

Exercise Training Results in Reduced Arrhythmic Burden and a Decrease in the Expression of Connexin 43 Phosphorylation at Serine 368 in Response to Acute Myocardial Ischemia

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Abstract

PURPOSE: Exercise training (ET) has been well documented to elicit antiarrhythmic benefits, although the molecular mechanisms behind these effects have not been established. The gap junction protein connexin 43 (Cx43) is highly expressed in the left ventricle (LV) and maintains efficient cell-cell coupling with evidence indicating that phosphorylation of Cx43 at S368 may increase arrhythmic susceptibility during acute myocardial ischemia (MI). We hypothesized that spontaneous wheel running in rats would reduce cardiac arrhythmic susceptibility in response to acute MI with a concomitant reduction in phosphorylation of Cx43 at S368.

METHODS: Female Sprague-Dawley rats were housed in cages with continuous access to spontaneous running wheels ($n=6$) or in normal sedentary (SED) cage condition ($n=6$) for 8 weeks to elicit ET. ET rats attained a peak running distance of approximately 13km per day after a few weeks and slightly declined over the remainder of the protocol. Following ET, under Inactin anesthesia rats underwent 20 minutes of acute MI through ligation of the left coronary artery during which electrocardiographic data were obtained. Cardiac arrhythmias were classified according to Lambeth Convention guidelines and quantified by an established scoring method. At the end of the protocol the soleus muscles and heart were removed, dissected, weighed and flash frozen. Cx43 expression and phosphorylation of the LV was examined through standard Western Blotting methods.

RESULTS: Citrate synthase activity of the soleus muscle (ET: 14.5 ± 0.5 vs. SED: 11.7 ± 1.1 $\mu\text{mol/g/min}$) was significantly ($p < 0.05$) higher in ET rats, verifying physiological adaptation to the ET. ET rats had a significantly lower arrhythmic burden (ET: 72 ± 30 vs. SED: 302 ± 55 a.u.) in response to acute MI. Furthermore, there was a significant decrease in phosphorylation of Cx43 specifically at S368 in the ET (0.7 ± 0.2 a.u.) animals when compared to SED (1.6 ± 0.2 a.u.), while other phosphorylation sites were unaffected.

CONCLUSION: Spontaneous wheel running exercise results in decreased arrhythmic response to acute MI, which is also associated with reduced expression of Cx43 at S368.

Methods

- Exercise training elicited through housing in cages with access to spontaneous wheel running exercise for 8 weeks.
- Performed acute myocardial ischemia (AMI)
 - Animals were anesthetized with Inactin (1-3mg/kg i.v., 0.05 ml initial dose). Supplemental doses (0.05 ml, i.v.), artificially ventilated, and core body temperature maintained
 - Ligation of the left coronary artery, and second ligation above marginal branch of left descending coronary artery via a left thoracotomy
 - Maintained ischemia for 20 minutes
 - Immediately removed the heart at end of ischemia
- Recorded arrhythmias using standard lead II electrocardiogram
 - Identification (5) & scoring (6) of arrhythmias (Figure 1)
- Tissue removal
 - Animals were deeply anesthetized
 - Removed and weighed the left ventricle (LV) and soleus hindlimb muscle of each animal (Table 1)
- Left ventricle lysed in a detergent-containing buffer and phosphatase inhibitor
- Western Blotting for Cx43 expression
 - Lysate resolved by SDS-PAGE and transferred to membranes
 - Membranes probed with antibodies for total Cx43 and phosphospecific S368-Cx43, as well as GAPDH as a loading control
 - Membranes visualized by chemiluminescence
- In a separate group of intact animals, ischemic area was quantified
 - Evan's blue dye was injected following AMI and allowed to circulate
 - Tissue that did not take up the dye was considered ischemic, and could be visualized (Figure 2)
 - Ischemic area calculated as a percentage of total LV area

Introduction

- A large body of evidence indicates that chronic exercise training can reduce the occurrence of cardiac arrhythmias and sudden cardiac death in individuals susceptible to these disease states (4).
- The cellular mechanisms of the antiarrhythmic effects of exercise training are largely unknown.
- Gap junctions in the heart allow for normal transport of ions through the cell membrane, facilitating cell-cell communication to maintain a normal cardiac rhythm.
- Normal conduction through gap junction proteins can be altered by shifts in phosphorylation at various residues (1).
- Phosphorylation at S368 results in a decrease in channel conductivity (2).
- Studies have shown that acute myocardial ischemia (AMI) causes an increase in phosphorylation at S368 of Cx43 (in addition to inducing cardiac arrhythmias), suggesting that this site is involved in an increased predisposition to arrhythmias (3).
- Due to these interactions, we investigated phosphorylation and expression of Cx43 in response to AMI, and the role of Cx43 phosphorylation in arrhythmic susceptibility in sedentary and exercise trained animals.

Arrhythmia Scoring

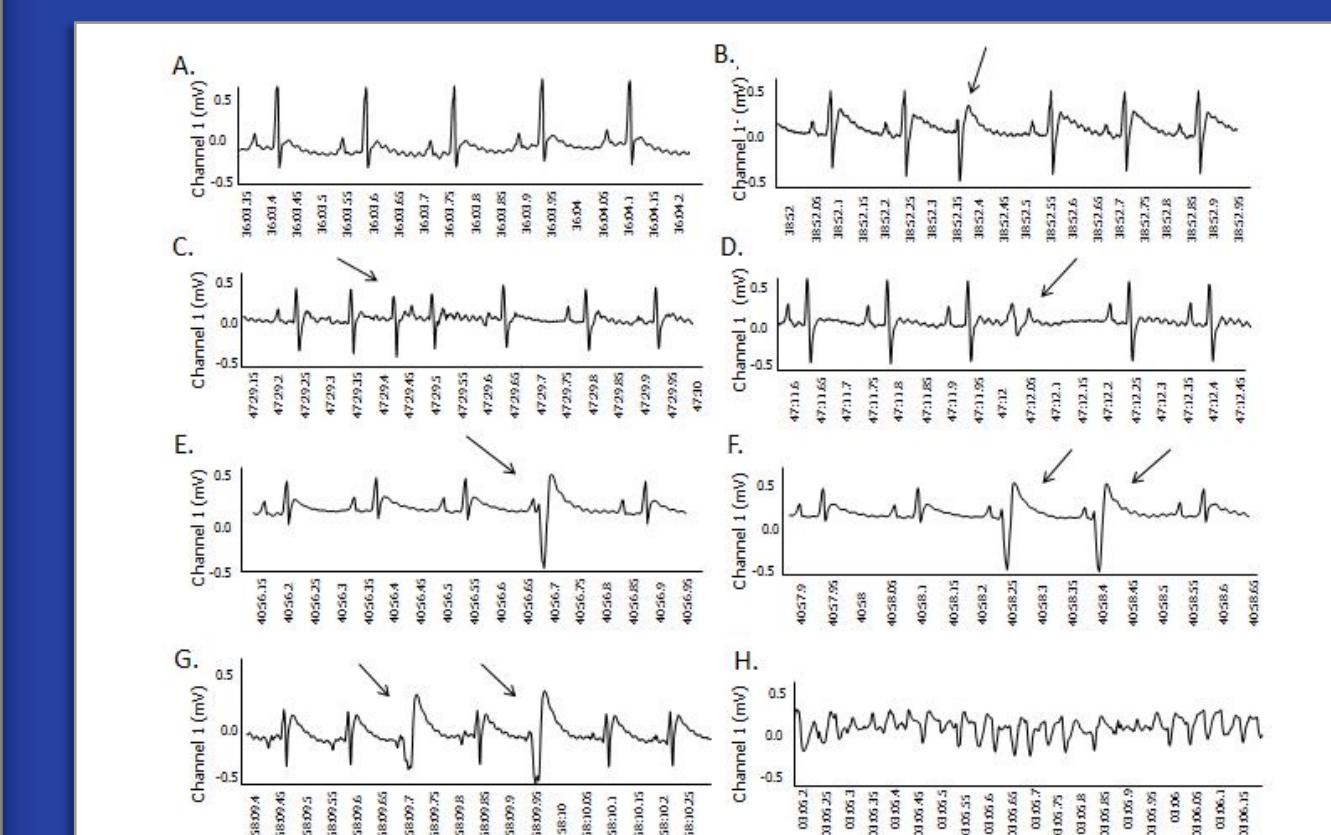


Figure 1: Identification and scoring of arrhythmias occurring during the 20-minute ischemic period. A. normal sinus rhythm B. supraventricular beat (SVB=1) C. supraventricular tachycardia (SVT=2) D. atrio-ventricular block (AVB=1) E. premature ventricular contraction (PVC) F. salvos (two-three consecutive PVCs=2) G. bigeminy (alternating sinus beats and PVCs=3) H. ventricular fibrillation (VF=4).

Results

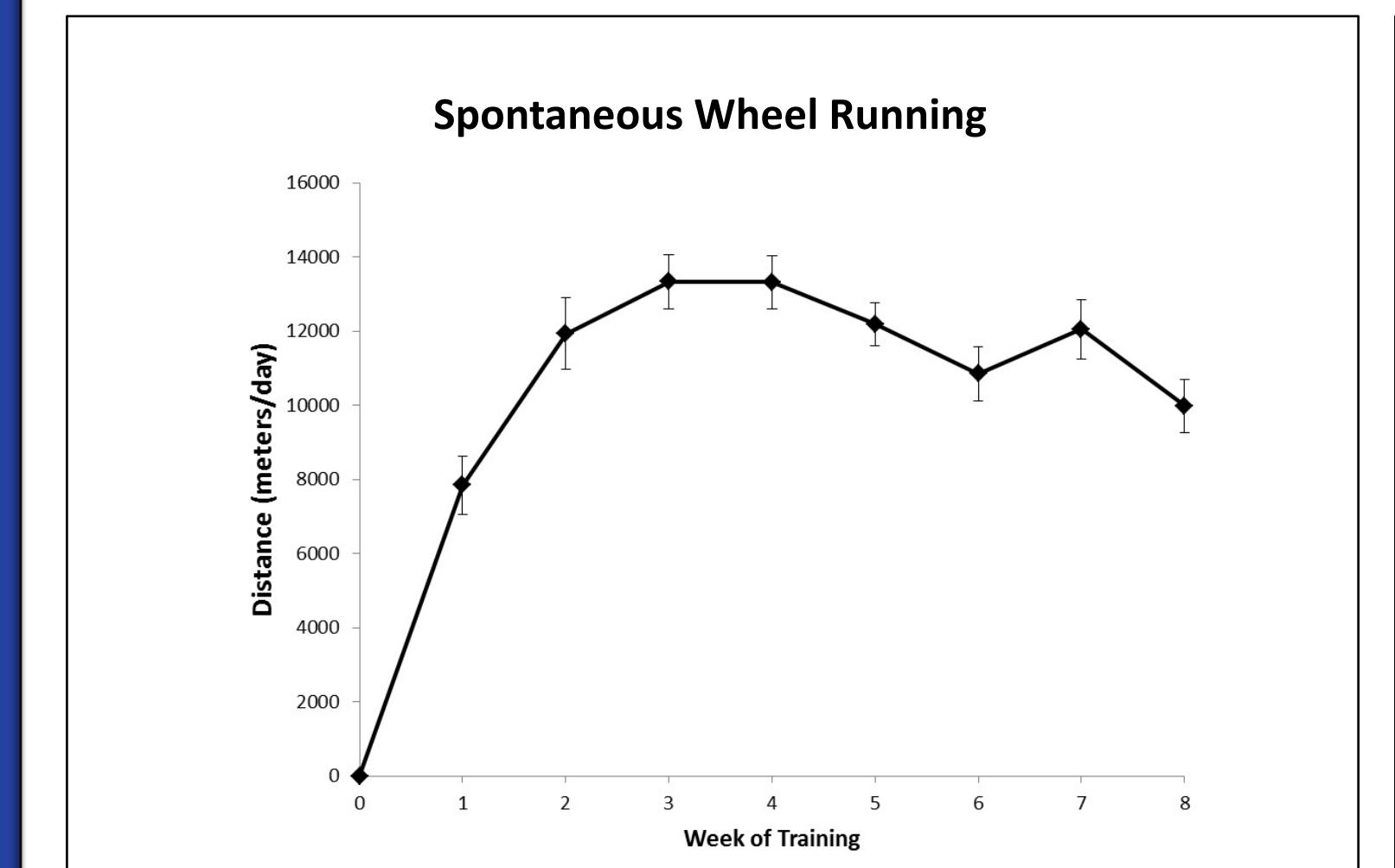


Figure 3: Meters ran per 24 hour period by animals undergoing voluntary exercise training. Over the 8 week training protocol, animals peaked at 3-4 weeks, with an average of ~13 km over each 24 hour period. This curve is typical for female rats, with a slow, slight decrease in running distances over the remainder of the training protocol. Values expressed as weekly means \pm SEM, $n=15$.

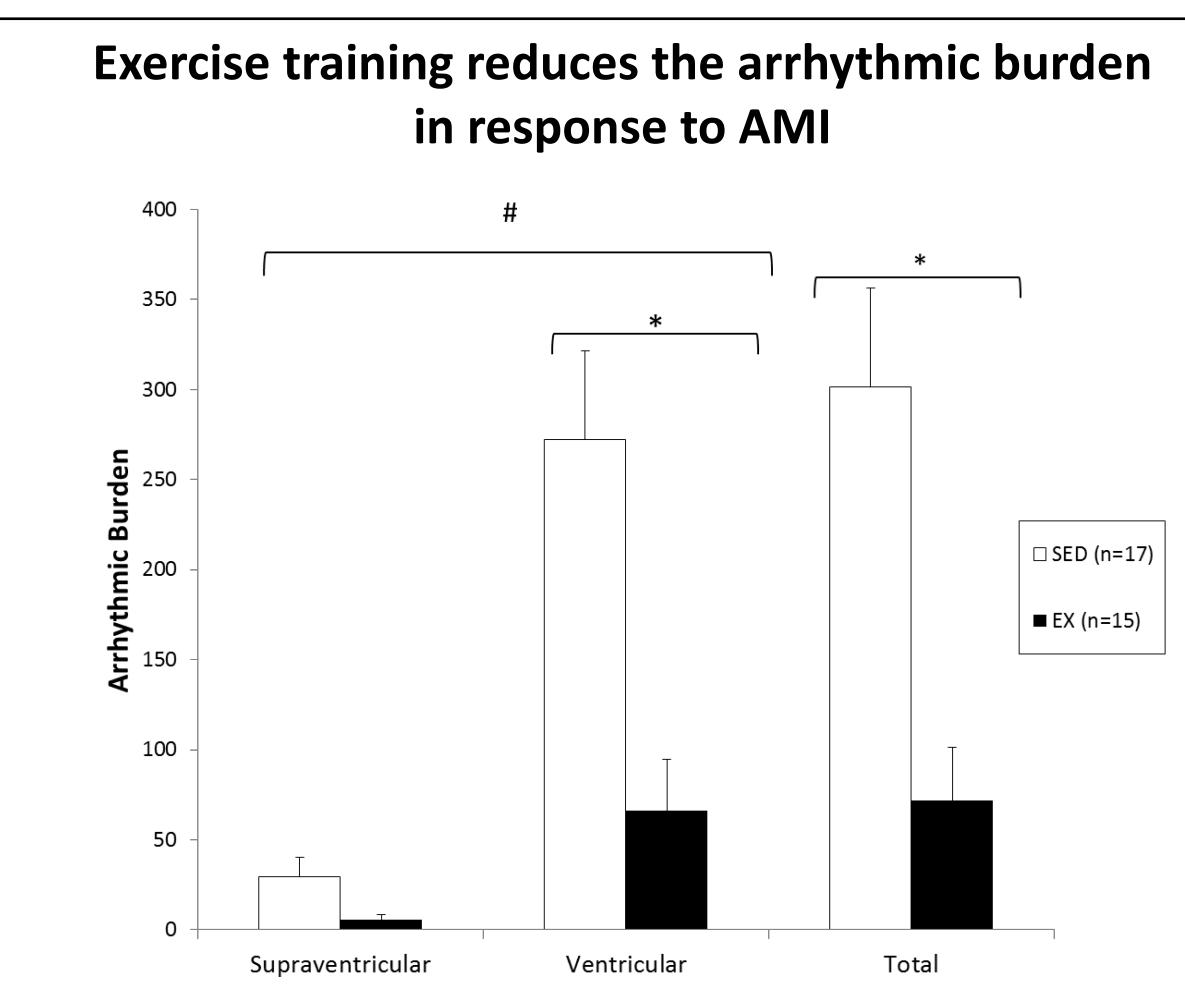


Figure 4: Data comparing the arrhythmic burden in response to ischemia between sedentary (SED) and exercise trained (EX) animals, as determined using established scoring methods. Arrhythmic burden can be broken down further into supraventricular, ventricular, and total as shown above. Exercise animals had a decreased incidence of ventricular arrhythmias, as well as a significantly reduced total arrhythmic burden. Additionally, there were significantly more ventricular-originating arrhythmias than supraventricular arrhythmias in response to AMI. # $p < 0.05$ significant main effect for arrhythmic classification * $p < 0.05$ vs SED

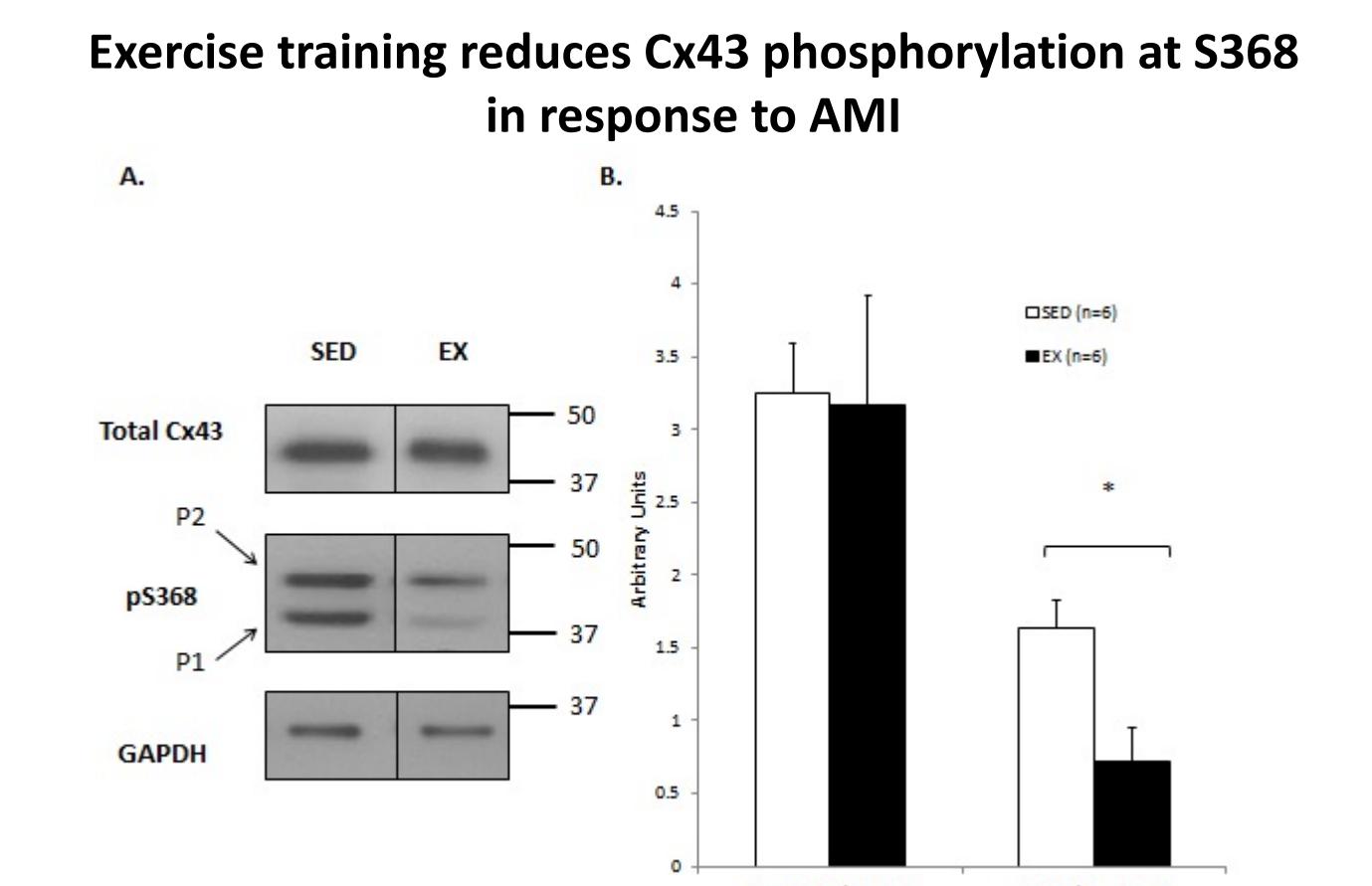


Figure 5: Cx43 protein expression and phosphorylation at S368 in left ventricle lysate of sedentary (SED) and exercise trained (EX) animals in response to acute myocardial ischemia. Panel A shows a representative Western blot analysis of the left ventricle from a SED and an EX animal. Panel B, the quantification of the mean values from these blots, shows the ratio of Total Cx43 to GAPDH (loading control), and pS368 adjusted to the Total Cx43 protein expression following AMI. While Total Cx43 expression was not different between groups, EX animals had significantly decreased expression of phosphorylated S368. * $p < 0.05$

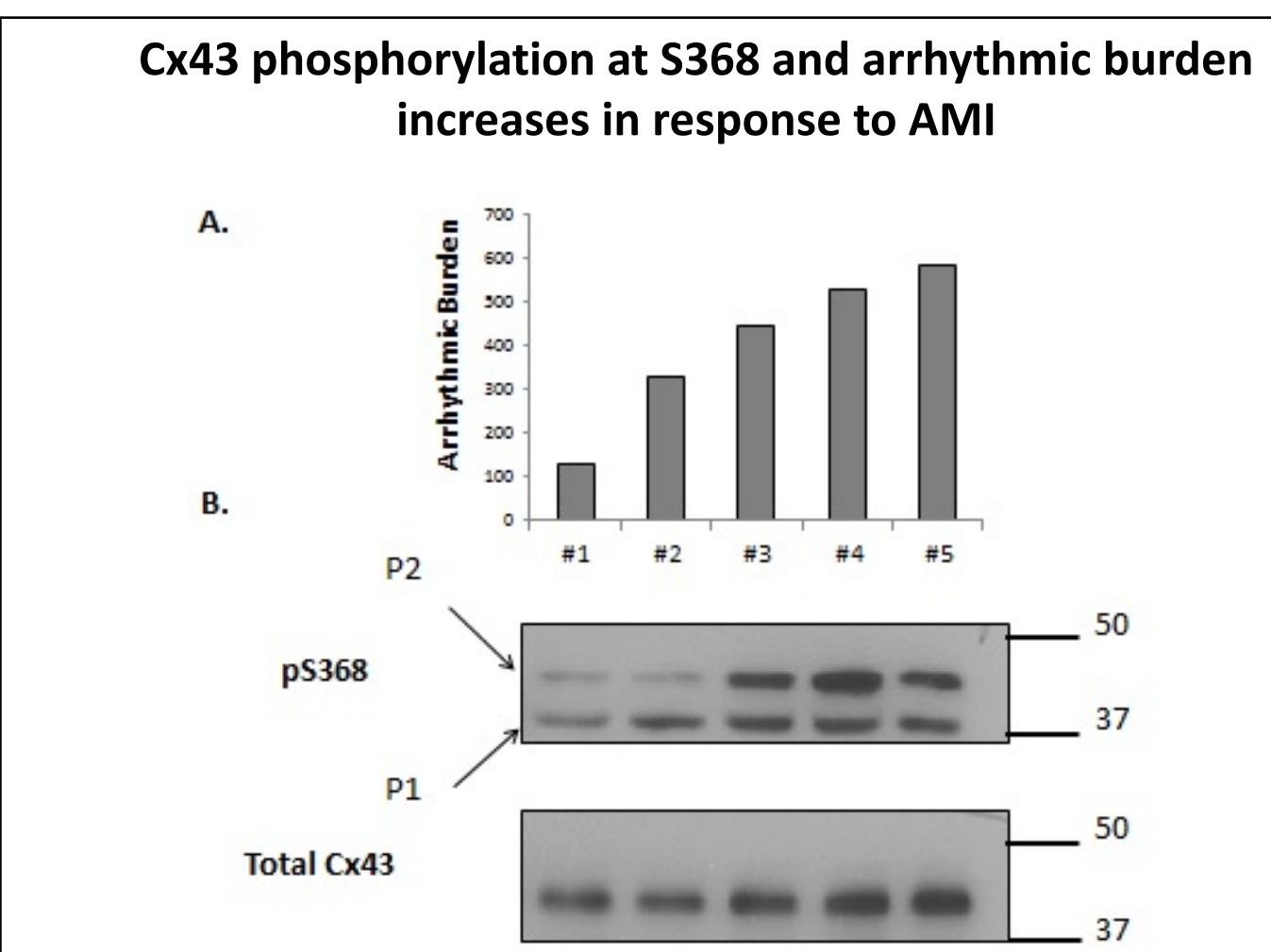


Figure 6: Panel A shows the arrhythmic burden for each of five separate sham-operated, sedentary animals (#1-5). Panel B shows the Western blot illustrating S368 phosphorylation and Total Cx43 expression in these five animals

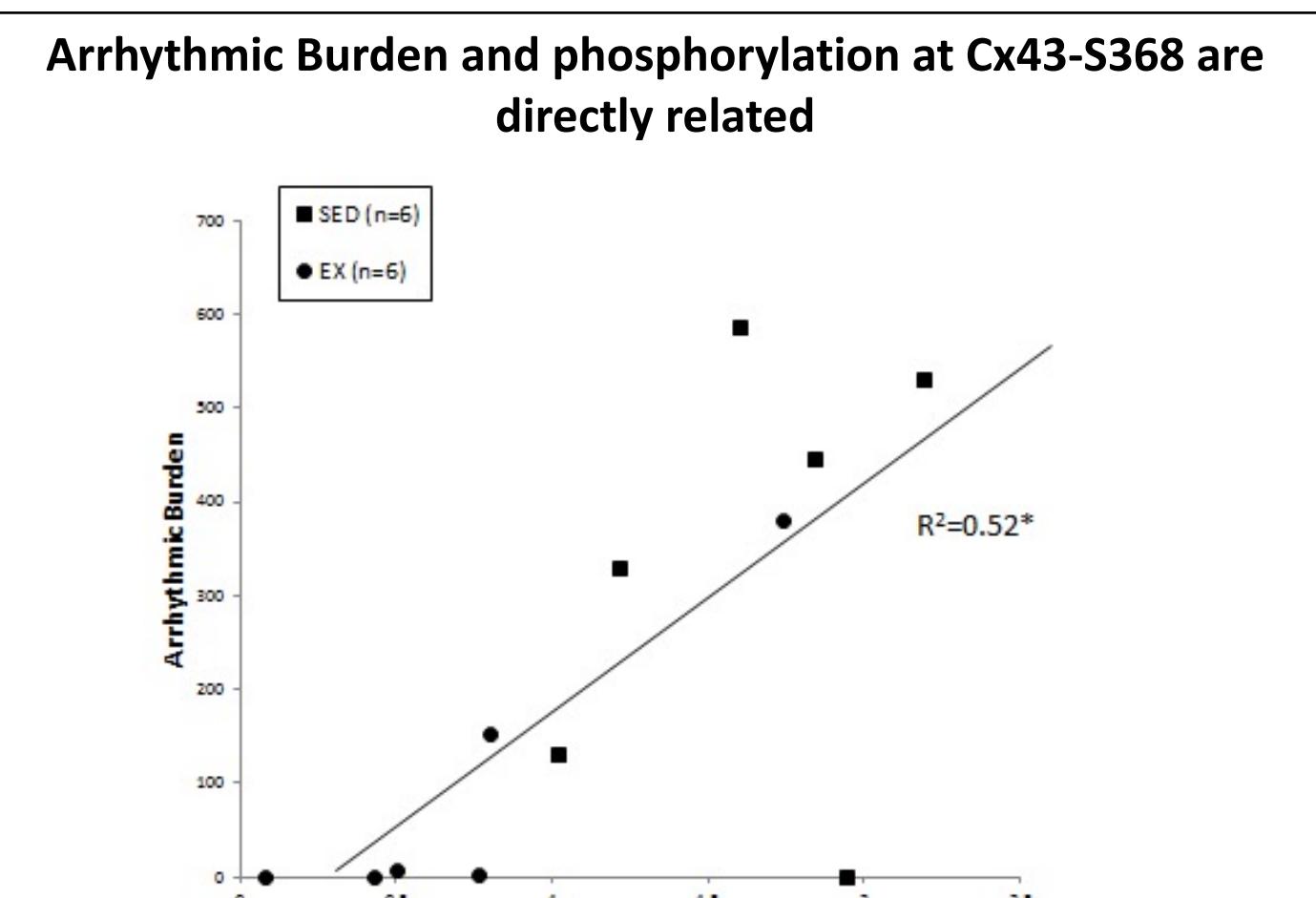


Figure 7: Linear regression analysis of the relationship between the arrhythmic burden of sedentary (SED) and exercise trained (EX) animals in response to AMI and the ratio of pS368/Total Cx43. A significant, direct relationship exists between the arrhythmic burden and the level of phosphorylation at S368. * $p < 0.05$

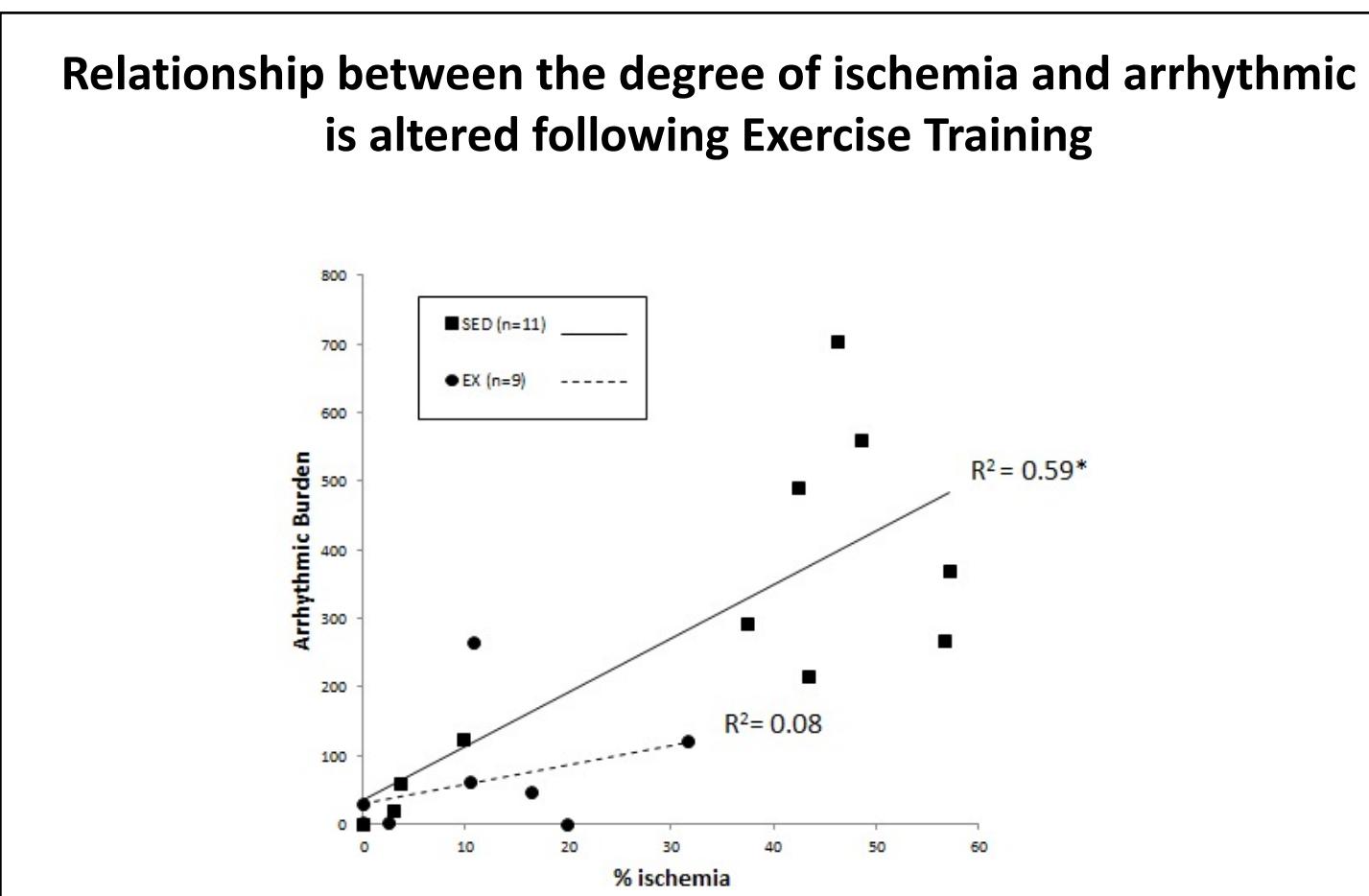


Figure 8: Linear regression analysis of the relationship between arrhythmic burden in response to AMI and the resulting % ischemia area. This analysis showed a direct and significant relationship between the arrhythmic burden and the degree of ischemia induced. Note that the relationship is broken into the sedentary (SED) and exercise trained (EX) groups, which demonstrates the differing slopes between these two groups. * $p < 0.05$

Summary

- Spontaneous wheel running resulted in a high volume of exercise training and significant physiological adaptations to exercise training in female, Sprague-Dawley rats.
- Exercise training reduced the arrhythmic burden in response to AMI as compared to sedentary animals.
- AMI resulted in a direct relationship between the increase in Cx43 phosphorylation at S368, and the arrhythmic burden.
- Similarly, there was a direct relationship between the degree of ischemia and the arrhythmic burden in response to AMI, however this relationship was altered in exercise trained animals such that at a given level of ischemia there was a lower arrhythmic burden in exercise trained animals.

Conclusions

- A high volume of running wheel exercise results in protection from cardiac arrhythmias in response to AMI in female rats.
- There was a directly relationship between phosphorylation of Cx43 at S368 and the arrhythmic burden in response to AMI, with exercise training resulting in less expression S368 Cx43 expression.
- These data taken together suggest that exercise training may induce cardioprotective effects during myocardial ischemia by attenuating Cx43 phosphorylation at S368.

Hypothesis

We hypothesized that there would be a direct relationship between Cx43 phosphorylation at S368 and the arrhythmic burden in response to acute myocardial ischemia. Additionally we hypothesized that exercise training would reduce the arrhythmic burden in response to AMI and attenuate the expression of phosphorylated Cx43 at S368.

AMI Quantification

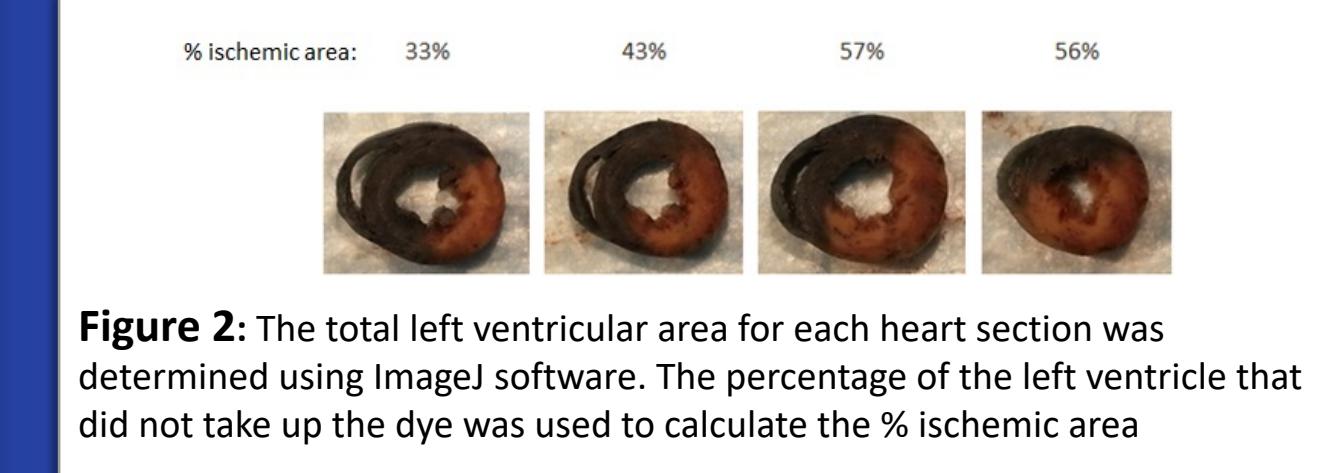


Figure 2: The total left ventricular area for each heart section was determined using ImageJ software. The percentage of the left ventricle that did not take up the dye was used to calculate the % ischemic area

Table 1: Verification of Physiological Adaptation to Exercise Training

	Body wt, g	LV wt, mg	LV/BW, mg/g	Soleus Citrate Synthase, $\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$	Heart Rate, bpm	Blood Pressure, mmHg
SED	257 \pm 6 ($n=17$)	625 \pm 14	2.5 \pm 0.04	12.1 \pm 0.6	348 \pm 8 ($n=17$)	107 \pm 3 ($n=17$)
EX	251 \pm 5 ($n=15$)	723 \pm 20	2.9 \pm 0.06*	15.2 \pm 0.5*	327 \pm 7 ($n=15$)	102 \pm 6 ($n=15$)

Data comparing body weight, heart weight to body weight ratio, citrate synthase activity of the soleus, baseline heart rate and blood pressure (anesthetized) between sedentary (SED) and exercise trained (EX) animals. * $p < 0.05$ vs SED

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