Saying Yes to Exercise and NO to Cardiac Injury

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Clinical studies demonstrate benefits of exercise in both preventing cardiovascular disease in the general population and mitigating existing disease in cardiovascular patients.1–3 Systemic effects of exercise on skeletal muscle and peripheral vessels as well as metabolism and insulin sensitivity undoubtedly contribute to these benefits. However, growing evidence from animal studies suggests that exercise also modulates intrinsic cardiac signaling mechanisms that contribute to its benefits. These benefits are probably best documented in models of ischemic injury but also appear to extend to heart failure in at least some experimental4 and clinical5,6 settings.

The effects of physical conditioning on ischemic injury can occur in as little as 3 to 5 days in experimental animal models.5 Interestingly, the protective effects of exercise are not limited to the immediate postexercise time period as in ischemic preconditioning but can persist for days afterward.6 In the current issue of Circulation Research, Calvert et al7 investigated the role of nitric oxide (NO) metabolites and β3-adrenergic receptor (β3-AR) signaling in this context through elegant studies in wild-type and genetically modified mouse models. Their work provides new insights into the mechanisms underlying exercise-induced cardioprotection and underscores the potential therapeutic relevance of these pathways.

Exercise increases expression of a variety of potentially protective proteins. These include heat-shock proteins (HSPs), both HSP27 and HSP90, as well as stress-related HSP72.8,9 Whereas overexpression of these proteins confers protection of mitochondria from myocardial ischemia-reperfusion injury (MI/R), the cardioprotective effects of exercise can also occur without significant elevations in HSPs.10 Other proposed cardioprotective proteins include upregulation of both mitochondrial and sarcoplasmal ATP-sensitive potassium channels, catalase, superoxide dismutase and ER stress proteins.11 Exercise also induces activation of PI3-Kinase/Akt signaling, which is required for physiological hypertrophy12,13 and mediates cardioprotection.14,15 However, none of these changes have been demonstrated in the dominant factor mediating the cardioprotective effects of endurance exercise and many of these changes return to baseline when exercise is discontinued even while the cardioprotection persists.

Strong evidence supports the cardioprotective effects of NO and its metabolites, including nitrite and nitrosothiols.16 Calvert et al7 set out to determine the role of NO and its metabolites in the cardioprotective effects of exercise, both early and after exercise is discontinued. Using a model of voluntary exercise in mice for a period of 4 weeks followed by varying periods of rest, the authors were able to confirm significant protection from MI/R after transient left coronary occlusion. In concordance with previous reports, there was a reduction in infarct area after ischemia-reperfusion injury occurring at the end of the training period, and this cardioprotective effect persisted up to 7 days after exercise was discontinued.7 The authors noted substantial increases in cardioprotective pathways including CuZnSOD and phosphorylated AMP-activated protein kinase (AMP-kinase) immediately after the period of exercise. However, these changes were not maintained 1 week after exercise was discontinued, making them unlikely to play a role in the persistent cardioprotection observed at this time point. In contrast, the authors found a nearly 2-fold increase in endothelial NOS synthase (eNOS) activity that persisted up to 1 week after the cessation of exercise. Increased eNOS activation was mediated by differential effects on activating (Ser1177) and inhibitory (Thr495) phosphorylation targets within eNOS without a change in overall expression of eNOS, iNOS, or nNOS. Evidence over that last several years has indicated that NO and its metabolites including nitrate are protective in MI/R.17,18 This effect has even been seen with increases in dietary nitrites,19 which underscores the ability of nitrite to mediate many of the effects attributed to NO, including vasodilation and protein nitrosylation.20 To further support the hypothesis that stimulation of eNOS in the heart is an important contributor to the cardioprotective effects of exercise, the authors examined both circulating and cardiac levels of NO metabolites, including nitrite, nitrate, and nitrosothiols. Exercise increased both circulating plasma and cardiac tissue levels of nitrite and nitrosothiols, which remained elevated 1 week after exercise was discontinued. Nitrate levels were not elevated immediately after the exercise period but were increased 1 week after exercise cessation. Together these data suggest a model whereby exercise acutely induces phosphorylation and activation of eNOS with increased nitrite and nitrosothiol levels, leading to subsequent accumulation of nitrates that can be reduced to nitrites.

In separate experiments, 1 week of voluntary exercise was followed by either 1 or 4 sedentary weeks. Whereas the cardioprotective benefits of exercise in MI/R were again seen 1 week after exercise cessation, they were lost by 4 weeks. Nitrite, nitrate, and nitrosothiols had all returned to baseline levels by this time point, consistent with their hypothesized...
role in exercise-induced cardioprotection. Interestingly, although serum nitrite and nitrate levels in human subjects who participated in regular endurance training were similar to those in nontrained individuals, nitrosothiol levels were higher in trained subjects, providing some evidence that exercise activates similar pathways in humans.

To formally test the role of eNOS in exercise-induced cardioprotection, Calvert et al examined the effects of exercise in eNOS-deficient mice (eNOS−/−). Four weeks of voluntary exercise did not induce cardioprotection against MI/R in eNOS−/− mice. Interestingly, eNOS−/− mice did not exercise as much as wild-type mice. Although the lack of a cardioprotective effect is consistent with the hypothesized role of eNOS activation and NO metabolites, these experiments do not fully exclude the possibility that the lower level of exercise achieved was insufficient to induce cardioprotection for some other reason. Even if we accept the plausible explanation that the absence of eNOS precludes cardioprotection specifically through the loss of NO metabolites, it is worth noting that this model does not identify the cellular source of eNOS that is important for these effects. The authors appropriately note the importance of vascular endothelium in the response to exercise and suggest that this may be the site of dynamic eNOS regulation and NO generation. However, cardiomyocytes also express eNOS that could contribute to the observed effects of exercise. Nevertheless, taken together, these data provide strong evidence that eNOS activation increases NO metabolites both in the plasma and taken together, these data provide strong evidence that eNOS activation increases NO metabolites both in the plasma and within the heart and that these metabolites are important mediators of both the early and sustained cardioprotection seen with exercise.

Calvert et al then turned to question of what mediates eNOS activation in exercise. On the basis of previous reports that the β3-AR is upregulated in exercising humans and that β3-AR stimulation causes a negative inotropic effect through induction of eNOS and vasodilation-mediated decreases in preload, the authors hypothesized that circulating catecholamines signaling via β3-AR could contribute to exercise-induced eNOS activation and cardioprotection. Indeed, circulating levels of epinephrine and norepinephrine as well as β3-AR levels increased after 4 weeks of exercise. In β3-AR-deficient mice (β3-AR−/−), not only did exercise fail to produce phosphorylation/activation of eNOS but it paradoxically led to a dramatic reduction in eNOS ser-1177 phosphorylation as well as a reduction in nNOS. No significant change was seen in circulating or cardiac NO metabolites after exercise in β3-AR−/−. Importantly, infarct size was substantially increased after MI/R in β3-AR−/− mice. With this striking result, the authors conclude that exercise stimulates in both circulating catecholamines and β3-AR expression as upstream mediators of cardiac eNOS activation. β3-AR null mice—as with the eNOS−/− mice—did not exercise to the same extent as wild-type mice (3 to 4 km/d versus ∼7 km/d for wild-type). Such confounders are not uncommonly seen with voluntary exercise in germline genetic models and leave open the possibility that failure to exercise adequately contributes to the phenotypes observed. However, considered as a whole, this study makes a compelling argument for an important role of β3-AR–eNOS signaling in the generation of both the early and sustained cardioprotection seen with exercise training.

Interestingly, the pathway implicated in this work may intersect with those previously identified in exercise-induced physiological hypertrophy and protection from heart failure. Although not directly examined in this work, PI3-Kinase/Akt signaling is not only activated in exercise and required for physiological hypertrophy but is an important mediator of eNOS Ser-1177 phosphorylation. PI3-Kinase/Akt signaling is also upstream of recently described transcriptional networks, including C/EBPβ and CITED4, which are favorably modulated by exercise and mitigate progression of heart failure. Although there are obviously important differences between heart failure and ischemia-reperfusion injury, it appears likely that a common and interconnected signaling network could contribute to the benefits of exercise in both settings. Further delineating the common and distinct mechanisms mediating benefit in these settings will be of great interest for future studies.

Understanding the mechanisms underlying the benefits of exercise could also have important practical implications. Previous work using metabolomic profiling of exercise-induced changes in human plasma demonstrated that metabolites not only provide independent markers of exercise performance but can have important biological effects. In the current study, exercise produced measurable increases in NO metabolites in plasma of mice and humans. Identification of markers signaling the salutary effects of exercise could help identify patients most likely to benefit from exercise and optimize those benefits. Even more enticing is the possibility that such studies could ultimately lead to pharmacological approaches that might mimic the effects of exercise to mitigate ischemic injury or heart failure. In the current study, Calvert et al found that a single dose of epinephrine led to rapid and dose-dependent increases in eNOS Ser-1177 phosphorylation. This acute experiment provides additional support for the author’s model linking catecholamine signaling via β3-AR to eNOS activation and is obviously not intended as a therapeutic strategy. Although catecholamines could contribute to the benefits of exercise, they have well-recognized adverse effects in other settings, such as heart failure. Manipulating downstream effectors may be a more promising therapeutic direction, as suggested by the authors’ previous work. It probably will take quite a while before we can fully exploit these pathways as therapeutic targets. In the meantime, Calvert et al provide important new insights into mechanisms mediating the benefits of exercise and how these can be sustained—as well as one more reason to keep exercising.

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References


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