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**Title**: The Effect of Endurance Exercise Training on Heart Rate Variability in Animals Susceptible to Sudden Cardiac Death: Cardioprotection Does Not Solely Result From Enhanced Cardiac Vagal Regulation

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**Running Title:** Exercise training improves cardiac vagal regulation

## Abstract

Low heart rate variability (HRV) is associated with an increased susceptibility to ventricular fibrillation (VF). Exercise training can increase HRV (an index of cardiac vagal regulation) and could, thereby, decrease the risk for VF. To test this hypothesis, a 2-minute coronary occlusion was made during the last minute of a 18-min submaximal exercise test in dogs with healed myocardial infarctions; 20 had VF (S, susceptible) 13 did not (R, resistant). The dogs then received either a 10-week exercise program (S n=9, R n=8) or an equivalent sedentary period (S n=11, R n=5). HRV was evaluated at rest, during exercise, and during a 2 min occlusion at rest, before and after the 10-week period. Pre-training, the occlusion (Occ) provoked significantly ($p<0.01$) greater increases in HR (S, 54.9±8.3 vs. R, 25.0±6.1 beats/min) and greater reductions in HRV (S, -6.3±0.3 vs. R, -2.8±0.8 ln $ms^2$) in the susceptible dogs as compared to the resistant animals. Similar response differences between susceptible and resistant dogs were noted during submaximal exercise. Training significantly reduced the HR and HRV responses to the occlusion (HR, 17.9±11.5 beat/min; HRV, -1.2±0.8, ln $ms^2$) in the susceptible dogs; similar response reductions were noted during exercise. In contrast, these variables were not altered in the sedentary susceptible dogs. Post-training, VF could no longer be induced in the susceptible dogs, while four sedentary susceptible dogs died during the 10-week control period and the remaining 7 animals still had VF when tested. Atropine decreased HRV but only induced VF in 1 of 8 trained susceptible dogs. Thus, exercise training increased cardiac vagal activity, which was not solely responsible for the training-induced VF protection.

**Key Word**s: exercise, parasympathetic nervous system, ventricular fibrillation, myocardial ischemia, myocardial infarction.

## Introduction

Reductions in cardiac parasympathetic control have been associated with an increase risk for sudden death (44). Kleiger and co-workers (3, 28) found that the patients recovering from myocardial infarctions with the smallest heart rate variability [a non-invasive index of cardiac parasympathetic regulation, (6, 40, 47)] had the greatest risk of dying suddenly. The relative risk of mortality was 5.3 times greater in patients with a R-R interval variability less than 50 ms compared to patients with variability greater than 100 ms. This initial observation has been confirmed in numerous clinical studies (1, 21). For example, the ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) group found that post-myocardial infarction patients with either low heart rate variability or reduced baroreflex sensitivity had a much greater risk of sudden death than those with well preserved cardiac vagal function (31).

Similar results have been obtained in animal studies. We previously reported that heart rate variability (HRV) was much lower in animals susceptible to ventricular fibrillation compared to animals resistant to these malignant arrhythmias (7, 12, 18, 22). In particular, the susceptible animals exhibited a much greater reduction (withdrawal) of vagal activity in response to either submaximal exercise (7, 18, 22) or acute myocardial ischemia (12, 18, 22) than did the resistant dogs. In a similar manner, bilateral vagotomy (13) or the cholinergic antagonist atropine increased arrhythmia formation (14), while cholinergic agonists or electrical stimulation of the vagus nerves increased ventricular fibrillation threshold, antagonized the effects of sympathetic stimulation, and decreased the incidence of ventricular fibrillation (4, 27, 29, 48). These data demonstrate that subnormal cardiac parasympathetic regulation increases the risk for malignant arrhythmias while interventions that enhance cardiac vagal function can protect against ventricular fibrillation. However, in order to be an effective antiarrhythmic therapy, an

intervention must not only increase baseline vagal activity but also must maintain this enhanced activity when the heart is stressed, as during myocardial ischemia. Indeed, low doses of cholinergic antagonists paradoxically increased the baseline cardiac vagal activity (30) but failed to maintain this increase in HRV when the heart was stressed by either submaximal exercise or a coronary artery occlusion (18). As a consequence, this intervention proved to be ineffective in the prevention of lethal arrhythmias induced by acute myocardial ischemia (18, 25).

It is well established that regular exercise can improve cardiac autonomic balance (increasing parasympathetic while decreasing sympathetic regulation of the heart) (5, 42). In both man and animals, heart rate at submaximal-workloads is lower in trained individuals compared to sedentary controls (5, 42), while the presence of a resting bradycardia is frequently used to confirm that training has been effective (17, 34, 45). Exercise training programs can increase HRV in patients recovering from myocardial infarction (32, 33, 35, 39) and may reduce the incidence of sudden death and arrhythmias in both man and animal models (8, 23, 32, 37, 38, 41). In a similar manner, meta-analysis of 22 randomized trials of rehabilitation with exercise after myocardial infarction found that exercise training elicited both significant reductions in re-infarction and in the incidence of sudden death (38). In animals, regular exercise either reduced the electrical current necessary to induce ventricular fibrillation (37, 41), or the susceptibility to ventricular fibrillation induced by myocardial ischemia (8, 24). However, the contributions of changes in cardiac autonomic balance to the protection afforded by exercise training were not extensively examined in these studies and remain largely to be determined. Although exercise training has been shown to increase baseline HRV in animals prone to lethal arrhythmias (24), the effects of this intervention on HRV when the heart is stressed (i.e., during exercise or coronary occlusion) remain to be determined.

Therefore, it was the purpose of this study to investigate the effects of exercise training on HRV and susceptibility to ventricular fibrillation using a conscious canine model of sudden death. Specifically, the hypothesis that exercise training would increase HRV even during physiological stress (exercise or acute ischemia) and, thereby, could prevent ventricular fibrillation induced by myocardial ischemia was tested.

The present study demonstrates that exercise training improves cardiac autonomic function such that cardiac vagal regulation is maintained even when the heart is stressed by either exercise or acute myocardial ischemia in animals with healed infarctions. Furthermore, exercise training completely suppressed ventricular fibrillation induced by myocardial ischemia. However, as atropine pre-treatment did not re-introduce lethal arrhythmias in these dogs, the exercise-induced protection from ventricular fibrillation did not result solely from enhanced cardiac vagal regulation.

## Methods

The principles governing the care and use of animals as expressed by the Declaration of Helsinki, and as adopted by the American Physiological Society, were followed at all times during this study. In addition, the Ohio State University Institutional Animal Care and Use Committee approved all the procedures used in this study.

### Surgical Preparation

Sixty heartworm-free mongrel dogs weighing 15.4 – 24.5 kg (19.1±0.4 kg) were used in this study. The animals were anesthetized and instrumented as previously described (7, 12, 18, 22). Briefly, using strict aseptic procedures, a left thoracotomy was made in the fourth intercostal space. The heart was exposed and supported by a pericardial cradle. The left circumflex coronary artery was dissected free of the surrounding tissue. Both a 20 MHz pulsed Doppler flow transducer and a hydraulic occluder were then placed around this vessel. Two pairs of silver coated copper wires were also sutured on the epicardial surface of the heart and used to obtain a ventricular electrogram (from which heart rate and various indices of HRV were measured, see below). One pair of electrodes was placed in the potentially ischemic area (lateral left ventricular wall, an area perfused by the left circumflex artery) and the other pair in a non-ischemic region (right ventricular out flow tract). A two-stage occlusion of the left anterior descending artery was then performed approximately one-third the distance from its origin in order to produce an anterior wall myocardial infarction. This vessel was partially occluded for 20 minutes and then tied off.

**Heart Rate Variability Protocols**

The studies began 3-4 weeks after the production of the myocardial infarction (see flow chart, figure 1). First, over the period of 3 to 5 days, the dogs learned to run on a motor driven treadmill. The cardiac response to submaximal exercise was then evaluated as follows: exercise lasted a total of 18 minutes with workload increasing every 3-minutes. The protocol began with a 3-min warm-up period, during which the dogs ran at 4.8 kph at 0% grade. The speed was then increased to 6.4 kph, and the grade increased every 3-min (0, 4, 8, 12, & 16%). The submaximal exercise test was repeated three times (one/day). Prior to the 3$^{rd}$ submaximal exercise test, a catheter was placed percutaneously in a cephalic vein to administer the muscarinic antagonist, atropine sulfate (50 μg/kg) 3-min before the end of the exercise period. On a subsequent day, with the dogs lying quietly unrestrained on a table, a 2-min left circumflex coronary occlusion was made. At least 48-hrs after the completion of these studies, the animals were tested for susceptibility to ventricular fibrillation using an exercise plus ischemia test that is described in the following section. Heart rate, left circumflex blood flow, and HRV were monitored continuously throughout the exercise or occlusion studies. These studies were repeated after the completion of the 10-week exercise training or the 10-week sedentary time period.

**Exercise plus Ischemia Test: Classification of the Dogs**

The susceptibility to ventricular fibrillation was tested as previously described (7, 12, 18, 22) (figure 1). Briefly, the animals ran on a motor driven treadmill while workload progressively increased until a heart rate of 70% of maximum (approximately 210 beats/min) had been achieved. During the last minute of exercise, the left circumflex coronary artery was occluded, the treadmill stopped, and the occlusion maintained for an additional minute (total occlusion time = 2 min.). The exercise plus ischemia test reliably induced ventricular flutter that rapidly

deteriorated into ventricular fibrillation. Therefore, large metal plates (11 cm diameter) were placed across the animal's chest so that electrical defibrillation (Zoll M series defibrillator, Zoll Medical, Burlington, MA) could be achieved with a minimal delay but only after the animal was unconscious (10-20 s after the onset of ventricular fibrillation). Of the 60 animals that underwent surgery, 21 animals could not be tested either due to death within 72-hrs of the myocardial infarction (n=14, 23.3%) or occluder failure (n=7). Thus, the exercise plus ischemia test was performed on 39 of the original 60 animals. The occlusion was immediately released if ventricular fibrillation occurred. Twenty-six dogs developed ventricular fibrillation (susceptible) while the remaining 13 did not (resistant). Three susceptible animals were not successfully defibrillated and, as such, were not available for additional studies. This exercise plus ischemia test, using the same exercise intensity, was repeated after the completion of a10-week exercise training or a 10-week sedentary time period (see below).

**Exercise Training Protocol**

The susceptible (n=23) and resistant dogs (n=13) were randomly assigned to either a 10-week exercise training period (susceptible n=9, resistant n=8) or an equivalent sedentary period (susceptible n=14, resistant n=5) (figure 1). The dogs in the exercise-training group ran on a motor-driven treadmill for 10 weeks, 5 days/week at approximately 70-80% of maximum heart rate. The exercise intensity and duration progressively increased as follows: 1$^{st}$ week, 20 min at 4.8 kph/0% grade; 2$^{nd}$ week, 40 min at 5.6 kph/10% grade; 3$^{rd}$ week, 40 min at 6.4 kph/10% grade; 4$^{th}$ week, 60 min at 6.4 kph/10% grade; 5$^{th}$ week, 60 min at 6.4 kph/12% grade; 6$^{th}$ week, 75 min at 6.4 kph/12% grade, 7$^{th}$ week, 90 min at 6.4 kph/12% grade; 8$^{th}$ – 10$^{th}$ weeks, 90 min at 6.4 kph/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 kph). The dogs in the sedentary

group wereplaced in transport cage for equivalent time periods but without exercise. All 17 animals (both the susceptible and resistant dogs) in the exercise group successfully completed the training program. Four dogs in the susceptible sedentary group died spontaneously between the 6$^{th}$ and the 10$^{th}$ week of the sedentary period and were eliminated from the study. The exercise plus ischemia test could not be repeated in three of the sedentary susceptible animals due to failure of the coronary artery occluder and were also eliminated from the study (figure 1). Finally, the exercise plus ischemia test was repeated after the administration of the muscarinic antagonist atropine sulfate (50 μg/kg, i.v. bolus 3-min before the occlusion) in the exercise-trained susceptible dogs (n=8).

**Citrate Synthase Assay**

The effects of exercise on skeletal muscle oxidative capacity were evaluated by measuring citrate synthase activity in the diaphragm. After the completion of the 10-week exercise training or 10-week sedentary studies, the animals were anesthetized (sodium pentobarbital, 50 mg/kg, i.v., Abbot laboratories, North Chicago, IL). The heart was rapidly removed and tissue samples frozen in liquid nitrogen and stored in a -80° C freezer. At the same time, a small piece of the diaphragm was also placed in liquid nitrogen. The citrate synthase activity of this skeletal muscle was assayed using the modified technique described by Srere (44). The skeletal muscle was harvested at least 48 hrs after the last exercise session.

**Echocardiography Studies**

Left ventricular free wall thickness was determined by echocardiography 3-4 weeks after surgery (i.e., myocardial infarction) and at the end of the 10-week exercise training or the 10-week sedentary period. These studies were performed before the animals were classified by the exercise plus ischemia test. Briefly, the dogs were lightly sedated with acepromazine (0.5

mg/kg, i.m.; Ft. Dodge Animal Health, Ft. Dodge, IA) prior to the studies. A conventional M-mode echocardiogram was obtained using a Sonos 1000 system (Hewlett Packard, Palo Alto, CA) with a 5.5 MHz transducer.

**Data Analysis**

All data are reported as mean ± SEM. The data were digitized (1 kHz) and recorded using a Biopac MP-100 data acquisition system (Biopac Systems, Inc., Goleta, CA). Heart rate variability was obtained using a Delta-Biometrics vagal tone monitor triggering off the electrocardiogram R-R interval (Urbana-Champaign, IL). This device employs the time-series signal processing techniques as developed by Porges to estimate the amplitude of respiratory sinus arrhythmia (40). Details of this analysis have been described previously (6). Data were averaged over 30s intervals either during exercise or the coronary occlusion. The following three indices of heart rate variability were determined: Vagal Tone Index, the high frequency (0.24 to 1.04 Hz) component of R-R interval variability; R-R interval range, the difference between the longest and shortest R-R interval for the same 30s time period; and standard deviation of the R-R intervals for the same 30s time period. The heart rate response to the exercise plus ischemia test was averaged over the last 5s before the occlusion, and at the 60s time point (or ventricular fibrillation onset) after occlusion onset.

The data were compared using ANOVA for repeated measures (NCSS statistical software, Kaysville, UT). For example, the effect of exercise training on the HRV data (heart rate; vagal tone index, i.e., 0.24 to 1.04 Hz component of the R-R interval variability; SD of R-R interval; and R-R interval range) were analyzed using a three way ANOVA [group (2 levels) x pre-post (2 levels) x exercise level (7 levels), or occlusion time 6 levels)] with repeated measures on two factors (pre-post and exercise level, or occlusion time). Comparisons between

the susceptible and resistant dogs were made using a two factor (group x exercise level, or occlusion time) ANOVA with repeated measures on one factor (exercise level, or occlusion time). A similar two factor (group x pre-post) ANOVA with repeated measures on one factor (pre-post) was used to evaluate the effects of the interventions on left ventricular systolic wall thickness. Since repeated measures ANOVA depends on the homogeneity of covariance, this sphericity assumption (i.e., the assumption that the variance of the difference scores in a within-subject design are equal across the groups) was tested using Mauchley's test (23). If the sphericity assumption was violated, then the F-ratio was corrected using Huynh-Feldt correction (23). If the F ratio was found to exceed a critical value ($p<0.05$) then the difference between the means was determined using Scheffe's test. The effect of exercise training on the incidence of ventricular fibrillation was evaluated using Fisher's Exact test. Finally, citrate synthase activity data (exercise trained vs. sedentary) were evaluated using Student's t-test.

## Results

### Confirmation of Exercise Training

There was no significant difference in body weight between the sedentary and trained animals for either the resistant (trained 20.4±1.1, sedentary 19.6±1.8 kg) or the susceptible (trained 20.0±0.6, sedentary 19.8±0.8 kg) dogs. However, left ventricular systolic wall thickness was significantly larger ($P<0.025$) in both susceptible (pre-training, 10.0 ± 0.6 versus post-training, 11.1 ± 0.4 mm; wall thickness increased by 10.1±4%) and resistant (pre-training, 8.8 ± 0.5 versus post-training, 9.4 ± 0.5 mm; wall thickness increased by 8.0±2.6%) animals following training but was unchanged in the sedentary dogs (susceptible pre-sedentary 9.8 ± 0.4; post-sedentary 9.6 ± 0.3 versus resistant pre-sedentary 10.1 ± 0.7; post-sedentary 10.7 ± 0.4 mm), indicating that the training had produced a small ventricular hypertrophy. In the susceptible dogs, exercise training provoked significant ($P<0.0025$) reductions in the peak heart rate response to exercise (pre-training 209.0 ± 7.4 versus post-training 184.4 ± 5.3 beats/min) that were accompanied by significant increases in R-R interval variability (e. g., vagal tone index, $p<0.04$; pre-training 1.0 ± 0.3 versus post-training 2.4 ± 0.3 ln $ms^2$) while these variables did not change in the sedentary animals (peak exercise response: HR, pre-sedentary 209.4 ± 6.0 versus post-sedentary 211 ± 5.3 beats/min; vagal tone index pre-sedentary 1.2 ±0.1 versus post-sedentary 1.5 ± 0.3). Similar but smaller changes were noted for the exercise trained resistant dogs (peak exercise response: HR, pre-training 200.2 ± 3.4 versus post-training 178.4 ± 7.4 beats/min; vagal tone index, pre-training 2.4 ± 0.3 versus post-training 2.8 ± 0.4 ln $ms^2$). Finally, citrate synthase activity was significantly ($P<0.02$) higher in skeletal muscle obtained from exercise trained (n=10, 11.6±1.0 μM/ml/min) as compared to sedentary (n=10, 7.5±1.4 μM/ml/min) dogs. As there were no differences between resistant and susceptible dogs, these

data were pooled for the analysis. These data confirm the exercise-training program was effective; that is, there was a significant skeletal muscle and cardiac adaptation induced by the training program.

**Pre-training Heart Rate Variability Responses**

The heart rate and HRV responses to submaximal exercise before training are displayed in figure 2. Submaximal exercise elicited significant (both $p<0.001$) increases in heart rate for both the susceptible and the resistant dogs. Furthermore, the heart rate increase was significantly (group x exercise level, $p<0.01$) greater in the susceptible dogs (peak heart rate change from pre-exercise values, susceptible 91.8 ± 2.0 versus resistant 84.8 ± 3.1 beats/min) as compared to the resistant dogs. The exercise-induced increases in heart rate were accompanied by significant (all, $P<0.001$) reductions in all three markers of HRV (vagal tone index, i.e., 0.24 to 1.04 Hz component of the R-R interval variability; SD of R-R interval; and R-R interval range). Greater reductions (group x exercise level, $p<0.001$) were again noted in the susceptible animals as compared to the resistant dogs (figure 2).

The contribution of cardiac parasympathetic regulation to the HRV response to submaximal exercise was also evaluated by the intravenous injection of atropine during the last exercise level. Atropine provoked larger increases ($p<0.01$) in heart rate (resistant, 28.2±3.6 versus susceptible, 17.9±2.8 beats/min), and reductions in the HRV indices (e.g., vagal tone index: resistant, -2.1±0.2 versus susceptible -1.1±0.3 ln $ms^2$) in the resistant animals as compared to the susceptible dogs. In fact, heart rate and HRV were no longer different between groups after the atropine treatment.

The heart rate and HRV responses to the coronary occlusion at rest before training are displayed in figure 3. The coronary artery occlusion elicited significantly (group x occlusion time, $p<0.001$) larger increases in heart rate in the susceptible as compared to the resistant dogs. The coronary occlusion induced increases in heart rate were accompanied by larger reductions (group x occlusion time, $p<0.001$) in all three HRV indices (vagal tone index, i.e., 0.24 to 1.04 Hz component of the R-R interval variability; SD of R-R interval; and R-R interval range) in the susceptible animals as compared to the resistant dogs.

**Post-training Heart Rate Variability Responses**

The heart rate and HRV responses to submaximal exercise for the susceptible dogs after the 10-week training or 10-week sedentary period are displayed in figure 4. Submaximal exercise elicited significant increases in heart rate ($p<0.001$) for both the sedentary and trained susceptible dogs. However, the heart rate increase was significantly (group x exercise, $p<0.001$) greater in the sedentary dogs as compared to the exercise-trained animals. The exercise-induced increases in heart rate were also accompanied by significant (all $p<0.001$) reductions in all three markers of HRV (vagal tone index, i.e., 0.24 to 1.04 Hz component of the R-R interval variability; SD of R-R interval; and R-R interval range). Once again, greater reductions (group x exercise, $p<0.01$) were noted in the sedentary animals as compared to the exercise-trained dogs (figure 4). Similar, but smaller, differences were noted between the sedentary and trained resistant dogs (data not shown). After exercise training, the susceptible and resistant dogs exhibited similar heart rate and HRV responses to exercise; that is, no statistically significant differences were noted when comparing these animals (figure 5).

The contribution of cardiac parasympathetic regulation to the HRV responses to submaximal exercise was also evaluated after either the 10-week exercise training or 10-week sedentary time period by the intravenous injection of atropine during the last exercise level. Atropine injection elicited larger increases ($p<0.01$) in heart rate and greater reductions ($p<0.01$) in HRV in the susceptible trained (HR, 36.8±3.2 beats/min; VT –2.3±0.3 ln $ms^2$) compared to the susceptible sedentary dogs (HR, 23.7±5.4 beats/min; VT, -1.1±0.5 ln $ms^2$).

The heart rate and HRV responses to the coronary occlusion at rest after either the 10-week exercise training or 10-week sedentary period are displayed in figure 6. The coronary artery occlusion elicited significantly (group x occlusion time, $p<0.001$) larger increases in heart rate in the sedentary animals as compared to the exercise-trained dogs. The coronary occlusion induced increases in heart rate were accompanied by larger reductions (group x occlusion time, $p<0.001$) in the HRV (vagal tone index, i.e., 0.24 to 1.04 Hz component of the R-R interval variability; and R-R interval range) indices in the sedentary susceptible compared to the susceptible trained dogs (figure 6). After training, the response to the coronary occlusion in the resistant and susceptible dogs was indistinguishable (figure 7). Thus, after the completion of exercise training, the susceptible animals exhibited a "resistant" HRV response pattern consistent with an increase in cardiac parasympathetic regulation. In contrast, the HRV response in the susceptible sedentary dogs did not change over time.

**Effect of training on susceptibility to ventricular fibrillation**

The exercise plus ischemia test was repeated after the completion of the either the 10-week exercise training period (susceptible n=9, resistant n=8) or 10-week sedentary period (susceptible n=7, resistant n=5). The heart rate responses to the coronary occlusion are displayed in figure 8. The coronary occlusion elicited significant ($p<0.0002$) increases in heart rate in both

the sedentary and the exercise-trained susceptible animals but did not alter heart rate in the resistant dogs. Both the pre-occlusion and occlusion heart rate were lower ($p<0.04$) in the susceptible exercise-trained animals as compared to the sedentary dogs. However, the change in heart rate elicited by the coronary occlusion was similar in both groups either before (sedentary 31.0±8.5, trained 32.4±11.1 beats/min) or after the 10-week period (sedentary 32.5±7.4, trained 29.3±11.9 beats/min). In addition, the exercise plus ischemia test provoked a similar ST segment depression in the sedentary and exercise trained susceptible dogs both before (sedentary –4.8 ± 1.2 versus exercise trained –4.7 ± 0.4 mm) and at the end of the 10-week period (sedentary –4.8 ± 1.7 versus exercise trained –4.8 ± 0.4 mm). When considered together, these data suggest that the coronary occlusion elicited a similar ischemic response before and at the end of the 10-week sedentary or 10-week exercise training period.

Exercise training significantly (Fisher's exact test $p=0.0014$) reduced the incidence of ventricular fibrillation in the trained susceptible animals protecting all 9 animals tested. In marked contrast, ventricular fibrillation was induced in all 7 sedentary susceptible dogs. In addition, 4 susceptible animals died spontaneously during the 10-week sedentary period. The exercise plus ischemia test failed to induce ventricular fibrillation in any of the resistant dogs in either the sedentary or the exercise trained groups.

The exercise plus ischemia test was repeated after pre-treatment with atropine in the susceptible exercise-trained dogs. Atropine significantly ($p<0.01$) increased heart rate (36.8±3.2 beats/min) and reduced HRV (–2.3±0.3 ln $ms^2$) both before and during the coronary occlusion (figure 9). Despite these changes, this intervention only re-introduced ventricular fibrillation, or any other arrhythmia, in 1 of 8 dogs tested.

## Discussion

The present study demonstrates that: A) exercise or acute myocardial ischemia provoked greater increases in heart rate that were accompanied by greater reductions in three HRV markers (indices of cardiac vagal regulation) in animals subsequently shown to be susceptible to ventricular fibrillation as compared to animals resistant to malignant arrhythmias; B) endurance exercise-training reduced heart rate and increased HRV even when the heart was stressed by either exercise or acute ischemia in the susceptible animals, such that the cardiac autonomic regulation was similar to that noted in resistant dogs; C) exercise-training completely suppressed ventricular fibrillation induced by acute myocardial ischemia protecting all 9 susceptible dogs that completed the 10-week exercise program. In marked contrast, an equivalent sedentary period not only failed to protect any of the susceptible dogs that completed the 10-week period, but 4 dogs died spontaneously during this period; D) treatment with the cholinergic antagonist, atropine, provoked large increases in heart rate and reductions in HRV yet failed to re-introduce arrhythmias in the majority of the trained susceptible animals, triggering ventricular fibrillation in only 1 of 8 dogs tested. These data suggest that exercise training can restore a more normal cardiac autonomic balance by increasing cardiac parasympathetic regulation and also reduce the incidence of malignant arrhythmias. However, this protection did not solely result from the training-induced enhanced cardiac vagal control. To the best of our knowledge, these findings represent the first demonstration that exercise training can improve cardiac parasympathetic control in diseased hearts even when the heart is stressed by acute myocardial ischemia or by an episode of submaximal exercise.

**Heart Rate Variability and Susceptibility to Ventricular Fibrillation**

Heart rate variability has gained widespread acceptance as a non-invasive marker of cardiac parasympathetic regulation (47). An ever-growing number of clinical studies have established a firm link between low HRV and a greater propensity for sudden death (1, 3, 6, 21, 28, 31, 40, 47). In agreement with the present study, we previously reported HRV was reduced by myocardial infarction and the dogs with the greatest reduction were also more susceptible to ventricular fibrillation (7, 12, 18, 22) than those dogs in which cardiac vagal regulation was not impaired by the ischemic injury. In particular, the susceptible animals exhibited a much greater reduction (withdrawal) of vagal activity in response to either submaximal exercise (7, 18, 22) or acute myocardial ischemia (12, 18, 22). When considered together, these clinical and experimental studies clearly suggest that reductions in cardiac parasympathetic regulation play an important role in the development of sudden cardiac death. Thus, one would predict that interventions that alter cardiac parasympathetic control should also alter susceptibility to ventricular fibrillation.

**Exercise Training and Susceptibility to Ventricular Fibrillation**

Exercise training can alter autonomic neural balance by both increasing cardiac parasympathetic and decreasing sympathetic activity (5, 42). In both man and animals the heart rate at submaximal workloads is reduced in trained individuals compared to sedentary controls (5, 42). Furthermore, acetylcholine content and cholineacetyl transferase activity is increased in the hearts of trained rats (15). In man, exercise training can increase HRV in patients recovering from myocardial infarction (32, 33, 35, 38, 39). In the present study, HRV was higher in trained dogs compared to the sedentary time-control animals. Importantly, the enhanced HRV was maintained even when the heart was challenged by either exercise or acute ischemia (see below).

Furthermore, atropine elicited much greater increases in heart rate in the exercise-trained animals than was noted for the sedentary dogs, data that are consistent with training-induced increases in the cardiac vagal regulation. Thus, endurance exercise training can elicit changes in cardiac autonomic control that could, in turn, protect against ventricular fibrillation.

Regular exercise is also associated with a lower risk for arrhythmias and sudden death in both man and animals (5). For example, Bartels et al. (2) found that the incidence of sudden cardiac death was inversely related to the level of regular physical activity; that is, sedentary individuals had the highest rate of sudden death (4.7 deaths per $10^5$ person-years) while those in the most active group had the lowest (0.9 deaths per $10^5$ person-years). Furthermore, meta-analysis of 22 randomized trials of rehabilitation with exercise after myocardial infarction found that exercise training elicited both significant reductions in the re-infarction rate and the incidence of sudden death (38). There was an overall reduction in cardiac mortality of 20% (due largely to the reduction in sudden death), a reduction that is comparable to the mortality reductions noted for β-adrenoceptor antagonists (19, 26).

Experimental studies also report that exercise training decreases the risk for arrhythmias (5, 8, 24) or the electrical current necessary to induce ventricular fibrillation (37,41). In agreement with the present study, Billman et al. (8) and Hull et al. (24) reported that daily exercise prevented ventricular fibrillation induced by acute ischemia in dogs with healed anterior wall myocardial infarctions, a protection that was accompanied by an improved baroreflex sensitivity (8) and an increase in baseline HRV (24). However, neither group examined the effects of training on autonomic regulation in response to physiological stressors such as exercise or acute ischemia. Improvement in the autonomic balance at rest might not provide sufficient protection against arrhythmias when the heart is stressed during dynamic situations. Indeed, the

observation that low doses of cholinergic antagonists paradoxically increased the level of cardiac vagal activity (31) led to the proposal that this treatment could provide an acceptable means of enhancing cardiac parasympathetic activity in patients (10, 49). However, Halliwill et al. (18) and Hull et al. (25) both demonstrated that, although low doses of cholinergic antagonists increased baseline cardiac vagal activity (R-R interval variability), this treatment failed to prevent ventricular fibrillation induced by myocardial ischemia. Halliwill et al. (18) further demonstrated that the enhanced baseline vagal activity was not maintained when the heart was stressed by either exercise or myocardial ischemia. As such, it was not surprising that this therapy failed to prevent ventricular fibrillation. It would appear that in order to be an effective antiarrhythmic therapy, an intervention must not only increase baseline vagal activity but, more importantly, must also maintain this enhanced activity when the heart is stressed.

The present study confirms and extends these previous studies. Training enhanced cardiac vagal control (as noted by increases in HRV) while an equivalent sedentary time period did not change cardiac parasympathetic regulation. The restoration of a more normal cardiac parasympathetic regulation, however, was not solely responsible for the protections associated with exercise training. The cholinergic antagonist atropine did not re-introduce malignant arrhythmias in the majority of animals, inducing ventricular fibrillation in only of 1 of 8 dogs tested. Other factors must play a more central role in the protection that results from training. Exercise training also alters sympathetic regulation. We previously reported that the susceptible dogs, in addition to reduced parasympathetic control, exhibit an enhanced $\beta_2$-adrenoceptor responsiveness (9). Therefore, training could also improve β-adrenoceptor balance by decreasing the enhanced $\beta_2$-adrenoceptor and could, thereby, remove the trigger for malignant

arrhythmias during myocardial ischemia. The effects of exercise training on $\beta_2$-adrenoceptor responsiveness merit further investigation.

**Limitations of the study**

There are a few limitations with the present study that could affect the interpretations of the results. First, dogs have extensive coronary collateral vessels (36) that exercise training may (43) or may not (11) increase. Therefore, exercise training-induced increases in coronary collateral circulation could contribute to the protection noted in the susceptible dogs. However, acute myocardial ischemia provoked similar increases in heart rate in the susceptible sedentary (pre 31.0±8.5 beats/min) and in the susceptible trained (pre 32.4± 1.1 beats/min) dogs before, or after the 10-week period (post-sedentary 32.5±7.4, post-trained 29.3± 1.9 beats/min). Thus, the ischemic stimulus that provoked the heart rate increases was similar before and after exercise training. In addition, the exercise plus ischemia test provoked a similar ST segment depression in the sedentary and exercise trained susceptible dogs both before (sedentary –4.8 ± 1.2 versus exercise trained –4.7 ± 0.4 mm) and at the end of the 10-week period (sedentary –4.8 ± 1.7 versus exercise trained –4.8 ± 0.4 mm). When considered together, these data suggest that the coronary occlusion elicited a similar ischemic response before and at the end of the 10-week sedentary or exercise training period.

Second, myocardial infarction size could also contribute to the differences noted between susceptible and resistant dogs; animals with larger infarctions would be expected to have poorer ventricular function and a higher risk for ventricular fibrillation. Myocardial infarction size was not measured in the present study (the hearts were removed for in vitro studies). Therefore, we performed a retrospective analysis of animals in which infarction size had been determined and

found that the susceptible dogs had larger infarctions (susceptible, n=93, 17.7±0.9%, resistant n=50, 12.6±1.4%). As the exercise program did not begin until after the myocardial infarction was healed (at least 4 weeks after the induction of the infarction), it seems unlikely that training would reduce the infarction size in these animals.

Third, exercise training reduced the peak heart rate achieved during either exercise or acute myocardial ischemia. By decreasing metabolic demand, a lower heart rate per se could reduce the risk for arrhythmias. However, atropine pre-treatment elicited large increases in heart rate, increases that exceeded the maximum heart rate values induced by the coronary occlusion before training, yet only modestly increased the arrhythmia frequency. Thus, heart rate reductions, alone, cannot be responsible for the training-induced protection from ventricular fibrillation.

Fourth, it must be acknowledged that in the present study, cardiac vagal regulation was only indirectly evaluated using various measures of HRV. This study did not measure the parasympathetic nerve activity directly. However, previous investigations have verified that heart rate variability provides an accurate representation of parasympathetic function (16). Additionally, in the current study, atropine effectively eliminated the heart rate and HRV differences noted between the susceptible and resistant dogs; that is, heart rate increased and HRV fell to zero in both groups after atropine treatment. These data are consistent with an atropine-induced inhibition (removal) of cardiac vagal regulation. Therefore, it is reasonable to conclude that the method used in the present study provided reliable indirect measurements of cardiac parasympathetic regulation.

Finally, it is well established that both respiratory rate and tidal volume can alter HRV (20). As such, differences in the respiratory response following exercise training could indirectly

contribute to the differences in the cardiac vagal indices in the susceptible and resistant animals. Respiratory parameters were not measured in this study due to the profound panting response induced by exercise in both groups of animals (before and after exercise training). It is possible that, despite the panting, respiratory rate or tidal volume was altered in the trained animals. However, the coronary occlusion at rest did not elicit any obvious change in respiration (or induce panting) either before or after training, yet HRV was profoundly increased in the exercise-trained susceptible animals. Furthermore, we previously demonstrated that exercise elicited similar respiratory rate changes in resistant and susceptible dogs and that panting did not alter HRV (7, 18, 22). It seems unlikely that training induced changes in respiration can explain HRV increase or the protection from ventricular fibrillation.

In conclusion, the present study demonstrates that exercise training improves cardiac autonomic function such that cardiac vagal regulation is maintained even when the heart is stressed by either exercise or acute myocardial ischemia in animals with healed infarctions. Furthermore, exercise training completely suppressed ventricular fibrillation induced by myocardial ischemia. However, as atropine pre-treatment did not re-introduce lethal arrhythmias in these dogs, the exercise-induced protection from ventricular fibrillation did not result solely from enhanced cardiac vagal regulation. The mechanisms by which exercise training improved cardiac vagal regulation and prevented ventricular fibrillation remain to be determined.

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## Disclosures

There are no conflicts of interest or disclosures to report.

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## Figure Legends

**Figure 1**. A flow chart illustrating the sequences of events in this study. Three – four weeks after myocardial infarction, the dogs were classified as susceptible or resistant to ventricular fibrillation (VF) with an exercise plus ischemia test. The animals were then randomly assigned to either 10-week exercise training program or a 10-week sedentary period. The exercise plus ischemia test was repeated at the end of the 10-week period.

**Figure 2**. Heart rate and heart rate variability responses to submaximal exercise in animals susceptible or resistant to ventricular fibrillation. Exercise elicited significantly greater increases in heart rate that were accompanied by greater reductions in the various indices of cardiac vagal regulation in the susceptible animals as compared to the resistant dogs. * $P<0.01$ susceptible versus resistant, Exercise levels: 1 = 0 kph/0% grade, 2 = 4.8 kph/0% grade, 3 = 6.4 kph/0% grade, 4 = 6.4 kph/4% grade, 5 = 6.4 kph/8% grade, 6 = 6.4 kph/12% grade, 7 = 6.4 kph/16% grade.

**Figure 3**. Heart rate and heart rate variability responses to a 2-min coronary occlusion in animals susceptible or resistant to ventricular fibrillation. The coronary occlusion elicited much larger increases in heart rate that were accompanied by greater reductions in the various indices of cardiac vagal regulation in the susceptible animals as compared to the resistant dogs. $P<0.01$ susceptible versus resistant. Pre = last 30 seconds before the coronary occlusion, Post = 1 min following coronary occlusion release (i.e., average over 30s to 60 s post release).

**Figure 4**. The effect of the 10-week exercise training or 10-week sedentary period on the heart rate and the heart rate variability responses to submaximal exercise in animals susceptible

ventricular fibrillation. Exercise elicited significantly smaller increase in heart rate and smaller reductions in the various indices of cardiac vagal activity in the exercise-trained dogs as compared to animals that received a similar sedentary period. The post-training response in the susceptible exercise-trained dogs was no longer different from that noted for the resistant (exercise trained or sedentary) dogs. * $P<0.01$ exercise-trained versus sedentary, Exercise levels: 1 = 0 kph/0% grade, 2 = 4.8 kph/0% grade, 3 = 6.4 kph/0% grade, 4 = 6.4 kph/4% grade, 5 = 6.4 kph/8% grade, 6 = 6.4 kph 12% grade, 7 = 6.4 kph/16% grade.

**Figure 5.** The effect of exercise training on the heart rate and heart rate variability responses to submaximal exercise in animals susceptible or resistant to ventricular fibrillation. Note that there no longer were any differences between the susceptible and resistant animals. Exercise levels: 1 = 0 kph/0% grade, 2 = 4.8 kph/0% grade, 3 = 6.4 kph/0% grade, 4 = 6.4 kph/4% grade, 5 = 6.4 kph/8% grade, 6 = 6.4 kph 12% grade, 7 = 6.4 kph/16% grade.

**Figure 6.** The effect of the10-week exercise training or 10-week sedentary period on the heart rate and the heart rate variability responses to a 2 min coronary occlusion in animals susceptible to ventricular fibrillation. The coronary occlusion elicited significantly smaller increase in heart rate and smaller reductions in the various indices of cardiac vagal regulation in the exercise-trained dogs as compared to animals that received a similar sedentary period. The post-training response in the susceptible exercise trained animals was no longer different from that noted for the resistant (either exercise trained or sedentary) dogs. * $P<0.01$ exercise-trained versus sedentary . Pre = last 30 seconds before the coronary occlusion, Post = 1 min following coronary occlusion release (i.e., average over 30s to 60 s post release).

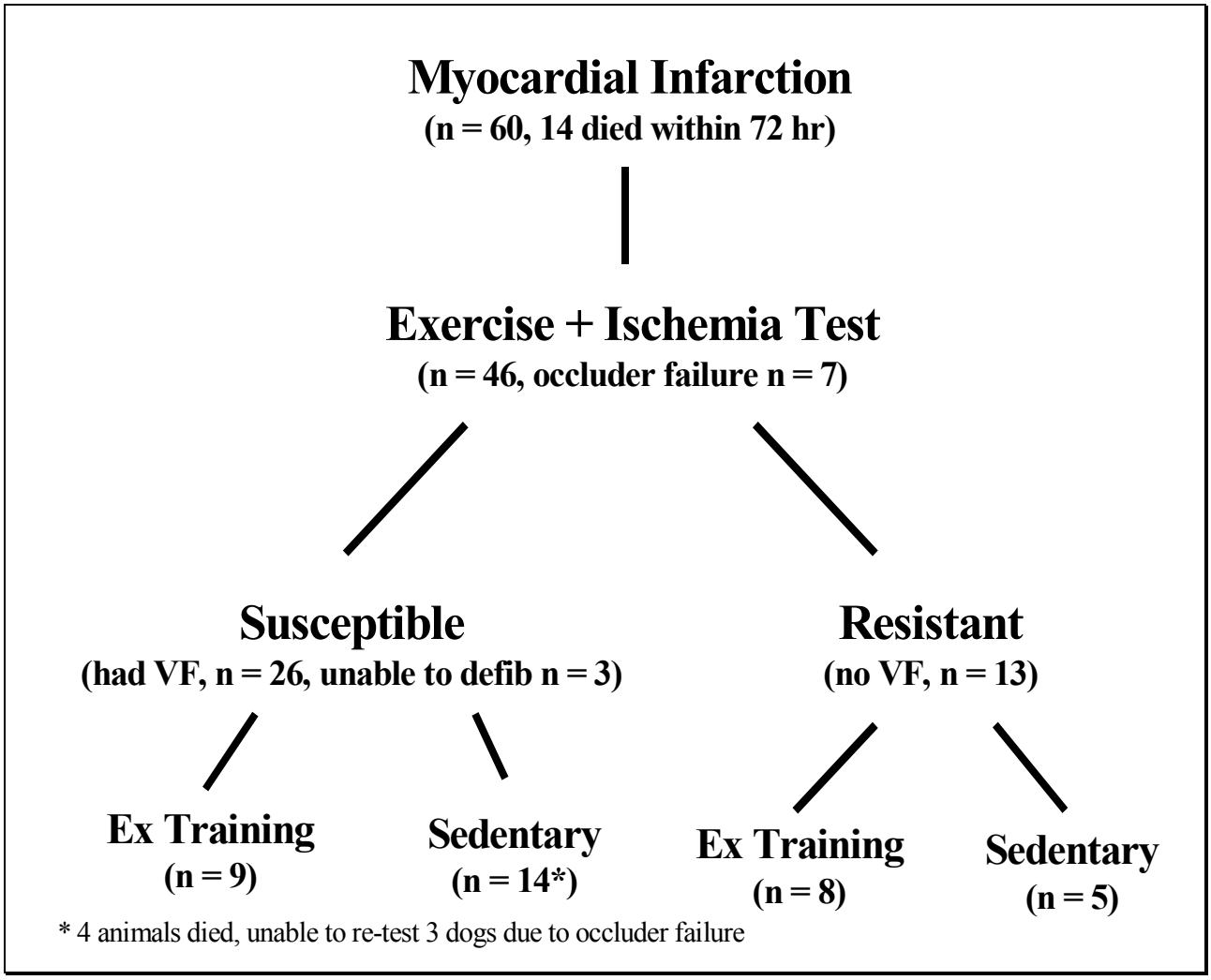
**Figure 7.** The effect of exercise training on the heart rate and heart rate variability responses to a 2-min coronary occlusion in animals susceptible and resistant to ventricular fibrillation. Note

that after exercise training there no longer were any differences between the susceptible and resistant dogs. Pre = last 30 seconds before the coronary occlusion, Post = 1 min following coronary occlusion release (i.e., average over 30s to 60 s post release).

**Figure 8**. The heart rate responses to the exercise plus ischemia test in sedentary and exercise trained animals susceptible and resistant to ventricular fibrillation. The coronary occlusion elicited a significant heart rate increase in the both the sedentary and exercise-trained susceptible animals. Heart rate before and during the coronary occlusions was reduced in the exercise-trained susceptible animals. * $P<0.01$ pre-occlusion versus occlusion, # $P<0.01$ sedentary versus exercise-trained.

**Figure 9**. The effect of atropine on the heart rate responses to the exercise plus ischemia in exercise-trained susceptible dogs. Atropine (50 μg/kg, i.v. given as a bolus 3 minutes before the coronary occlusion) elicited a large increase in heart rate. * $P<0.01$ pre-occlusion versus occlusion, # $P<0.01$ No drug versus atropine.

Figure 1.



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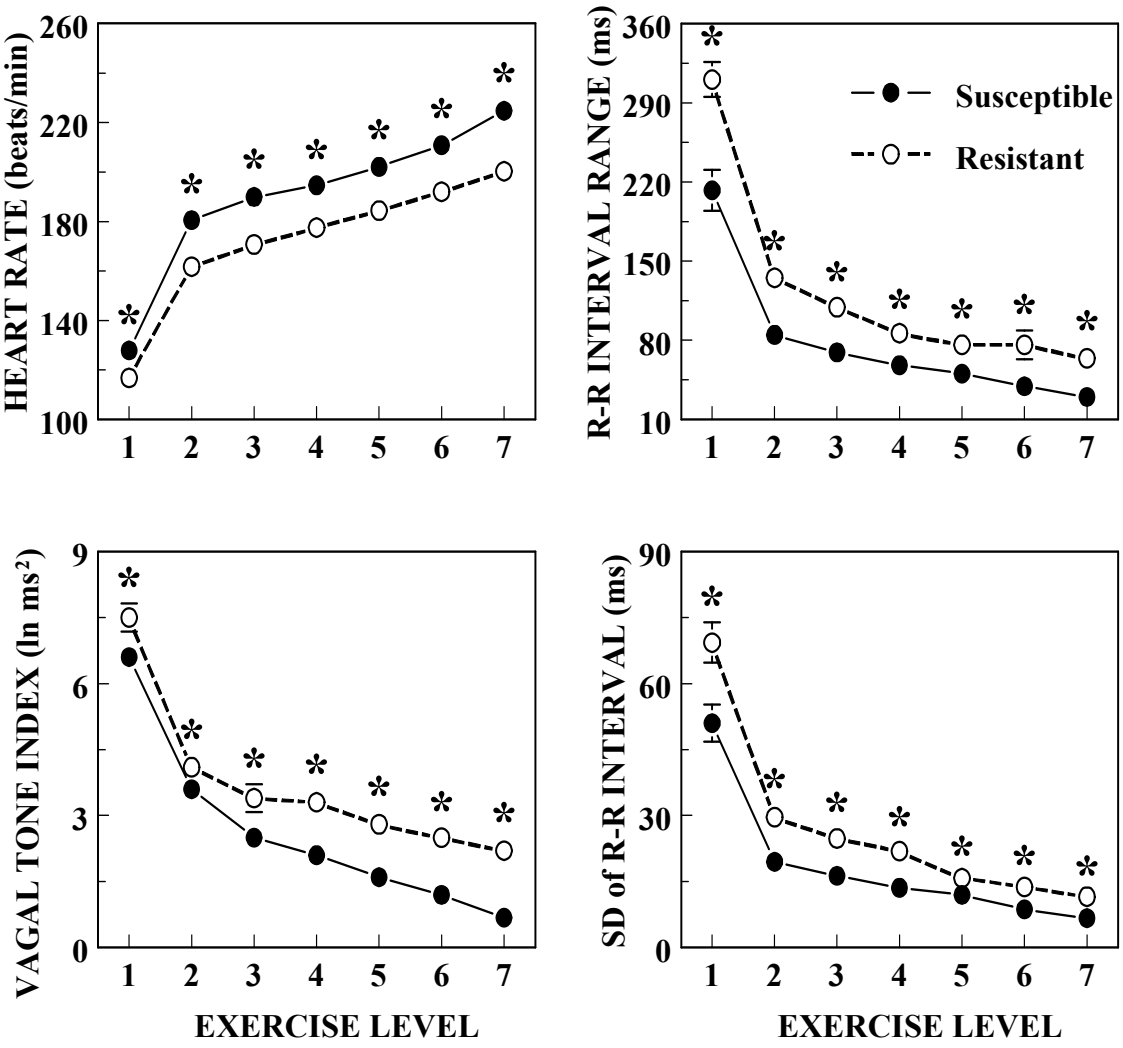

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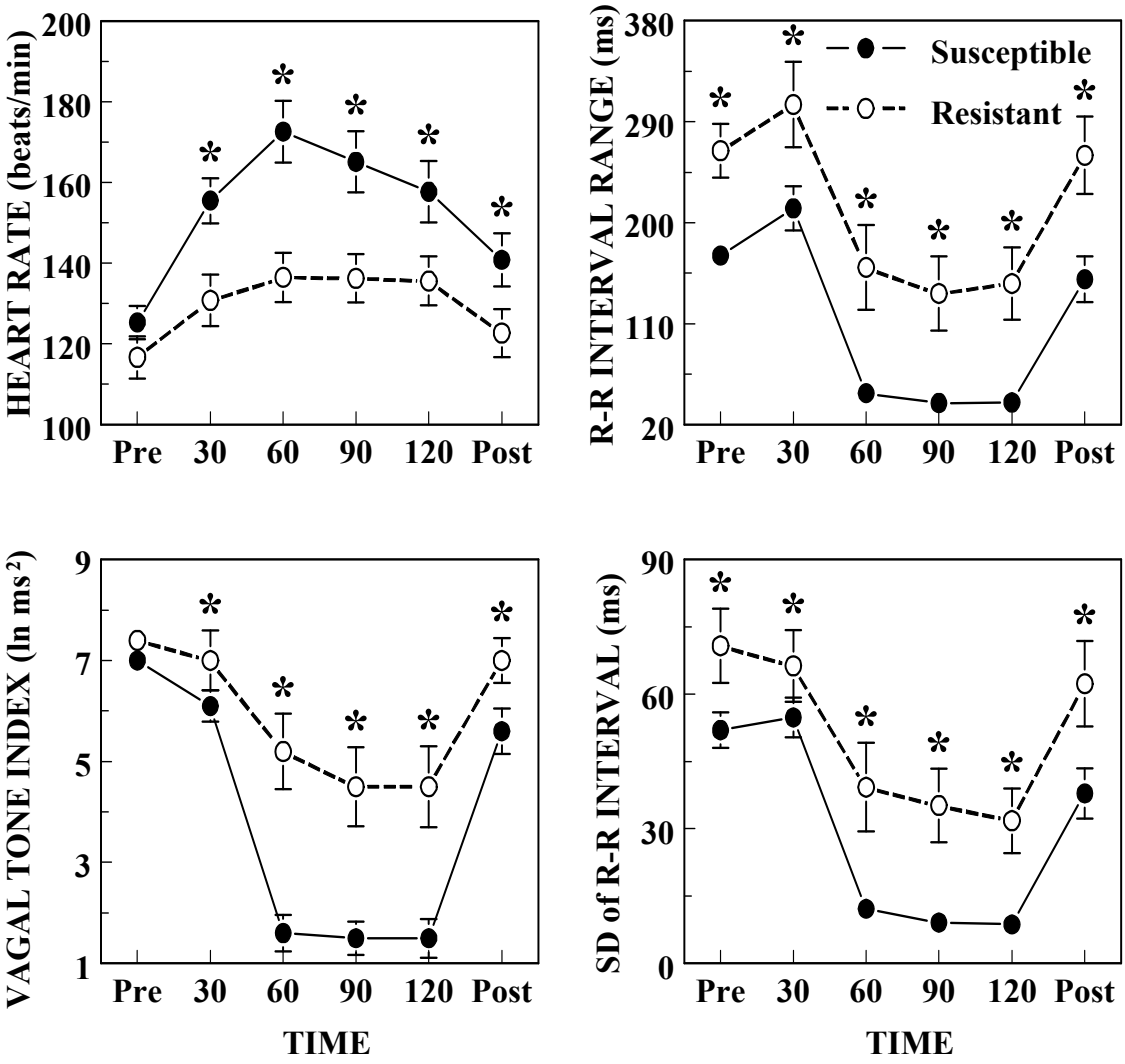

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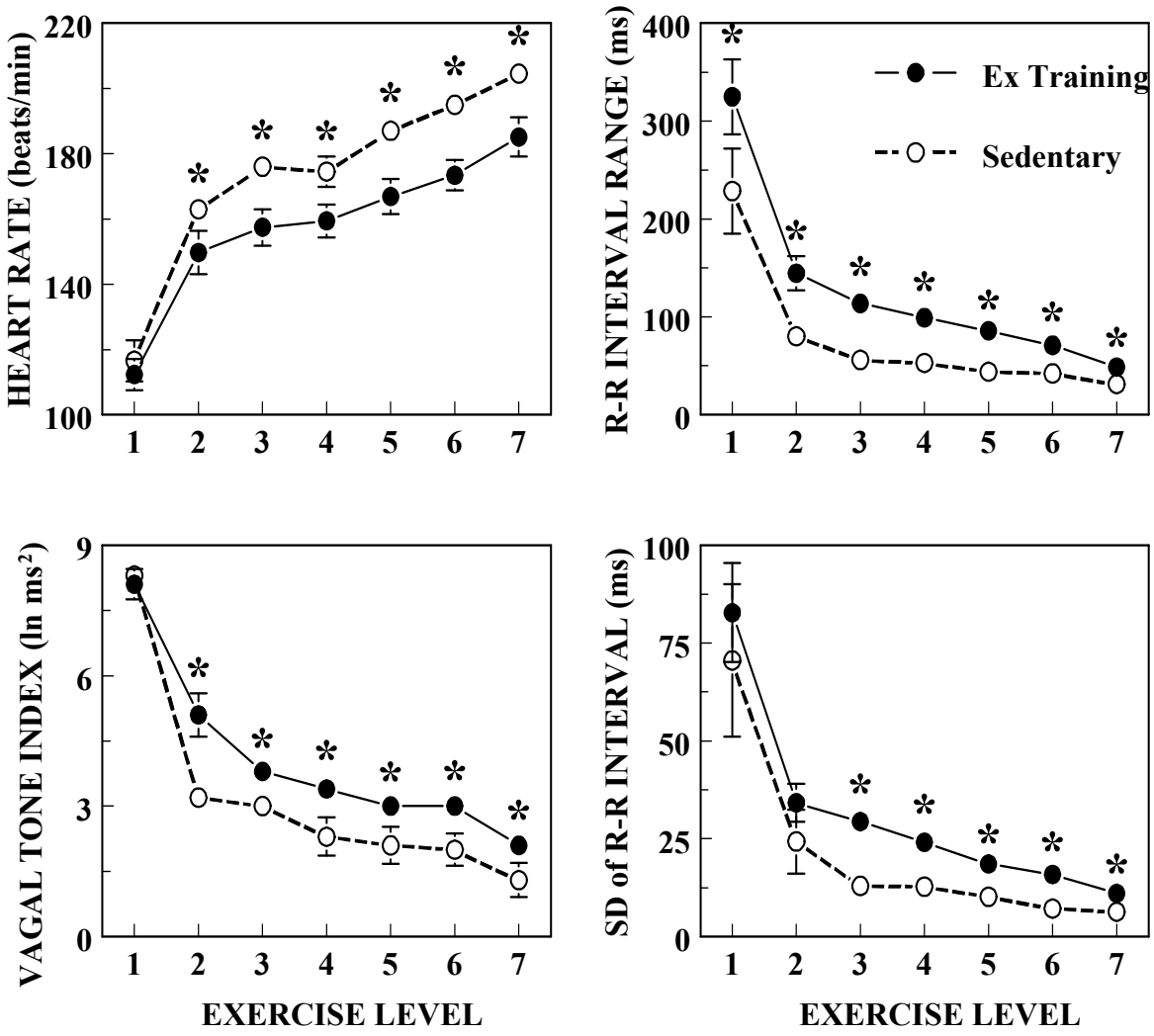

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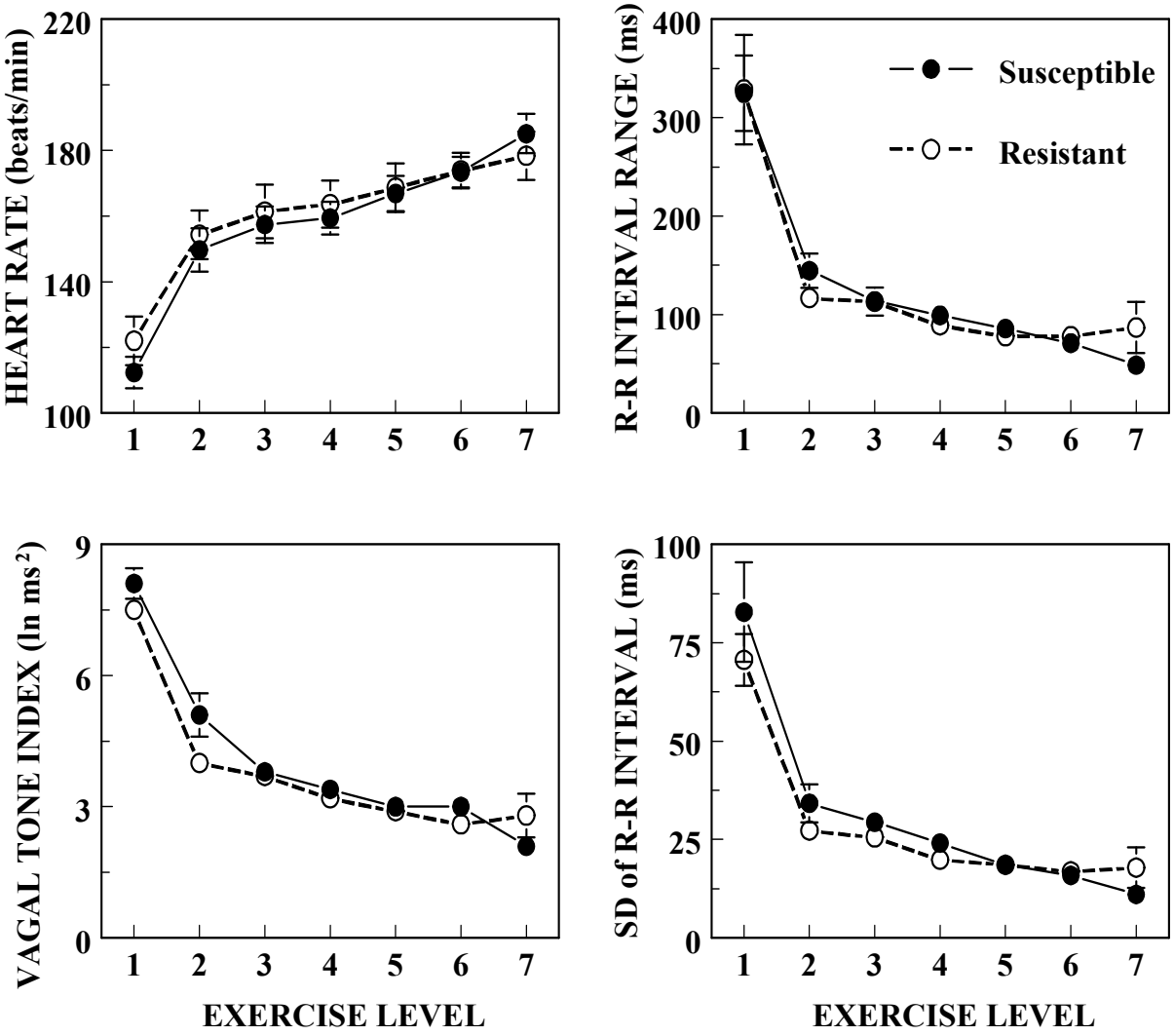

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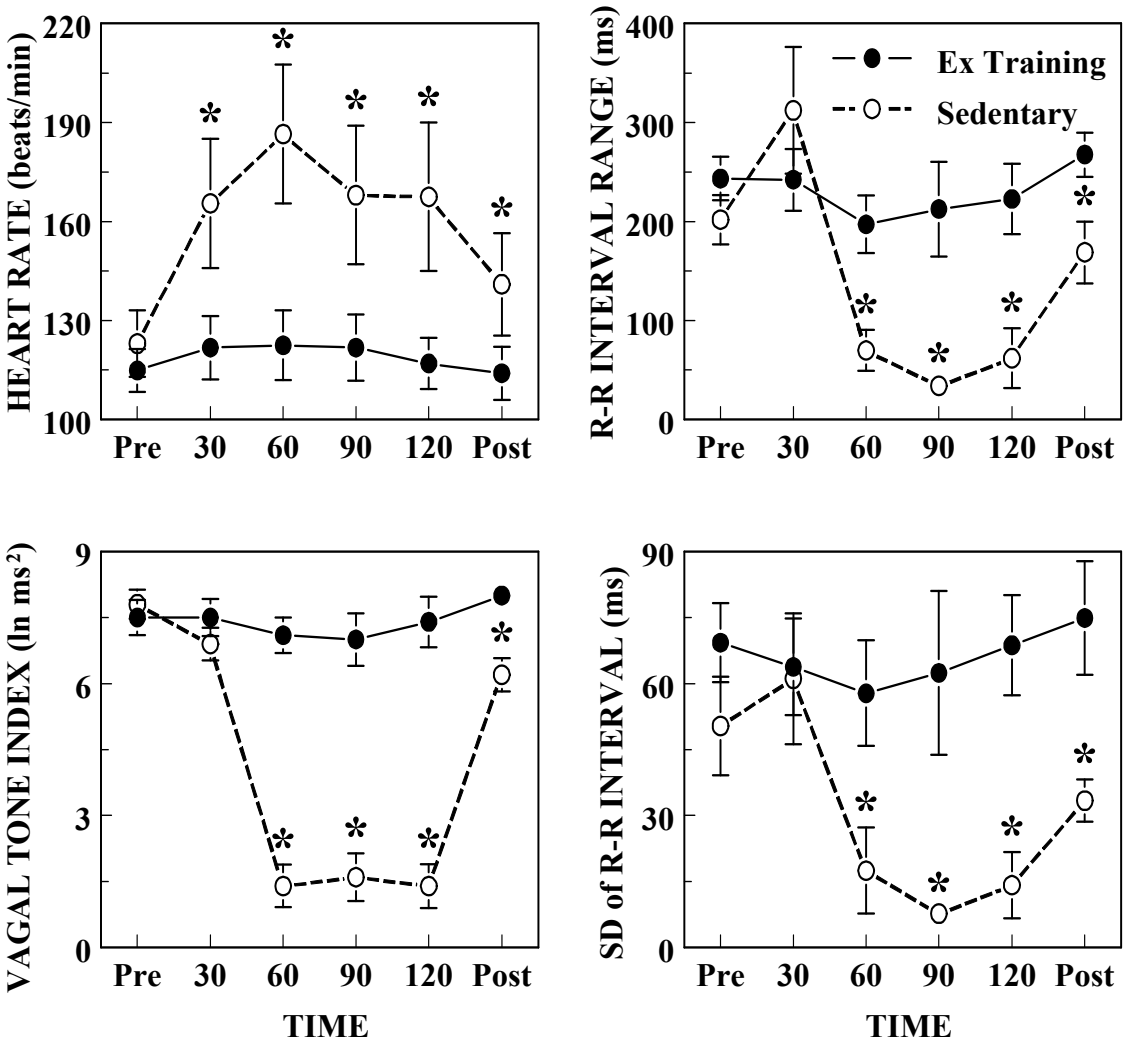

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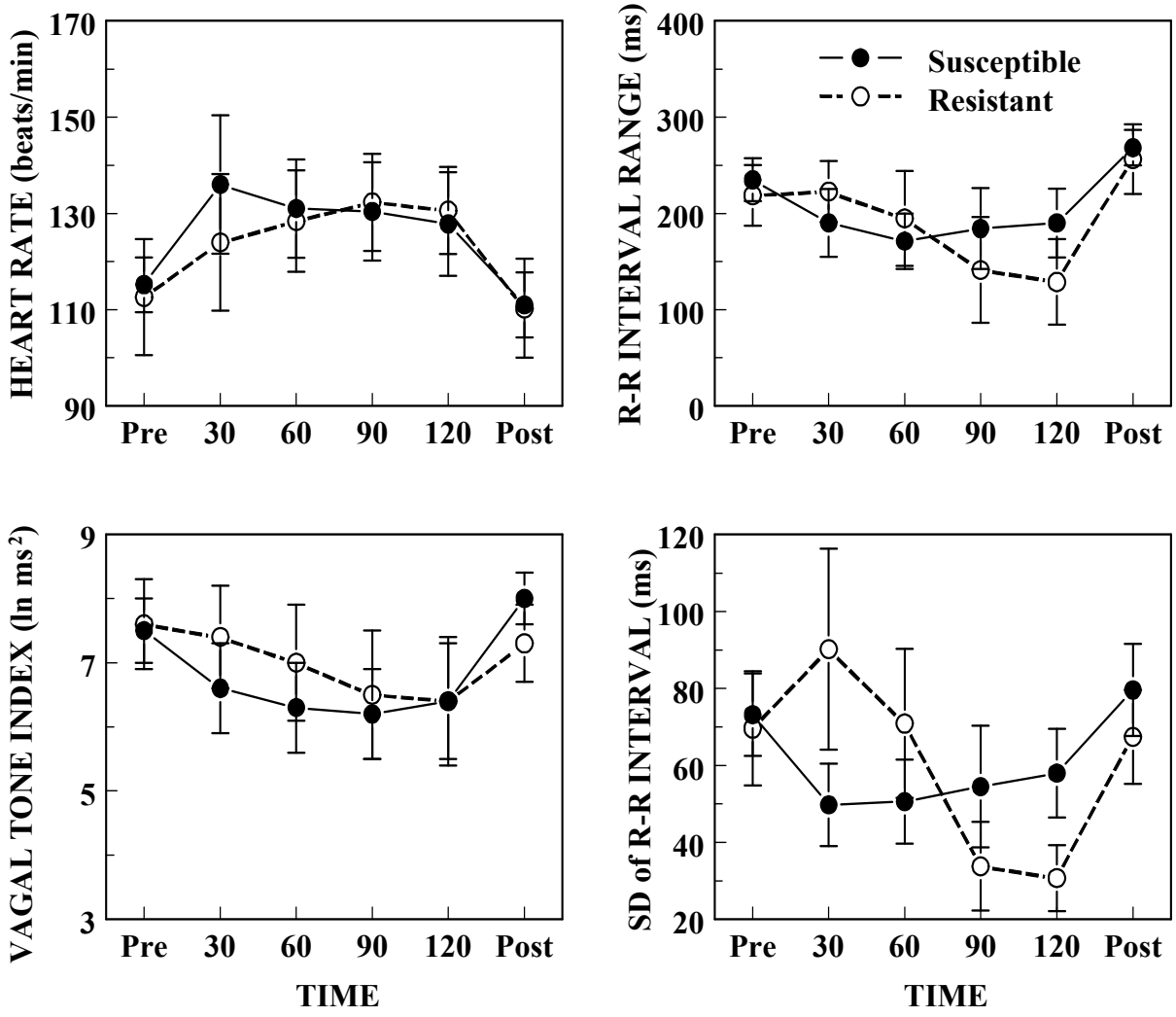

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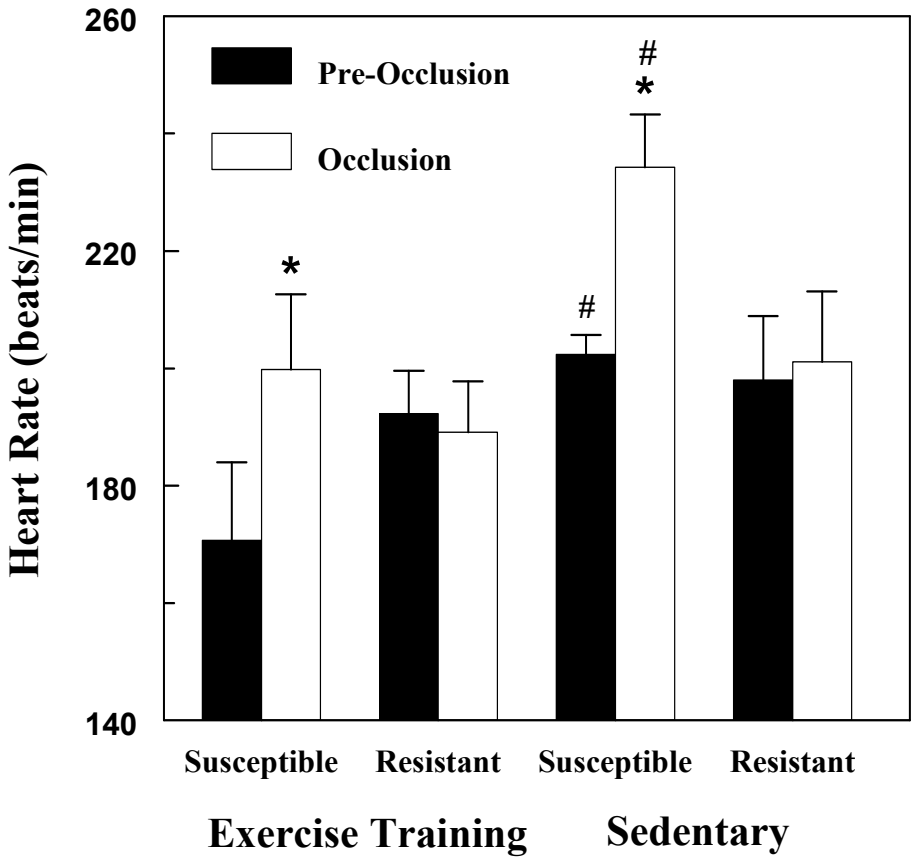

Figure 9.