Effect of exercise training and myocardial infarction on force development and contractile kinetics in isolated canine myocardium

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Canan BD, Haizlip KM, Xu Y, Monasky MM, Hiranandani N, Milani-Nejad N, Varian KD, Slabaugh JL, Schultz EJ, Fedorov VV, Billman GE, Janssen PM. Effect of exercise training and myocardial infarction on force development and contractile kinetics in isolated canine myocardium. J Appl Physiol 120: 817–824, 2016. First published January 28, 2016; doi:10.1152/japplphysiol.00775.2015.—It is well known that moderate exercise training elicits a small increase in ventricular mass (i.e., a physiological hypertrophy) that has many beneficial effects on overall cardiac health. It is also well known that, when a myocardial infarction damages part of the heart, the remaining myocardium remodels to compensate for the loss of viable functioning myocardium. The effects of exercise training, myocardial infarction (MI), and their interaction on the contractile performance of the myocardium itself remain largely to be determined. The present study investigated the contractile properties and kinetics of right ventricular myocardium isolated from sedentary and exercise trained (10–12 wk progressively increasing treadmill running, begun 4 wk after MI induction) dogs with and without a left ventricular myocardial infarction. Exercise training increased force development, whereas MI decreased force development that was not improved by exercise training. Contractile kinetics were significantly slower in the trained dogs, whereas this impact of training was less or no longer present after MI. Length-dependent activation, both evaluated on contractile force and kinetics, was similar in all four groups. The control exercise-trained group exhibited a more positive force-frequency relationship compared with the sedentary control group while both sedentary and trained post-MI dogs had a more negative relationship. Last, the impact of the β-adrenergic receptor agonist isoproterenol resulted in a similar increase in force and acceleration of contractile kinetics in all groups. Thus, exercise training increased development of force but slowed contractile kinetics in control (noninfarcted animals), actions that were attenuated or completely absent in post-MI dogs.

contractility; Frank-Starling mechanism; force-frequency relationship; heart failure

NEW & NOTEWORTHY

Exercise training in dogs leads to enhanced force development and slower contractile kinetics, whereas myocardial infarction largely negates these changes.

MODERATE EXERCISE CAN ELICIT an increase in ventricular mass (i.e., physiological hypertrophy) (20) that has many beneficial effects on overall cardiac health. It is also well established that exercise training consistently provokes reductions in heart rate, i.e., training bradycardia (7, 38). However, the effect of exercise training on contractile performance is less clearly known. Previous studies have yielded conflicting results; exercise training decreased (37), or increased (19), ventricular indexes of contractility. These apparent discrepancies may be due to differences in the intensity of training, and/or the conditions used to measure contractility, or the breed of animals (39). Most indexes of ventricular contractility may not necessarily reflect the changes in the actual quality of the contracting myocardium. Ventricular contractility is significantly impacted by the quantity (hypertrophy) of myocardium on one hand and systemic parameters (afterload, heart rate, and β-adrenergic baseline drive) on the other. It remains incompletely understood what the impact of moderate exercise is on the actual functioning myocardial muscle tissue, per se, when these confounding factors of hypertrophy, heart rate, β-adrenergic drive, and pre- and afterload are controlled.

When myocardial infarction (MI) damages part of the ventricle, the remaining myocardium undergoes remodeling in an attempt to compensate for the loss of viable functioning myocardium (32). This remodeling can result in altered performance of the myocardium. Although MI typically results in a depression of most global indexes of ventricular contraction (15), the effects of MI at the tissue level are less clear, since tissue function cannot be readily extrapolated from these global indexes due to the inhomogeneous wall geometry and compensatory hypertrophy induced by MI.

Therefore, it was the purpose of the present study to evaluate the effects of exercise training on right ventricular (RV) myocardial tissue obtained from animals with and without healed left ventricular (LV) myocardial infarctions. In particular, the hypothesis that exercise training would improve RV contractile performance in control but not in infarcted dogs was tested. Force-frequency, length-tension, and β-adrenergic receptor responses were evaluated using RV trabeculae isolated from the hearts of sedentary and exercise trained dogs with and without healed LV myocardial infarctions.

METHODS

Origin of tissue. All of the animal procedures were approved by the Ohio State University Institutional Animal Care and Use Committee and conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH publication no. 85-23, revised 1996). All dogs were primarily used for other studies over the past 11 years (8, 11, 13, 14, 21, 29). RV free myocardial wall sections were obtained for the current studies at the time of death. All dogs (n = 40, mixed breed) were free of heartworms, 2–3 yr old, and weighing ~20 kg. The animals were anesthetized and instrumented as previously described (4, 6, 13).
The exercise training protocol has been previously described (9, 10, 12). Briefly, the dogs (n = 18) ran on a motor-driven treadmill for 10–12 wk, 5 days/wk at ~70–80% of estimated maximum heart rate. The heart rate response to exercise was determined for each animal (the average of the 3 exercise tests) as previously described (10) before the onset of the exercise training program. The exercise training protocol was designed to achieve the target heart rate as previously described (10). Briefly, the exercise intensity and duration progressively increased as follows: 1st wk, 20 min at 4.8 km/h and 0% grade; 2nd wk, 40 min at 5.6 km/h and 10% grade; 3rd wk, 40 min at 6.4 km/h and 10% grade; 4th wk, 60 min at 6.4 km/h and 10% grade; 5th wk, 60 min at 6.4 km/h and 12% grade; 6th wk, 75 min at 6.4 km/h and 12% grade; 7th wk, 90 min at 6.4 km/h and 12% grade; 8th-12th wk, 90 min at 6.4 km/h and 14% grade. Each exercise session included 5-min warm-up and 5-min cool-down periods (running at a low intensity, 0% grade and speed, 4.8 km/h). The dogs in the sedentary group (n = 22) were placed in a transport cage for equivalent time periods but without exercise.

A cohort of animals (n = 15, 9 sedentary and 6 trained) underwent MI surgery. Briefly, the left anterior descending coronary artery was ligated in two stages that previous studies have shown results in an anterior wall MI that impacts ~16% of ventricular mass (4). The exercise training (or equivalent sedentary) period began 4 wk after the induction of a myocardial infarction. Myocardial infarction was not measured in the present study. However, previous studies demonstrate that MI size is quite variable depending on the native coronary collateral vessel in each dog. However, the left anterior ligation elicits, on average, a MI of ~16% of LV mass (4). All dogs adapted easily to the treadmill running without having to use any adverse stimuli (i.e., shock avoidance as is common for rodent studies). All dogs completed the training program without any complications, protocol modifications, or any discernable differences from noninfracted animals. A detailed histological analysis and comparison of the MI between animals susceptible to and resistant to ventricular fibrillation (VF) has been published previously (31).

Trabecula studies. The animals were deeply anesthetized with pentobarbital sodium (30–50 mg/kg iv), intubated, and placed on a respirator. The chest was opened via a sternotomy, and the heart was rapidly removed (elapsed time from anesthesia to heart removal <1 min). The heart was also harvested without removal from the pericardium. Immediately after excision, the coronary arteries were flushed with cold cardioplegic solution (high potassium), and the RV free wall was removed. This free wall was stored in a second cardioplegic solution and transported to the dissection lab in ~5 min. This modified Krebs-Henseleit solution was bubbled with 95% O2-5% CO2 and contained (in mM): 137 NaCl, 5 KCl, 0.25 CaCl2, 20 NaHCO3, 1.2 NaH2PO4, 1.2 MgSO4, 10 dextrose, and 20 2,3-butanedione mono-o-xime (BDM) at pH of 7.4. Per dog, up to six nonbranching linear RV trabeculae were dissected. The average dimensions were 311 ± 33 μm wide, 210 ± 22 μm thick, and 2.85 ± 0.17 mm long, with no differences (per ANOVA) between the groups (n = 85 muscles total, from n = 40 dogs). Each of these muscles was used for the assessment of the effects of in vitro contractile function. Muscles were mounted in the setup as previously described (13, 21, 43) and stimulated at 1 Hz with a 3-ms duration and at 120% threshold voltage while being perfused with the same oxygenated Krebs-Henseleit solution, without BDM, and containing 2.0 mM Ca2+. All experiments were performed at 37°C to maximize translation of the results to the in vivo setting. Muscles were stretched until an increase in passive (diastolic) force was no longer accompanied by a substantial increase in developed force, representing optimal length, and were allowed time to equilibrate for 20–30 min. After stabilization, baseline assessments were recorded for developed force and kinetics. Next, the length-dependent activation was assessed by measuring developed force during steady state at 85, 90, 95, and 100% of optimal length. Next, at optimal length, the muscles were stimulated at 1, 2, 3, 4, and 5 Hz, and force was recorded when the muscle had equilibrated at each frequency.

After this protocol, the muscle was returned to 1 Hz. The third protocol assessed was for the β-adrenergic receptor responsiveness by the generation of an isoproterenol dose-response curve, obtained in semilog steps between 1 nM and 1 µM.

Force development was normalized to the cross-sectional area of the trabeculae (33) to allow for comparison between muscles of different diameters. Twitches were recorded at each experimental condition upon stabilization of developed tension. Data from n = 13 sedentary dogs, 12 trained dogs, 9 MI sedentary dogs, and 6 MI trained were collected and analyzed using custom-designed software (in LabView, National Instruments, Austin, TX).

Data analysis. Data from all the muscles were averaged to obtain one value for each dog, and, then, this value was used for all statistical comparisons between and within groups. All data are presented as means ± SE. Unless specifically stated otherwise, data were analyzed using a two-factor mixed-design ANOVA with or without inclusion of a repeated-measures variable. Post hoc comparison was made using the Tukey-Kramer Multiple-Comparison Test.

RESULTS

Confirmation of the training. As reported in previous studies on the trained dogs from which muscles were used in this study (7, 10–12, 30), exercise training elicited significant reduction in both baseline heart rate and the heart rate response to submaximal exercise that were accompanied by increases in the high-frequency component of heart rate variability (often used as indexes of cardiac parasympathetic regulation) (5). In addition, in previous studies on the trained dogs, exercise increased diaphragm citrate synthase activity, and exercise increased LV free wall systolic thickness by 8.0 ± 2.6% in animals resistant to VF and 10.1 ± 4.0% in dogs susceptible to VF (7). No data were obtained from the right ventricle (RV).

Baseline contractility and kinetics. First, the developed force at optimal length was assessed in four groups of muscles (Fig. 1A). Muscles from trained dogs showed a higher developed force compared with muscles isolated from sedentary animals (ANOVA, P < 0.05). In muscles from hearts that underwent MI, this training effect was no longer observed, whereas the factor MI (ANOVA, P < 0.05) caused a decrease in developed force in RV muscles. Timing of kinetics was impacted by training in no-MI muscles. Muscles from exercise-trained dogs had a significantly slower (ANOVA, P = 0.01) time-to-peak tension (TTP) and 50% (RT50) and 90% (RT90) relaxation time (Fig. 1B) compared with sedentary and sedentary MI groups. MI did not alter twitch kinetics in sedentary controls, but MI in trained muscles resulted in faster kinetics, most prominent in RT90 (141 ± 23 ms) that was virtually identical to nontrained MI values (142 ± 30 ms), and faster than in the non-MI-trained muscles (178 ± 46 ms). Maximal rate of force development (dF/dt) and force decline were assessed (Fig. 1C), and showed a depressed rate as a result from training, or MI, an effect that was additive with the MI trained group having the lowest rates. Note, the maximal rate of force development and decline is a composite of force and kinetics. To obtain a pure kinetic rate (unit/s), dF/dt was normalized by the developed force. The pure kinetic rate is shown in Fig. 1D. These rates closely match the absolute twitch timings (Fig. 1B); training slowed the kinetic rate, whereas MI negated the difference between muscles from sedentary and trained subjects.

Length-dependent activation. After determining optimal length, length-dependent activation was quantified by reducing the length of the muscle to 85% of optimal length (L55). At this
length, the muscle is typically taut; any further reduction in length will cause the muscle to buckle between the force transducer and microdisplacement screw. At this L85, the contractile parameters were allowed to stabilize, and data were recorded. Thereafter, the muscle was stretched to 90% of optimal length, and data again were collected after stabilization. Typical examples of original recordings for all four groups are shown in Fig. 2, A–D. This assessment of force was repeated for 95% of optimal length and then once more at optimal length. In Fig. 2E, length-dependent activation of developed force is shown for all four groups. As expected, length had an overall significant impact on both force (P < 0.0001) and RT50 (P < 0.0001). The statistical interaction component between the parameters force and length was, however, not different for the groups; other than a different level of force, the length dependency was not different in either training or MI groups. Twitch timing kinetics exhibited the same result; although there was a group-dependent difference in RT50, it did not interact with changed length (Fig. 2F). Length-dependent changes for TTP and RT90 very closely mimicked those of RT50 (data not shown). Combined length-dependent activation over the range of muscle length as would occur in vivo was not altered by training or MI for force or kinetics.

**Force-frequency relationship.** At optimal length, frequency of stimulation was varied from 1 to 5 Hz, encompassing the normal in vivo heart rate range (i.e., 60–300 beats/min) of the dog. At each frequency, the contractile parameters were allowed to stabilize before data collection. Typical examples for all four groups are shown in Fig. 3, A–D. As depicted in Fig. 3E, both the sedentary control and trained groups had a positive force-frequency relationship up to 3 Hz. At 4 and 5 Hz, which typically falls at the top of the physiological range, force development is slightly depressed, mainly due to elevated diastolic tension (data not shown). After MI, this force-frequency relationship was flatter (lesser increase from 1 to 3 Hz),
albeit not significantly. ANOVA indicated a significant effect of frequency on force \((P < 0.0001)\), and a significant effect for groups \((P = 0.046)\), but their interaction was not significant. In all groups, the effect of frequency on RT50 was significant (slower RT50 as length increased), as well as significantly different between groups. Sedentary, \(n = 13\); trained, \(n = 12\); MI sedentary, \(n = 7\); MI trained, \(n = 6\).

\*Significant difference between control trained and all other groups, \(P < 0.05\).
in a concentration-dependent manner (ANOVA, $P < 0.0001$; Fig. 4A). There was no significant difference in the response between groups (ANOVA, $P = 0.133$). Likewise, all groups responded with an acceleration of twitch kinetics (ANOVA, $P < 0.0001$); RT$_{50}$ was reduced in a dose-dependent way (Fig. 4B), whereas there was a significant group effect for RT$_{50}$ (ANOVA, $P = 0.046$). Changes for the TTP and RT$_{90}$ were very similar to those recorded for RT$_{50}$ (data not shown).

**DISCUSSION**

The main findings of this study are that 1) the RV myocardial tissue of exercise-trained canines, at baseline pacing frequency, is slightly stronger than myocardium from sedentary dogs, 2) the stronger contractile force is accompanied by slower kinetics, and 3) LV myocardial infarction largely negates these differences; in dogs with myocardial infarction, training did not lead to significantly
higher contractile force in RV myocardium, nor did it lead to slower kinetics.

**Baseline contractile parameters.** We observed a slight increase in active developed force in trained dogs at the baseline contraction of 1 Hz, at optimal length. Generally, training results in ventricular hypertrophy (17, 36), in which the increase in myocardial mass is typically mainly responsible for the increase in total ventricular force. In our trabeculae, all forces were normalized to cross-sectional area, and thus the increased force reflects an increase in specific force. The resting heart rate of the trained dogs is lower than the resting heart rate of sedentary dogs (4, 7, 9). Because the dogs, like all healthy mammals (28), have a positive force-frequency relationship, lower heart rates result in lower twitch force. Exercise training causes a mild ventricular hypertrophy, such that at the same pacing rate less force per cross-sectional area of tissue is generally needed for a baseline ventricular ejection compared with nontraining tissue. At optimal pacing rate, the difference between trained and sedentary dogs is no longer significant. Thus, the enhanced specific force at baseline is potentially due to changes in excitation-contraction coupling that are induced by training and not in maximal force-generating capacity. This would be in agreement with reports on myocardium isolated from exercise-trained rodents: myocytes increase contractility, most likely via an increase of sarcoplasmic reticular calcium ATPase protein levels or enhanced phospholamban phosphorylation, in trained rats (42), but this was not assessed in our study (see Limitations of the study).

Interestingly, the kinetics of contraction were slower in the myocardium from trained dogs: timing parameters (TTP, RT50, and RT90) were significantly slower. The interaction of the three measured timing parameters was not significantly impacted by group, indicating a general decrease in muscle kinetics as a result of exercise training, and not a specific effect either on contraction or on relaxation in the trained animals. This is in close agreement with previous reports from rodents in which there was a tight and significant correlation between contraction and relaxation kinetics (24). This decrease in kinetics of contraction/relaxation is likely related to the exercise training-induced remodeling of the myocardium that is required to maintain mechanical performance at the lower baseline heart rates of trained animals. Myocytes, isolated from trained rats, had a slightly lower calcium transient. This lower calcium transient, coupled with the higher force, was due to an increase in myofilament calcium sensitivity (42) and was also observed in infarcted animals (41). If a similar exercise-induced remodeling of the myocardium occurs in the dog, then slower kinetics would arise from this enhanced calcium sensitivity at a resting heart rate.

**Length-dependent activation.** All groups responded with an increase in developed force and a mild slowing down of twitch kinetics when muscle length was increased. This is in close agreement with previous studies on isolated mammalian myocardium under similar experimental conditions (27, 34, 35). In both MI groups, this response was not different, in agreement with findings that, even in severe human heart failure, length dependency of activation, part of the Frank-Starling mechanism, is typically unchanged (22, 40).

**Frequency-dependent activation and β-adrenergic stimulation.** At increasing stimulation rates, both non-MI groups responded with a similar positive force-frequency behavior. This finding is in close agreement with normal mammalian frequency-dependent activation: in all mammals, the force of contraction increases at the lower end of the in vivo heart rate range (28). The slower contraction kinetics at baseline in the trained group were absent at higher frequencies: because the acceleration of the trained myocardium with increasing frequency was greater than that of the nontrained tissue, the differences at baseline were no longer present at higher stimulation frequencies. A larger acceleration of relaxation timing is possible in trained dogs, mainly due to the slower rates at rest. The kinetics in all groups accelerated independently of developed force. Although force development slightly declined at 4 and 5 Hz in all groups, timing parameters continued to accelerate. This is in
close agreement with previous reports in different mammals (2, 33) where time-to-peak and relaxation times accelerated throughout the entire force-frequency range, largely independent of changes in developed force. Both training and MI had little or no impact on β-adrenergic responsiveness. All groups responded to isoproterenol in a dose-dependent manner by both increasing force and accelerating kinetics; a comparison with past similar experiments (13) reveals a similar quantitative and qualitative response to β-adrenergic stimulation.

Impact of myocardial infarction. In agreement with previous studies, myocardial infarction adversely altered myocardial contractile function, decreasing maximum force development, the rate of force development, and the relaxation rate (1, 16, 18). In contrast to the normal myocardium (i.e., tissue from noninfarcted animals), exercise training did not elicit changes in these contractile parameters such that, after MI, there was no longer a significant difference between the trained and sedentary group. These data suggest that myocardial infarction may limit the beneficial actions of exercise training on RV mechanical properties in this canine model. However, it must be emphasized that myocardial infarction did not reduce exercise capacity, since all dogs completed this rigorous training program. The mechanisms responsible for the mechanical adaptation to exercise training in the presence or in the absence of myocardial infarction remain to be determined.

Clinical impact. Exercise training is beneficial in many models, even after MI. We find that RV responds positively to training in animals with healthy hearts (i.e., no MI), whereas the exercise training-induced changes in mechanical performance are attenuated or absent in animals with MI. Thus, the impact of exercise on increasing amplitude of contraction and slowing kinetics of contraction is lost upon MI. It should be emphasized that exercise training has many other beneficial actions, even in animals with MI [e.g., increased electrical stability (3)], that may be more important than enhanced RV mechanical function. For example, exercise training has been shown to improve cardiac autonomic balance (increase parasympathetic and decrease cardiac sympathetic regulation) (3, 12, 23, 30), improve myocyte calcium handling (14) and attenuate repolarization abnormalities (14), and thereby almost completely suppress malignant arrhythmias in a well-characterized canine model of sudden cardiac death (3, 23, 30).

Limitations of the study. Only the RV was available for these experiments. Contractile parameters in the RV and LV are typically very similar in normal rats (25). The same holds true for normal mice done in our lab over the past decade, partially reviewed in Ref. 26, while RV-LV comparison data on isolated muscle performance in larger animals, particularly after an MI, are currently lacking. The larger pressure generated by the LV is a matter of LV mass, and not specific force, i.e., the pressure difference generated by the left vs. the right is proportional to the mass of the ventricles, not the quality of the myocardial muscle tissue. It is not surprising that training beneficiated the RV myocardium, since both ventricles in essence work in series. A LV infarction has been shown to impact RV muscle-level function in a rat animal model (18). Thus, our finding of a depressed force development in the RV after LV MI would be in line with other studies. However, we cannot exclude that other differences in RV vs. LV can potentially impact on a different LV response, or that the adaption of the LV and RV to exercise is different. In addition, the study was conducted over a 10-year period, and myocardium was preserved in long-term storage at (only) −80°C. Typically, liquid nitrogen storage is needed for phosphorylation studies of such samples; thus, we were unable to provide an unambiguous molecular analysis of calcium-handling proteins.

Conclusion. In conclusion, exercise training significantly altered RV myocardial contractile function in dogs. Most notable, exercise training elicited a small improvement in specific force at baseline, accompanied by a slowing of contraction/relaxation kinetics. However, this beneficial response to exercise training was absent in almost all assessed parameters following LV myocardial infarction. These data suggest that exercise training may not be able to compensate for the detrimental mechanical actions of myocardial infarction.

GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

REFERENCES
10. Billman GE, Kukielka M. Effects of endurance exercise training on heart rate variability and susceptibility to sudden cardiac death: protection is not

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