EVIDENCE FOR EXERCISE-INDUCED CARDIOPROTECTION

The ability of physical exercise to decrease the symptoms of coronary heart disease has been known for many years. Perhaps the first documented observation in this area was made by the eminent English physician, William Heberden, in the mid-18th century. Heberden, who coined the term angina pectoris, anecdotally noted that, “With respect to the treatment of this complaint, I have little or nothing to advance. . . . [although] I knew of one who set himself a task of sawing wood for half an hour every day, and was nearly cured” of subsequent bouts of angina (32). Countless studies since Heberden’s initial observation have noted that exercise can lower a number of risk factors for cardiovascular disease, including hypertension, hyperlipidemia, and obesity. Epidemiological evidence also indicates that humans who exercise regularly are more likely to survive an ischemic event (47), even in senescence (1), and that exercise itself could actually precondition the myocardium was first promulgated in the late 1970s.

In 1978, McElroy et al. (43a) demonstrated that hearts of rats exposed to swimming for 5 wk had ~30% smaller infarctions than the sedentary controls after a permanent coronary occlusion. For the ensuing two decades after the study of McElroy et al., little additional insight was provided in the exercise-induced preconditioning area. The majority of studies examining exercise and infarct size were in the context of postinfarction rehabilitation and remodeling. Subsequent to the emergence of preconditioning, renewed attention has been devoted to prevention of tissue damage after infarction, with recent research revisiting the protective effects of exercise in delaying cell death.

Since the late 1990s, a number of studies have shown that hearts from animals exposed to acute (1–7 days) exercise show significantly smaller infarcts than sedentary counterparts after the same index ischemia (Table 1 and/or Fig. 1). Using both in vivo and in vitro models of ischemia-reperfusion,
Infarct size reductions 24 h following short-term exposure to treadmill running have been observed in rat, mouse, and dog, with the most common model of exercise being forced treadmill running. The exercise-mediated protection varied from 20 to 80% reductions in infarct size compared with control, with the average protection across studies being about a 40% reduction (Fig. 1).

While the findings of short-term exercise studies are of considerable interest, the afforded protection is by no means unique; a large (and constantly growing) number of interventions can precondition the heart when administered acutely (see Refs. 11, 12, 70 for review). While mechanistic insight is gleaned from studies administering (pharmacological or non-pharmacological) preconditioning agents transiently, the clinical relevance of such strategies must be questioned, as humans rarely know when an ischemic event will occur and are unlikely to precondition themselves a priori. As such, optimal preconditioning strategies must be sustainable; in other words, the protection should be sustainable and last for months or years. Relating to sustainability, exercise preconditioning distinguishes itself from most other preconditioning treatments. For example, two such preconditioning agents, prior ischemia (8, 58, 64) and adenosine receptor agonists (7, 9, 63), both provide delayed preconditioning against subsequent ischemia. However, if administered repetitively, these treatment are no longer effective in providing cardioprotection (20, 66).

That exercise confers protection against ischemia-reperfusion injury that is sustainable over months was first demonstrated by

### Table 1. Experimental characteristics for exercise-induced cardioprotection studies to date

<table>
<thead>
<tr>
<th>Reference</th>
<th>Animal</th>
<th>Ischemia-Reperfusion</th>
<th>Exercise</th>
<th>Sex</th>
<th>Candidate Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akita (2)</td>
<td>mouse</td>
<td>in vivo</td>
<td>7-day run</td>
<td>male</td>
<td>NOS</td>
</tr>
<tr>
<td>Chicco et al. (19)</td>
<td>rat</td>
<td>in vitro</td>
<td>5-day run</td>
<td>male and female</td>
<td>sarcKATP, anti-ox, HSPs</td>
</tr>
<tr>
<td>Marini et al. (43)</td>
<td>rat</td>
<td>in vivo</td>
<td>14-wk run</td>
<td>male</td>
<td>anti-ox, HSPs</td>
</tr>
<tr>
<td>Chaves et al. (17)</td>
<td>rat</td>
<td>in vitro</td>
<td>10-wk run</td>
<td>male</td>
<td>anti-ox, HSPs</td>
</tr>
<tr>
<td>Brown et al. (14)</td>
<td>rat</td>
<td>in vitro</td>
<td>12-wk run</td>
<td>female</td>
<td>sarcKATP</td>
</tr>
<tr>
<td>Freimann et al. (21)</td>
<td>rat</td>
<td>in vitro</td>
<td>11-wk swim</td>
<td>male and female</td>
<td>sarcKATP, anti-ox, HSPs</td>
</tr>
<tr>
<td>Brown et al. (16)</td>
<td>rat</td>
<td>in vitro</td>
<td>5-day run</td>
<td>male and female</td>
<td>MnSOD</td>
</tr>
<tr>
<td>Brown et al. (15)</td>
<td>rat</td>
<td>in vitro</td>
<td>12-wk run</td>
<td>female</td>
<td>MnSOD</td>
</tr>
<tr>
<td>Hamilton et al. (28)</td>
<td>rat</td>
<td>in vivo</td>
<td>5-day run</td>
<td>female</td>
<td>MnSOD</td>
</tr>
<tr>
<td>Hoshida et al. (34a)</td>
<td>rat</td>
<td>in vivo</td>
<td>2-day run</td>
<td>male</td>
<td>MnSOD</td>
</tr>
<tr>
<td>Domonech et al. (20a)</td>
<td>dog</td>
<td>in vivo</td>
<td>1-day run</td>
<td>male</td>
<td>PKC</td>
</tr>
<tr>
<td>Yamashita et al. (67a)</td>
<td>rat</td>
<td>in vivo</td>
<td>1-day run</td>
<td>male</td>
<td>MnSOD</td>
</tr>
<tr>
<td>Yamashita et al. (68)</td>
<td>rat</td>
<td>in vivo*</td>
<td>5-wk swim</td>
<td>male</td>
<td>MnSOD</td>
</tr>
<tr>
<td>McElroy et al. (43a)</td>
<td>rat</td>
<td>in vivo</td>
<td>5-wk swim</td>
<td>male</td>
<td>collateral flow</td>
</tr>
</tbody>
</table>

NS, sex not specified; NOS, nitric oxide synthase; sarcKATP, sarcolemmal ATP-sensitive potassium channel; anti-ox, antioxidant enzymes; HSP, heat shock protein; MnSOD, manganese superoxide dismutase; PKC, protein kinase C. *Ischemic zone-at-risk was not defined.

![Fig. 1](http://example.com/fig1.png)

**Fig. 1.** Infarct size reductions (expressed as percentage reduction from the respective sedentary group) across a number of studies examining exercise-induced preconditioning. Solid and open bars represent studies where male and female animals were used, respectively. Shaded bars indicate that sex of the animal was not disclosed. Full citations are provided in the references section.
Brown et al. in 2003 (15). In this study, the infarct-sparing effects of exercise training were found in rats exposed to 3 mo of consecutive treadmill running. The sustainable nature of exercise cardioprotection now seems clear, with subsequent studies corroborating their initial results (14, 17, 21, 43) (Table 1 and/or Fig. 1). Given the potent capacity for exercise to elicit sustainable cardioprotection, identification of the mechanisms is warranted.

**CANDIDATE CELLULAR PROTEINS INVOLVED**

To better understand how exposure to exercise can provide such marked protection in delaying ischemic injury, it is necessary to shift focus to candidate cellular mechanisms involved in the protection. While a number of changes likely occur at the pre- and posttranslational level in hearts exposed to exercise, we will apply reductionist logic to narrow our attention to several key proteins and highlight recent exciting advances in this area.

Initial studies examining exercise protection focused on antioxidant enzymes and cellular stress proteins, specifically myocardial manganese superoxide dismutase (MnSOD) and the 72-kDa heat shock protein (HSP72). While several studies have noted increased MnSOD and HSP72 in myocardial tissue of trained animals (15, 28, 36, 51, 68), it has recently been shown that exercise-induced protection against several different indexes of ischemic injury can be attained without increased MnSOD (16, 62) or HSP72 (27, 65) expression. It seems likely that increased MnSOD and HSP72 are model specific and not central mediators or “end effectors” of exercise-induced protection.

There is now considerable evidence that potassium channels in the sarclemmal membrane are centrally involved in the protection afforded by exercise. Sarclemmal ATP-sensitive potassium (sarcKATP) channels were first discovered in heart and open in response to metabolic challenge (52). Specifically, channel gating is highly sensitive to the local balance of ATP (which promotes channel closure) and ADP (which stimulates channel opening) in the channel microdomain (reviewed in Ref. 3). Minimally comprised of four pore-forming [inwardly rectifying potassium 6.2 subunit (KIR 6.2)] and four accessory subunits (sulfonlurea receptor 2a), sarcKATP channels represent an important target in cardioprotective strategies (see Ref. 53 for review). Providing direct coupling between the energetic status of the cell to membrane excitability, sarcKATP channels are directly involved in the supply-and-demand matching scheme of biological tissues and are obligatory in reacting to metabolic stress such as ischemia. The vital role of sarcKATP channels in heart function is clearly indicated in humans, where sarcKATP channel mutations are accompanied by altered metabolic signaling and increased susceptibility to cardiomyopathy and arrhythmia (10, 57).

A clear, positive correlation exists between sarcKATP channel activity and protection from ischemia-reperfusion injury. Preconditioning, either by ischemia (26) or a number of other stimuli (7, 9, 22, 34, 45), has often been shown to depend on the activity of sarcKATP channels (although in many of these studies, a nonspecific blocker of KATP channels was used). sarcKATP channels also seem to be centrally involved in sex-specific cardioprotection. Female animals (including humans), which have frequently been observed to have smaller infarct sizes than males (6, 16, 19, 23, 37, 41, 44), have higher sarcKATP channel density (16, 61), with estrogen shown to upregulate sarcKATP channel subunit expression (60). The sex-specific protection mediated by sarcKATP channels has been confirmed in several studies where blocking the channels abolishes the protection intrinsic to female animals (19, 37). This important role for sarcKATP channel-mediated protection from infarction has led several laboratories to examine a putative role for sarcKATP channels in mediating exercise-induced protection.

There is growing evidence that sarcKATP channels are centrally involved in the protection afforded by exercise. Increased sarcKATP channel expression has been observed after just 1 or 5 days of exercise training and correlated with decreased infarction following ischemia (16). Notably, the increased sarcKATP channel expression is still present following months of exercise (14). Brown et al. (16) first indicated that the regulatory (sulfonylurea receptor 2) subunits of the channel, believed to be the rate-limiting factor in functional channel formation (61), were increased in hearts of rats exposed to acute exercise. Subsequent experiments have indicated that the increase in sarcKATP channel expression is even more marked following months of training, with increases in both the pore-forming and accessory subunits observed (14) (see Fig. 2 for representative blots). A central role of sarcKATP channels was again established when blocking sarcKATP channels abolished the protection elicited by both short-term (19) and chronic (14) exercise.

Further evidence indicating that sarcKATP channels are crucial for animals to adapt to the stress of exercise was observed in several studies by Terzic and colleagues. Zingman et al. (71) first exposed mice with genetically disrupted KIR 6.2 subunits to treadmill running and found that mice with dysfunctional sarcKATP channels were severely exercise intolerant compared with intact wild-type counterparts. KIR 6.2 knockout mice exercised at ~50% of the duration and maximal intensity than wild-type counterparts (71). Their finding was subsequently supported by Kane et al. (39), who observed that intact sarcKATP channels are required for the beneficial effects of a 1-mo swimming regimen. Mice lacking intact sarcKATP channels showed poor adaptation to the exercise stress, with sarcKATP knockout animals displaying significant cardiac damage and increased mortality on exposure to swimming. Taken together, there is clear evidence that sarcKATP channels play an important role in tolerating metabolic stress (reviewed in Ref. 40), and that the cellular capacity to handle metabolic stress is enhanced by exercise.

In addition to the sarcKATP channel, there is also an isofrom in the mitochondrion, although the molecular identity remains elusive to date. A number of studies have indicated that preconditioning from a variety of preconditioning agents is abolished by mitochondrial KATP channel blockade (7–9, 18, 34, 54–56, 58, 59, 63, 64). Interestingly, exercise training, the only one among these stimuli that has been shown to be sustainable over months, was not sensitive to inhibition by mitochondrial KATP channel blockade (14) (Fig. 2A). Importantly, we must note that virtually all of these studies examining the influence of mitochondrial KATP channel on mediating the protection used 5-hydroxydecanoic acid as a specific inhibitor of the mitochondrial KATP channel. Notwithstanding the lack of effect of this drug on exercise-induced protection, we must note that a number of researchers have questioned the
specificity of this compound (29–31), even noting that prolonged administration promoted intracellular calcium overload and severe mechanical dysfunction in normoxic rat hearts (14). While much more research is needed in the area of mitochondrial ion channels and exercise-induced protection, both the sustainable nature and 5-hydroxydecanoic acid-independent aspects of the protection represent a unique paradigm for preconditioning.

**sarcKATP CHANNELS AND CALCIUM HANDLING**

Given the clear role that sarcKATP channels play in exercise-mediated preconditioning, we will briefly comment on candidate mechanisms through which the KATP channels may provide protection. There is an emerging role for sarcKATP channels in maintaining myocardial calcium homeostasis, especially in conditions such as ischemia. Several separate investigations have noted an inverse relationship between the number of functional sarcKATP channels and the propensity for calcium overload in the face of metabolic stress (5, 25, 38, 60, 67, 71). Exactly how increased sarcKATP channels can decrease calcium overload has not been definitively resolved. A common postulation is that sarcKATP channel opening may shorten the action potential and subsequently decrease the calcium transient (50, 71), although it is important to note that other investigations have shown that the beneficial effects of sarcKATP channel openers can be obtained in the absence of noticeable shortening of the cardiac action potential (24, 69).

One putative scenario relating to the role of sarcKATP channels in decreasing injury may be as follows. At the onset of ischemia, the mitochondrial membrane potential depolarizes rapidly, quickly diminishing the ATP-generating capability of the heart. As sarcKATP channels are extremely sensitive to mitochondrial membrane potential (4), sarcKATP channel opening is likely to contribute to cessation of calcium cycling by decreasing the open probability of L-type calcium channels. By diminishing calcium cycling (and downstream contraction), decreased ATP usage due (indirectly) to KATP opening would promote a decline in energy demand to match the hindered supply. With prior exercise, increased channel density (24, 69) may enhance the metabolic sensing capability of the tissue, leading to decreased calcium overload and cell death in the face of cellular ischemia. While purely speculative, such a scheme would also be consistent with observations that exercise has also been shown to delay ischemic contracture (13, 15) and the depletion of myocardial ATP during ischemia (35). Clearly much more research in this exciting area is warranted before the mechanisms can be fully elucidated.

In summary, exercise-induced cardioprotection against ischemic injury appears to be very unique in that, unlike virtually every other known type of acquired cardioprotection, it is...
dependent on the operation of the sarcolemmal rather than the mitochondrial isoform of the myocardial \(\text{K}_{\text{ATP}}\) channel. More importantly from a clinical perspective, cardioprotection acquired through exercise is sustainable for long periods of time, a quality that appears to be lacking in other forms of acquired cardioprotection. Exercise also represents the most cost-effective preconditioning therapy, in contrast to putative pharmacological interventions. Clearly the potential clinical utility of exercise to mitigate the effects of myocardial ischemia is self-evident. Nevertheless, many key challenges remain. From a basic research perspective, these include the identification of the stimuli, the cellular signaling pathways, and the integrated cellular modifications that are invoked by exercise to establish the “protected” phenotype. From the vantage point of the clinician, key issues that remain to be clearly resolved include the determination of what intensity and duration of exercise are necessary to provide a cardioprotective adaptation. Equally challenging will be the elucidation of effective strategies to promote adherence to exercise prescription in at-risk patient populations. Indeed, much work lies ahead in this exciting and important field.

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