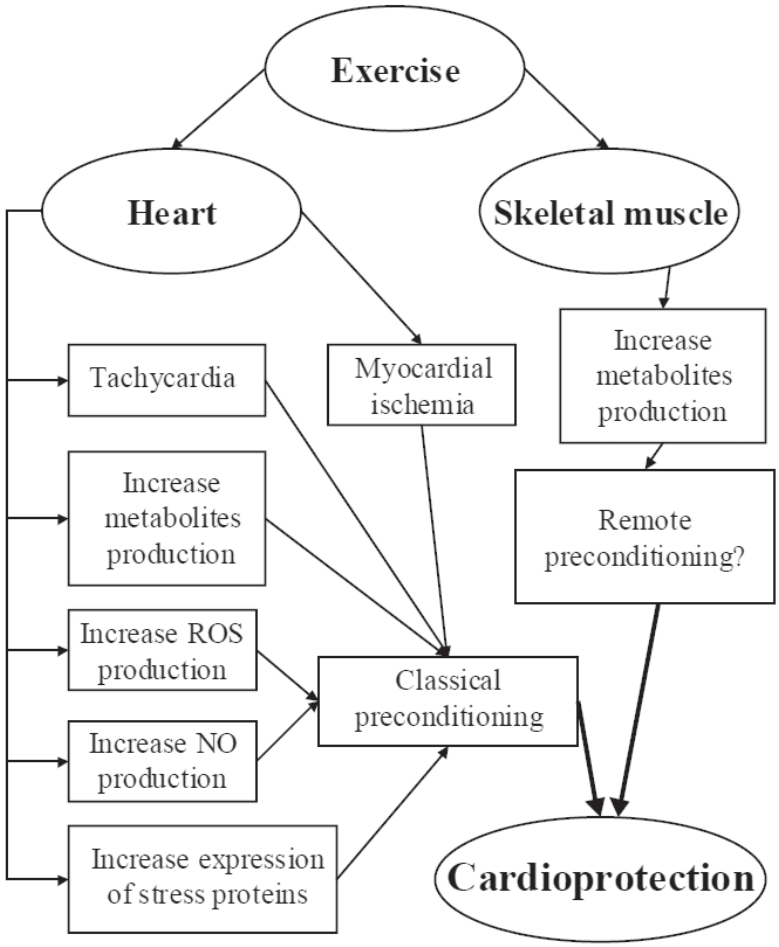

Fig. (2). Putative mechanisms responsible for exercise-induced preconditioning: exercise directly affects the heart by means of ischemia, tachycardia, NO, ROS, other metabolites, and stress protein production. Remote preconditioning is a further possible mechanism by which affects the heart. See text for more details.

not seem to be harmful and it may afford protection induced by IP [194]. Thus, it is possible to speculate that introducing short periods of ischemic training in these patients may be effective in inducing the IP phenomenon.

Further research is warranted to address all these issues. In any case, it appears that regular exercise should be recommended not only to obtain the beneficial effects on conventional cardiovascular risk factors, but also to achieve the preconditioning status [195, 196]. Currently, given the lack of a therapeutic target, the only available practical method of inducing ischemic preconditioning at the heart level is exercise training [197].

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

This study was supported by the University of Cagliari and the Italian Ministry of Scientific Research. The authors wish to thank Mr. Barry Mark Wheaton for his editorial assistance.

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Received: May 14, 2013 Revised: December 16, 2013 Accepted: March 28, 2014