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Age-Related Autonomic Regulation in Catecholaminergic Ventricular Tachycardia

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THE FLORIDA STATE UNIVERSITY

COLLGE OF HUMAN SCIENCES

AGE RELATED AUTONOMIC REGULATION IN CATECHOLAMINERGIC POLYMORPHIC VENTRICULAR TACHYCARDIA

By

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1. Abstract:

Catecholaminergic polymorphic ventricular tachycardia (CPVT), also called exercise-induced ventricular tachycardia is a hereditary disease that can cause sudden cardiac death in human patients. Interestingly, while patients with CPVT have normal function of the heart at rest, they show life-threatening arrhythmias during physical activity or acute emotional stress. Autonomic nervous system is known to have a role in arrhythmia pathogenesis, and its response changes with aging. However, little is known that how autonomic regulation with aging contributes to arrhythmia burdens in CPVT. Methods: To test our hypothesis, we examined ECG recording with a catecholaminergic challenge (isoproterenol, 3mg/kg + caffeine, 120mg/kg) in anesthetized CPVT mice at different age groups (6 weeks old, n=6; 12 weeks old, n=9; and 48 weeks old, n=6). Data expressed Mean±SE. Results: In CPVT mice, the frequency of arrhythmias were increased with aging (Premature ventricular contractions (PVCs)/min: 6 weeks, 24.8±14.67; 12 weeks, 17.7±15.65; 48 weeks, 115±51.52). The resting heart rate (HR) decreased (BPM: 6 weeks, 462±23; 12 weeks, 419±11; 48 weeks, 360±10*, *p <0.01) with aging, whereas the HR response after adrenergic stress was significantly increased (delta HR: 6 weeks, 1.3±0.04; 12weeks, 1.4±0.03; 48weeks, 1.5±0.04*, *p <0.05). The average RR interval also increased in advanced age (msec: 6 weeks, 130.2±6.27; 12 weeks, 144.2±3.68; 48 weeks, 168.7±5.69*, *p <0.01), suggesting a possibility of an increased autonomic regulation. Conclusion: We found that arrhythmia incidence worsens by lowering intrinsic HR, and possibly by a modulation of parasympathetic nerve activity in CPVT mice with advanced age.

2. Introduction:

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmia syndrome. This condition is characterized by stress-induced bidirectional or polymorphic ventricular tachycardia (VT) without any underlying functional or structural abnormalities at rest. [1] The present defect in CPVT is impaired intracellular calcium regulation from mutations in the genes: RyR2,[26] Casq2,[3,4] triadin,[27] and calmodulin.[28] Those suffering from CPVT are characterized by a normal baseline electrocardiogram, but display polymorphic or bidirectional ventricular tachycardia under stress conditions.[1] The more common, autosomal dominant form is linked to a mutation in the gene encoding the cardiac calcium release channel (Ryanodine receptor 2; RyR2).[2] The cardiac calsequestrin 2 (Casq2) mutation is a less common but more severe form caused by an autosomal-recessive mutation that affects the major calcium binding protein in the sarcoplasmic reticulum.[3,4,5] Sudden cardiac death has been reported in patients with both mutations.[6,7] Some patients with the Casq2 mutation have been shown to have complete loss of Casq2 function, yet maintain normal cardiac contractile function in the non-stressful conditions.[6] The mutation of the Casq2 gene is known to destabilize the RyR2 complex and cause stress-induced ventricular tachycardia in human patients.[8] The primary function of Casq2 was found to serve as a modulator of sarcoplasmic reticulum (SR) calcium release where it reduces SR calcium release and RyR2 open probability in conditions of high SR calcium conditions.[8] In this condition, catecholamines bind to β-adrenergic receptors, which causes the SR of cardiomyocytes to spontaneously release calcium via RyR2.[8] These catecholamine-induced premature SR calcium release and spontaneous calcium waves cause delayed after–depolarizations in myocytes which triggers additional heart beats called premature ventricular contractions (PVC) in hearts.[8,9] It was also found that while Casq2 gene knockout mice (Casq2-/)

showed normal function SR calcium storage, they were prone to premature spontaneous SR calcium release and triggered PVC beats leading to arrhythmias after an isoproterenol induced stress test.[8] An unexpected finding in one study showed a drastic increase in the SR calcium binding protein calreticulin. A protein normally found in the developing heart but decrease after birth.[37]

Since the Casq2 mutation alters SR calcium release[8], it is important to understand the role that calcium plays in the electrical excitation-contraction coupling of cardiomyocytes. During the depolarization of the phase of the action potential, calcium enters the cells via depolarization gated channels[39] on the plasma membrane. This influx of calcium triggers calcium release from the SR[39] via RyR2. This increase in intracellular calcium concentration allows for calcium to bind to troponin C[39], in turn exposing the active sites on the myofilament actin, allowing cross bridge formation and contraction to occur. For relaxation, calcium must be transported out of the cytosol[39], hereby ending the excitation coupling reaction.

The current standard treatment for patients with CPVT are beta-adrenergic receptor blockers, calcium channel blockers, and implantable cardioverter defibrillators (ICD).[10,11] The treatment of beta-adrenergic receptor blockers reduces the arrhythmia burden but are not completely effective[1] and often increases the mortality of patients. In more severe cases, patients will have an ICD to prevent sudden cardiac death, but if the device delivers an inappropriate shock it can increase the adrenergic stress in the circulation, and causes arrhythmias.[10,11]

In a study to find the underlying mechanism in CPVT, it was found that mice with RyR2 mutations also had "leaky" RyR2 channels.[8] One study that generated RyR^{+}/RyR^{R4496C} knock-in mice showed that after stress testing with epinephrine and/or caffeine, the mice displayed bidirectional and polymorphic VT, characteristic of patients with CPVT.[34] Additionally, the lack

of calsequestrin led to premature calcium leak from the SR, inducing triggered beats and eventually arrhythmias.[8] With very few long-term studies of CASQ2 CPVT patients, there is little known about the cardiac manifestations that could arise later in life. One of the few long term studies[50] follows a patient for 23 years, starting from the age of 13. Their study found a previously reported splice site mutation (c.532+1G>A (p.Gln67*)) on one allele and a novel stop mutation (c.199C>T) on the other. In the last 5 years, her ECGs have shown low QRS voltage in the precordial leads, increased QRS duration, and right bundle branch block. However, it is unclear if these changes are pharmacologically induced.

The autonomic nervous system is an important regulator of the electrophysiology of the heart. It is composed to two antagonistic divisions, the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). PNS stimulation, which is primarily dominant during resting conditions, works by decreasing heart rate via vagal impulses,[46] which decrease the spontaneous pacemaker activity of SA node.[47,48] The SNS is responsible for increasing heart rate and cardiac contractility, decreasing venous capacitance, and constricting resistance vessels.[44,45] In a healthy heart, the SNS influence shortens the action potential duration.[40] PNS stimulation, unlike the SNS, has a different effect on the atria and the ventricles. In the atria, PNS stimulation reduces the effective refractory period,[41,42] while in the ventricles, it prolongs the effective refractory period and action potential duration.[40,43] SNS stimulation of the heart is regulated by the cardiovascular reflex. [44,45]

New treatment studies have been focusing on certain class 1C, sodium channel blockers as an alternative therapy. One found that the class 1C antiarrhythmic drug flecainide, a known sodium channel blocker, suppressed spontaneous SR calcium release in Casq2 -/- mice by inhibiting RyR2.[12] Flecainide successfully prevented exercise-induced polymorphic or

bidirectional VT in conscious Casq2-/- mice.[12] A follow up study tested all class 1C antiarrhythmic drugs in addition to flecainide from the previous findings. Propafenone, one of the class 1C antiarrhythmic drugs tested, is used clinically as a racemic mixture of the R and S-enantiomers, and both are sodium channel blockers and RyR2 channel blockers. However, only S-propafenone has a moderate beta-adrenergic blocking activity.[13] The study found that propafenone also inhibited RyR2 calcium release and successfully prevented exercise-induced CPVT in Casq2-/- mice, and suppressed arrhythmogenic calcium waves.[14] The potency and efficacy of flecainide and propafenone on suppressing arrhythmogenic calcium waves was determined to be based on the RyR2 activity.[15] Increasing RyR2 activity increased the potency of flecainide and propafenone action.[15]

Recently, a study on catheter-based renal sympathetic denervation (RDN) which was used in place of antiarrhythmic drugs and cardiac ablation, found that RDN successfully reduced arrhythmia burden in patients with chronic heart failure and refractory ventricular arrhythmias.[25] This study demonstrated that the autonomic nervous system plays an important role in ventricular arrhythmias. In a study seeking an alternative therapy in suppressing CPVT symptoms, overdrive atrial pacing was found to successfully reduced catecholamine-induced VT in mouse models of CPVT, and exercise-induced VAs at peak exercise in patients with CPVT.[29]

CPVT patients also exhibit sinoatrial node (SA) dysfunction[23] which is innervated by the autonomic nervous system (ANS). Heart rate variability (HRV) is a non-invasive way to measure ANS function. It provides a way to indirectly measure ANS fluctuations via parasympathetic and sympathetic influence by recording electrocardiogram (ECG) data. The higher HRV would mean that the heart has wider physiological range by modulating the sympathetic and parasympathetic nervous systems under stress. The ANS plays a role in the regulation of the heart rate. The

parasympathetic and sympathetic systems of the ANS modulate vagal tone to control the rate of blood flow through the heart resulting in the regulation of heart rate (HR). There are three frequency components of HRV: high frequency (HF) and low frequency (LF) and very low frequency.[18] HF is respiration-linked and is mediated solely by the parasympathetic vagal activity, while LF reflects mainly sympathetic and partly parasympathetic activity.[31] The LF/HF ratio is used to determine sympathetic nervous system activity.[32]

The effects of aging on autonomic control of the cardiovascular system show impaired vagal control of HR with increasing age and significant reduction in the cardio vagal functional domain.[16] There is evidence that aging reduces overall autonomic control, with sympathetic predominance and vagal tone diminishing;[19] however, Casq2-/- mice have an increased vagal tone compared to wild type mice. [24] The age-related increase in sympathetic nervous system influence affects the heart by reducing the effective refractory period, increasing automaticity, and a raising the susceptibility to ventricular arrhythmias.[22] Age related cardiac changes in 35 weeks old $CASQ^{\Delta E9/\Delta E9}$ and $CASQ^{307/307}$ showed LV hypertrophy, atrial enlargement and reduced ejection fraction compared with wild type mice. [38]

A recent study on the effects of long-term exercise on Casq2-/- mice found that the autonomic adaptations that occur with long-term exercise had a dual role in triggering the catecholaminergic arrhythmias by decreasing HR and increasing SR calcium leak via the RyR2 in ventricular cardiomyocytes. [36] There was a decrease in sympathetic activity and increase in vagal activity, suggesting that both branches could have contributed to the decrease in HR. [36] The study noted that with the enhanced vagal response of the trained mice, their susceptibility to arrhythmia increased by debilitating robust HR. [36] Notably, they found that ß-adrenergic stimulation simultaneously increased parasympathetic effects in the trained Casq2-/- mice.

[36]Importantly, the deficient HR acceleration during sympathetic activation from chronotropic incompetence becomes even more evident after training. [36] The conclusion of the study determined that light recreational activity could be harmless or beneficial but must be regularly monitored.[36]

3. Hypothesis:

Heart rate plays an important role in catecholaminergic polymorphic ventricular tachycardia patients and mice, and the autonomic nervous system a one of the major mechanisms to control heart rate. Autonomic nervous stimulation and heart rate are known to change with age, but little is known about how these changes can affect the progression of CPVT in mice models. In this study we tested the hypothesis that age-related autonomic changes will exacerbate arrhythmia susceptibility in aged CPVT mice. To test our hypothesis, we had two specific aims as follows: 1. Aged CPVT mice will increase the incidence of ventricular arrhythmias after stress challenge. 2. Aged CPVT mice will decrease resting heart rate and heart rate variability.

4. Experimental Design

Animal Models of CPVT:

In this study, we used Casq2 -/- mice of four different age groups. The mice show relatively normal SR calcium release and contractile function, attributed to increases in SR calcium content, lower levels of triadin and junctin, and increased calcium induced SR calcium release.[8] The Casq2-/- mice also exhibit normal SR calcium storage but increased SR calcium leak, increasing their susceptibility to ventricular arrhythmias.[8] The groups used were ages 6

weeks, 12 weeks, 24 weeks, and 48 weeks consisting of n=9 for each group. The mice were kept in a 12-hour light-dark cycle and had free access to normal diet and water.

ECG Recording of Isoproterenol-induced Stress in Casq-/- Mice:

Prior to ECG recording the mice will be anesthetized with 2% isoflurane with loss of toe pinch reflex used to measure level of anesthesia. Surface ECG needles will be placed sub dermally in each limb. ECG data will record the baseline HR for five minutes and for an additional 25 minutes after the isoproterenol+caffeine challenge. To control body temperature of the mice during ECG measurement, a warm pad of 36˚C will be used.

Isoproterenol + Caffeine Challenge:

Isoproterenol (ISO) is a β-adrenergic receptor agonist, and it is used to induce ventricular arrhythmia in CPVT mice as known previously.[8] Caffeine is an RyR2 agonist that induces calcium release from intracellular stores and lowers the threshold for luminal calcium activation of the RyR2 channel.[35] Isoproterenol 3mg/kg has been shown to increase heart rate and induce ventricular tachycardia in CPVT mice as shown previously[14] so this is the concentration that will be used in this study. A high dose of caffeine (CAFF) (120 mg/kg) was used in addition to ISO to increase the open probability of the cells and further induce arrhythmia. In a study using RyR2 knock-in mouse model, the mice displayed bidirectional and polymorphic tachycardia after injection of 120 mg/kg of caffeine.[34] After five minutes of baseline HR measurement, the ISO+CAFF challenge was injected intraperitoneally.

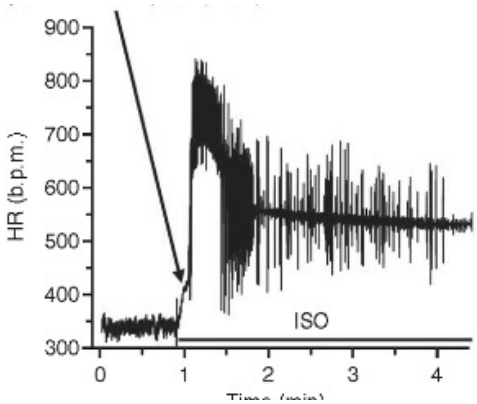


Figure 1: Heart rate response to ISO (1.5 mg kg^{-1}) challenge in a *Casq2*$^{-/-}$ mouse. As illustrated in the ECG trace, the rapid and irregular heart rate (HR) was the result of numerous repetitive ventricular extrasystoles (VE, arrows) and the induction of ventricular tachycardia (VT). b.p.m., beats per minute.[12]

Heart Rate Variability:

Heart rate variability (HRV) is a measure of beat to beat fluctuation that indicates the autonomic nervous system influence on heart rate.[22] High frequency (HF) is solely mediated by the parasympathetic nervous system while low frequency (LF) is mediated by both the sympathetic and parasympathetic systems.[20] Since it has been shown that short term frequency domain HRV analysis is more accurate than long term, a five minute increment was chosen.[18] Compared to a healthy individual, lower HRV can serve as an predictor of mortality and arrhythmic complications. Depressed HRV after a myocardial infarction has also been associated with increased cardiac mortality.[30]

Analysis

The LabChart software was used to record the ECG and measure HRV. A total of three parameters were used to determine age-related changes in autonomic regulation. Heart rate

changes from rest, before ISO+CAFF injection, peak, and end of measurement. The incidence of arrhythmias counted from 0-30 minutes. The heart rate variability measured in five-minute increments. The data obtained from the heart rate variability measurements was then used to measure the autonomic regulation of heart in mice. A T-Test was be performed to calculate the statistical significance of the differences of arrhythmia incidence between the different age groups of mice.

5. Limitations:

A limitation in this study is from the use of unconscious mice for ECG recording to obtain HRV data. All studies on HRV in mice models have used conscious mice to obtain their data. Our use of unconscious mice may have affected values obtained for HRV.

The ISO+CAFF challenge may increase the mortality rate due to the polymorphic and bidirectional ventricular tachycardia it may induce. This mortality rate may be of significant importance in the 6 weeks age group because of their high resting heart rate.

Although the conventional is thought is that LF power relates to sympathetic response, some studies believe that LF is related to baroreflex function. Patients with low baroreflex-cardiovagal slope (BRS) had low LF power compared to patients with normal BRS regardless of the status of cardiac sympathetic denervation.[33] When pharmacological agents were used to increase release of norepinephrine from sympathetic nerves, LF power only increased in patients with normal BRS.[33]

6. Results:

Heart Rate Response Increased in Aging CPVT Mice:

Heart rate plays an important role in CPVT. Little is known about how heart rate changes with age in CPVT mice. The resting HR decreased with aging, gradually lowering in each age group tested (6 weeks: 462 bpm, 12 weeks: 419 bpm, 48 weeks: 360 bpm). The decrease in resting HR, shown in Figure 9 was significant between 6weeks and 48 weeks (P=0.002) and 12 weeks and 48 weeks (P=0.002).

As shown in Figure 10, average peak HR in response to catecholaminergic challenge with ISO (3 mg/kg) and CAFF (120 mg/mg) was highest in 6weeks mice (619 bpm), we quantified the change in HR from rest to peak and found that the greatest increase was in 48 weeks mice (1.49). This increase was significant between 6 weeks and 48 weeks (P=0.03) and 12 weeks and 48 weeks (P=0.02).

Heart Rate Variability Changes in Aging CPVT Mice:

Heart rate variability (HRV) is a non-invasive way to measure ANS function. It provides a way to indirectly measure ANS fluctuations via parasympathetic and sympathetic influence by recording electrocardiogram (ECG) data. The LF/HF ratio is a mixture of sympathetic and parasympathetic activity. To further investigate the modulation of autonomic function, we tested several measures for heart rate variability in aged CPVT mice. As shown in Figure 8, in the time domain we analyzed RR interval, with increasing age, RR interval increased from 138ms in 6weeks to 158ms in 48weeks, but the results were not statistically significant. In the frequency domain show in Figure 7, we measured LF/HF ratio for each age group. 48 weeks showed the highest (0.65), while 12 weeks showed the lowest (0.36).

Total Incidence of Arrhythmia Increases with Age in CPVT Mouse Model:

CPVT Cas2-/- mice show catecholamine-induced premature SR calcium release and spontaneous calcium waves that cause delayed after–depolarizations in myocytes which triggers additional heart beats called premature ventricular contractions in hearts. The spontaneous SR calcium release can also lead to arrhythmias. The total number of arrhythmias increased significantly between 6 weeks and 48 weeks only mice (P=0.02) with an 85% increase and between 12 weeks and 48 weeks old mice (P=0.01) with a 99% increase in incidence as shown in Figure 6. Five minutes after anesthetizing, a catecholaminergic challenge was performed which induced a greater response in 48 weeks mice compared to 6 weeks and 12 weeks (P=0.03, P=0.02 respectively) as shown in Figure 10.

Ventricular tachycardia, a severe symptom of CPVT, was more prevalent in aged CPVT mice compared to the younger age groups. Of the 9 mice age 48 weeks tested 8showed ventricular tachycardia compared to 1/8 6 weeks and 2/8 12 weeks.

PR interval and QRS duration increased in Aging CPVT Mice:

To investigate if CPVT mice had altered cardiac conduction, we measured PR interval and QRS duration in Casq2-/- mice. PR interval duration increased in the aged CPVT mice. As shown in Figure 2, at rest, 48 weeks was significantly higher than both 6 weeks (P=0.002) and 12 weeks (P=0.009). At 20 min, 25min and 30min of measurement, 48 weeks (P=0.02, P=0.03, P=0.04) and 12weeks (P=0.009, P=0.02, P=0.048) PR interval were significantly higher than 6weeks.

QRS interval duration also increased in aged CPVT mice shown in Figure 4. At 5 minutes, 12 weeks was significantly longer than 6 weeks (P=0.02) and 48 weeks was longer than 6 weeks as well, but not statistically significant. At 20, 25, and 30 minutes of recording, 48 weeks was significantly higher than 6 weeks (P=0.02, P=0.02, P=0.02). At 25 minutes, 48 weeks was significantly higher than 12 weeks (P=0.02).

7. Discussion:

ANS regulation is altered in aged 48weeks CPVT mice compared with 6weeks an 12weeks mice. In this study we tested the hypothesis that aging alters autonomic regulation in CPVT Casq2 KO mice. We found that aging exacerbated arrhythmia susceptibility in aged mice, possibly due to a modulation of parasympathetic activity.

A previous study reported that a decrease in resting HR resulting from long-term training in Casq2-/- mice increased arrhythmia susceptibility in the trained mice by increasing SR calcium leak from RyR2 in ventricular myocytes [36] The enhanced vagal response of the trained Casq2-/- also increased arrhythmia susceptibility by debilitating robust HR. [36] These findings support our hypothesis that the aged CPVT mice, which had a lower resting HR (Figure 9) and increased incidence of arrhythmia (Figure 6), could possibly have altered parasympathetic activity.

Renal Denervation has recently proven to be an effective treatment for ventricular arrhythmias.[25] This connection between renal denervation and the reduction of ventricular arrhythmias demonstrates that the autonomic nervous system plays an important role in ventricular arrhythmias. This evidence supports our data that showed altered autonomic nervous system with aging, as demonstrated by decreased HR (Figure 9) and increased PR interval and QRS duration (Figure 2, 4), exacerbated arrhythmias susceptibility. It is previously known that

aging reduces HR and cardio vagal functional domain[16], with some studies showing that overall autonomic control is reduced.[19] Our data of reduced HR, shown in Figure 9, and increased change in HR in response to catecholaminergic challenge, shown in Figure 10, support previous findings of altered ANS function due to aging. We hypothesize that these changes in ANS function increase susceptibility to ventricular arrhythmias in aged CPVT Casq2-/- mice.

Based on our results, we found that 48 weeks mice have the lowest resting HR compared to 6 weeks and 12 weeks. CPVT mouse models[8] and human patients[23] have been shown to exhibit resting bradycardia. A recent study found that a low basal heart rate is a significant risk factor for CPVT.[49] This finding supports our data that found the 48 weeks had the most susceptibility to arrhythmia and the lowest resting HR. The increases in PR interval and QRS duration, shown in Figures 2 and 4, respectively, could be due to altered cardiac conduction. At rest and after response to catcholaminergic challenge, 48 weeks mice consistently showed increased PR interval, as shown in Figure 2. QRS duration in 48 weeks was drastically higher than 12 weeks and 6 weeks, most notably after catecholaminergic challenge. These data are consistent with a previous case study[50] who also found increased QRS duration in a CPVT patient beginning at 31 years old. They also found low QRS voltage in precordial leads and right bundle branch block[50] which could support our hypothesis that cardiac conduction was altered in aged CPVT mice.

The slowed conduction shown in increased PR interval (Figure 2) and QRS duration (Figure 4), could be due to an increase in phosphorylated C-jun N-terminal kinase (JNK) which has been shown in aging mice, humans, and rabbits.[53] In aged mice, on study found an increase in JNK phosphorylation, calcium wave frequency, and SR calcium load and leak.[54] These findings further support our data of increase susceptibility in aged mice, possibly due to increased JNK

activation with alters calcium handling causing calcium leaks which lead to arrhythmogenic intracellular calcium waves. [54]

Aged 48 weeks mice also exhibited a decrease in LF/HF ratio. This decrease in LF/HF ratio and increase in RR interval (Figures 7 and 8 respectively) as well as the decrease in resting HR (Figure 9), suggests that the autonomic nervous system is altered with aging in CPVT Caq2-/- mice. We hypothesize that this modulation is from a change in parasympathetic activity because of the lowered HR that the ages mice exhibit.

It is known that aging can increase reactive oxygen species (ROS) production. These increase in ROS production lead to oxidative stress that play an important role in the pathogenesis of ventricular arrhythmias. [51] Antioxidants such as tempol (a superoxide dismutase mimetic) and N-acetyl-L-cysteine, (an ROS scavenger) significantly reduced ventricular arrhythmias in Casq2-/- mice by reducing SR calcium leak and release. [52] Vidarabine, a cardiac adenylyl cyclase inhibitor, successfully reduced ventricular arrhythmias by inhibiting sympathetic activation induced cardiac ROS production in cardiac myocytes. These findings further link ROS production to an increase in arrhythmias susceptibility.[52] These findings may also explain how the increase in arrhythmias in aged Casq2-/- mice could be due to increased ROS production and oxidative stress.

A limitation of this study is that HRV was measured in unconscious mice. HRV value norms from the 1996 Task Force are based experiments that used conscious mice. Certain components of HRV such as HF are correlated with respiration rate, so the decrease in respiration rate caused by isoflurane could affect this. In future studies, we will use conscious mice to eliminate the possibility of isoflurane influence on HRV.

In conclusion, this study found that in Casq2-/- CPVT mice, the effects of aging exacerbate arrhythmias susceptibility. The aged mice exhibit lower resting HR, increased change in HR after catecholaminergic challenge, and increased PR interval and QRS duration after catecholaminergic challenge. All of these data, along with other mechanisms of aging such as increased ROS production, could be responsible for an altered autonomic nervous system, especially the parasympathetic nervous system, that leads to increased arrhythmias.

These findings are significant because very few studies have been done on the effects of aging on Casq2-/-CPVT mice. More studies are needed to improve treatment and monitoring of the disease progression.

Figure legends

Figure 1: Experimental Design. A: Age groups of Casq2-/- mice to be tested. B: Protocol for ECG recording and catecholaminergic stress challenge.

Figure 2: PR interval changes in aged CPVT Mice at rest and after stress challenge. Changes in PR interval between each age group. * vs. 6 weeks and # vs. 12 weeks p<0.5

Figure 3: Normalized PR interval changes in aged CPVT Mice at rest and after stress challenge. Changes in PR interval between each age group. * vs. 6 weeks and # vs. 12 weeks p<0.5

Figure 4: QRS duration changes in aged CPVT Mice at rest and after stress challenge. Changes in QRS duration between each age group. Values are expressed mean"±"SE. * vs. 6 weeks and # vs. 12 weeks p<0.5

Figure 5: Normalized QRS duration changes in aged CPVT Mice at rest and after stress challenge. Changes in QRS duration between each age group. Values are expressed mean"±"SE. * vs. 6 weeks and # vs. 12 weeks p<0.5

Figure 6: Total number of PVC in aged CPVT Mice at rest and after stress challenge. Incidence of PVC after stress challenge in each age group. Values are expressed mean"±"SE. * vs. 6 weeks and # vs. 12 weeks p<0.5

Figure 7: LF/HF ratio for each age group at rest. Values are expressed mean"±"SE. * vs. 6 weeks and # vs. 12 weeks p<0.5

Figure 8: Changes of RR interval for each age group. Values are expressed mean"±"SE. * vs. 6 weeks and # vs. 12 weeks p<0.5

Figure 9: Resting HR values for each age group. Values are expressed mean"±"SE. * vs. 6 weeks and # vs. 12 weeks p<0.5

Figure 10: Heart rate response after stress challenge in aged CPVT Mice. HR change after stress challenge for each age group (HR change: Peak HR- Resting HR). Values are expressed mean"±"SE. * vs. 6 weeks and # vs. 12 weeks p<0.5

Figure 11: HR increase in response to ISO injection. Watanabe et al, *Nat Med*, 2009

Figure 1.

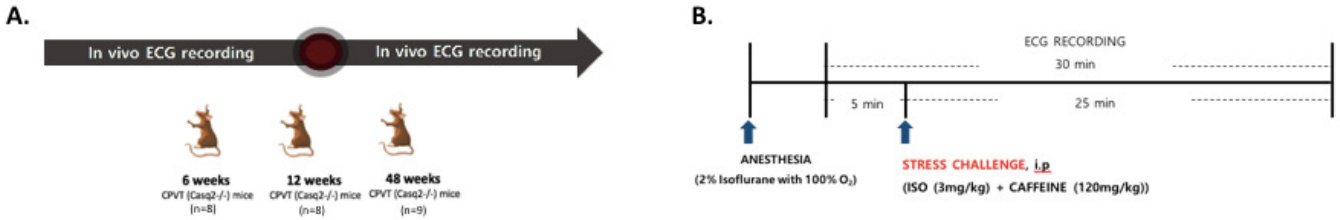
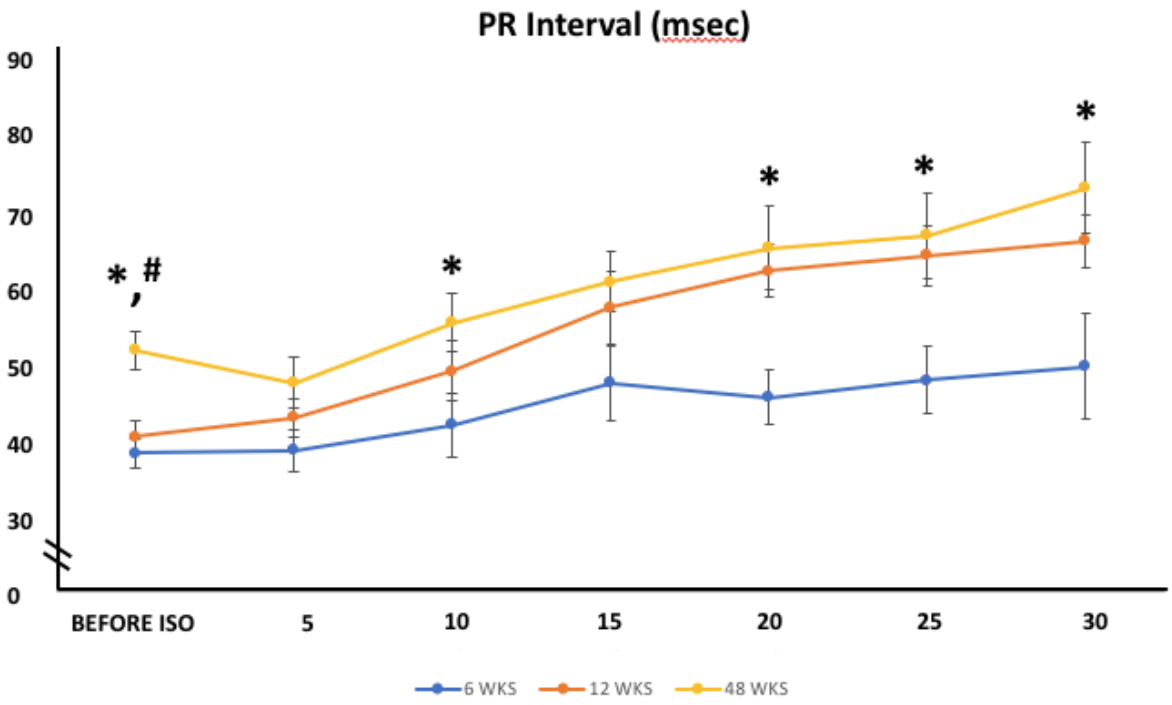

A.
In vivo ECG recording
In vivo ECG recording
6 weeks
CPVT (Casq2-/-) mice
(n=8)
12 weeks
CPVT (Casq2-/-) mice
(n=8)
48 weeks
CPVT (Casq2-/-) mice
(n=9)
B.
ECG RECORDING
30 min
5 min
25 min
ANESTHESIA
(2% Isoflurane with 100% O2)
STRESS CHALLENGE, i.p
(ISO (3mg/kg) + CAFFEINE (120mg/kg))

Figure 2.


PR Interval (msec)
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80
70
60
50
40
30
0
* , #
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*
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BEFORE ISO
5
10
15
20
25
30
6 WKS
12 WKS
48 WKS

Figure 3.

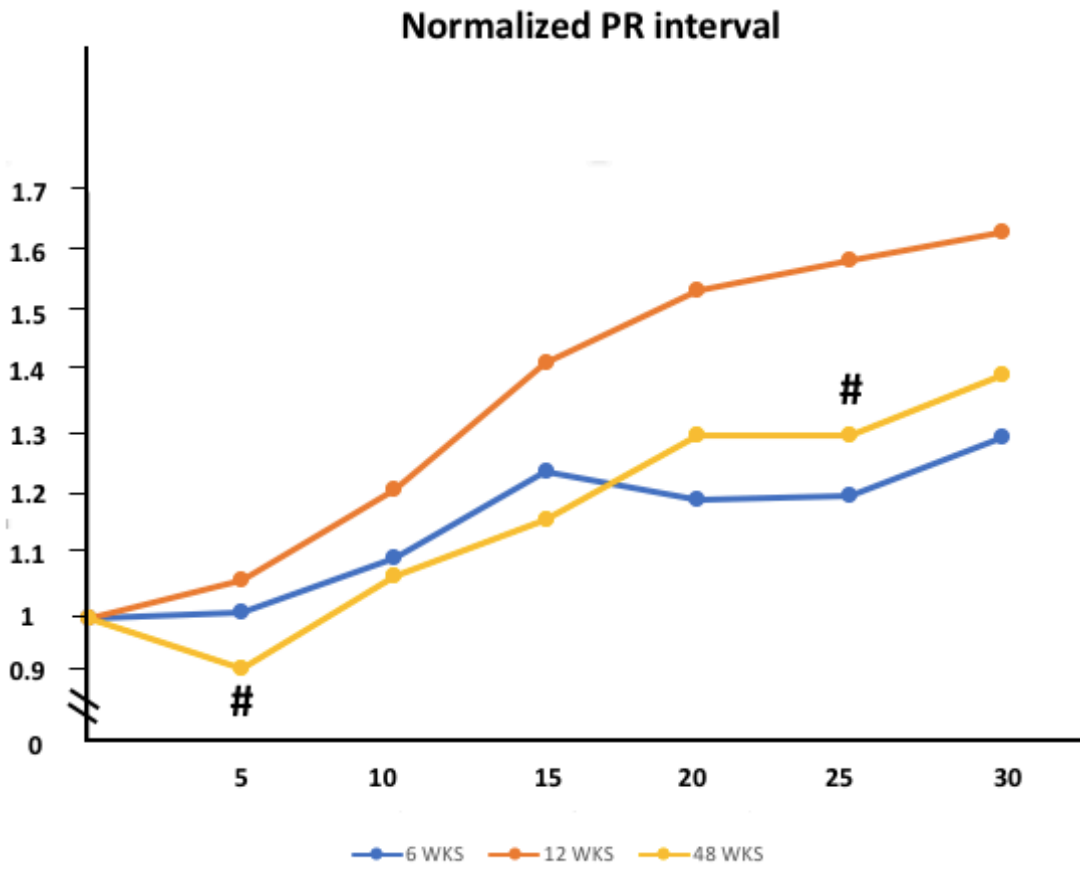
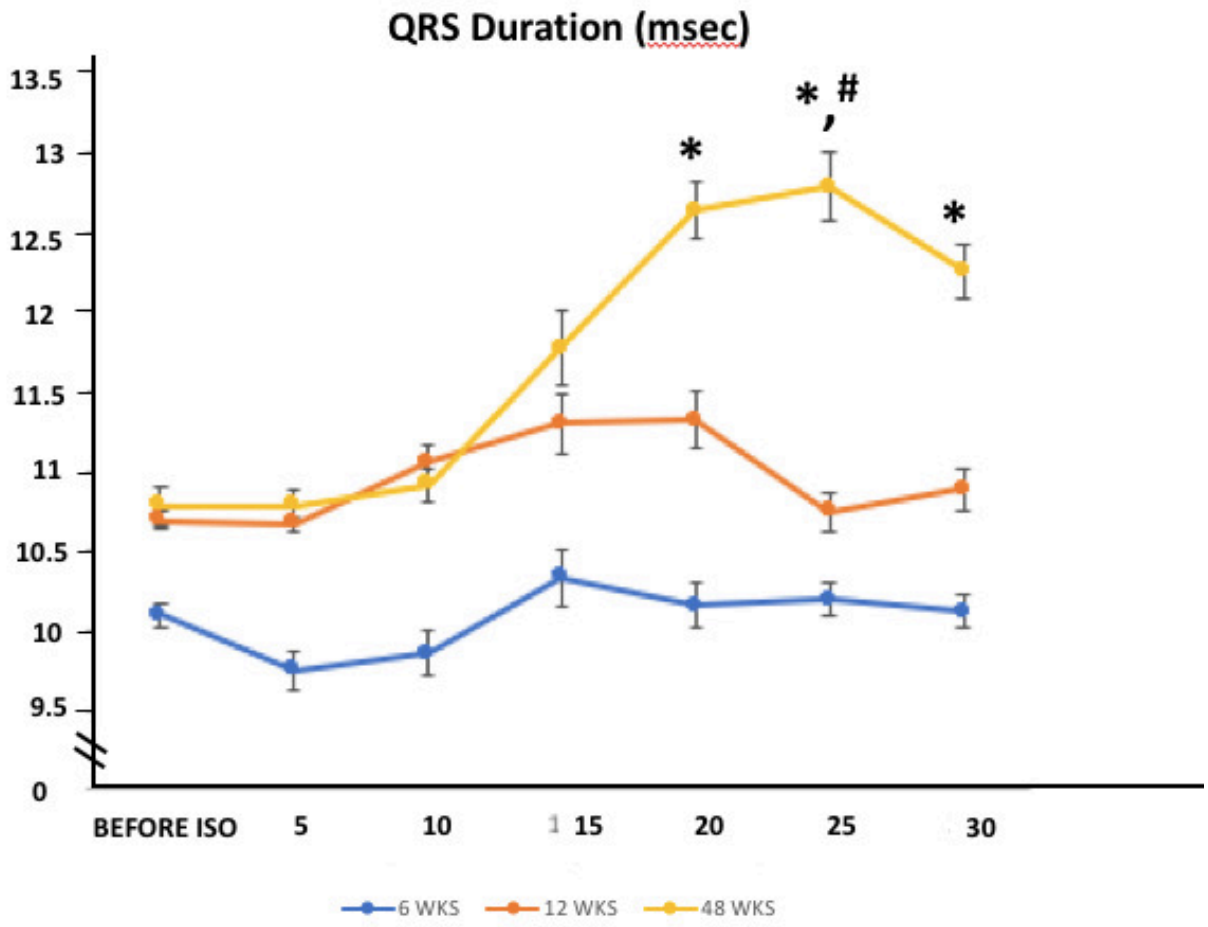

Normalized PR interval
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0
#
#
5
10
15
20
25
30
6 WKS
12 WKS
48 WKS

Figure 4.


QRS Duration (msec)
13.5
13
12.5
12
11.5
11
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5
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48 WKS

Figure 5.

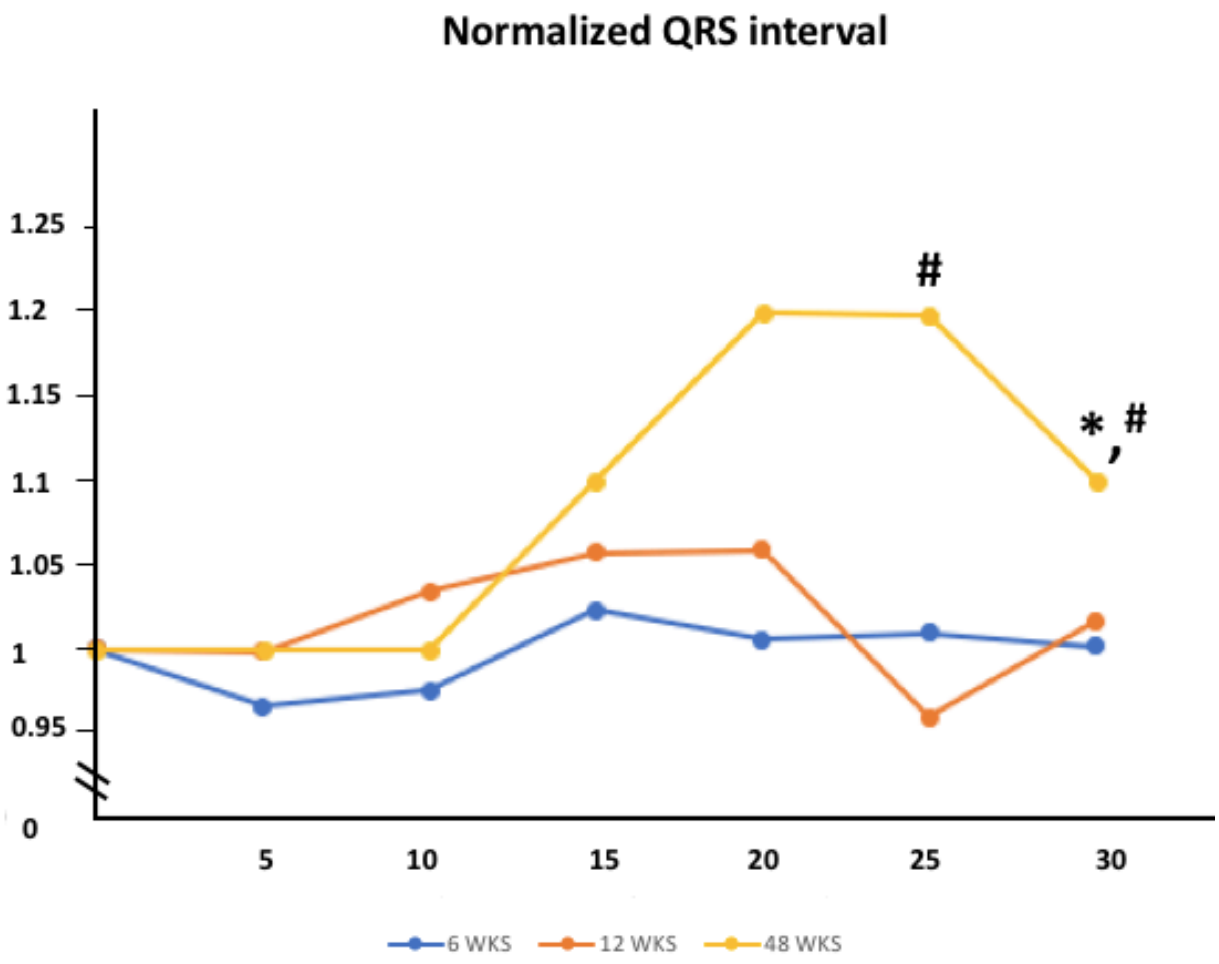
Normalized QRS interval
1.25
1.2
1.15
1.1
1.05
1
0.95
0
5
10
15
20
25
30
#
* , #
6 WKS
12 WKS
48 WKS

Figure 6.

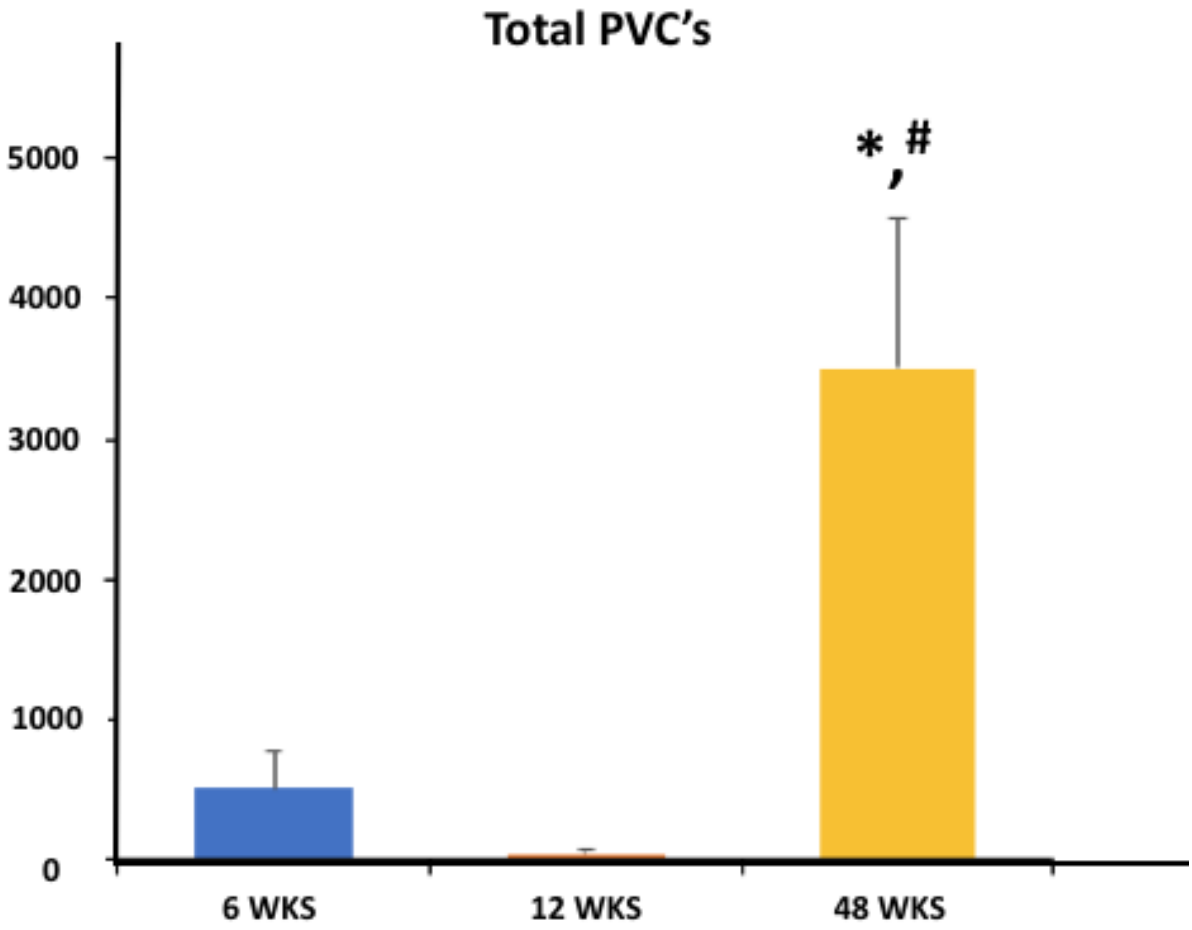

Figure 7.

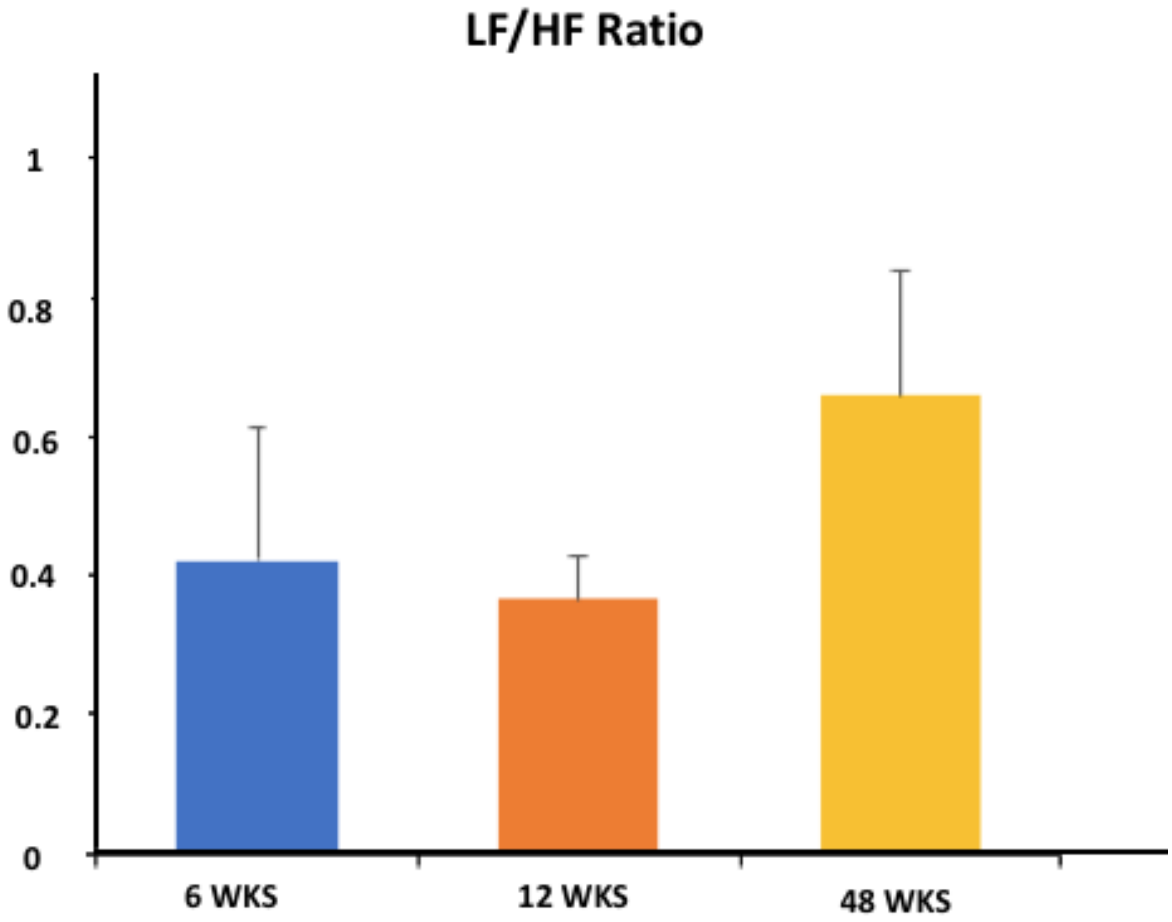

Figure 8.

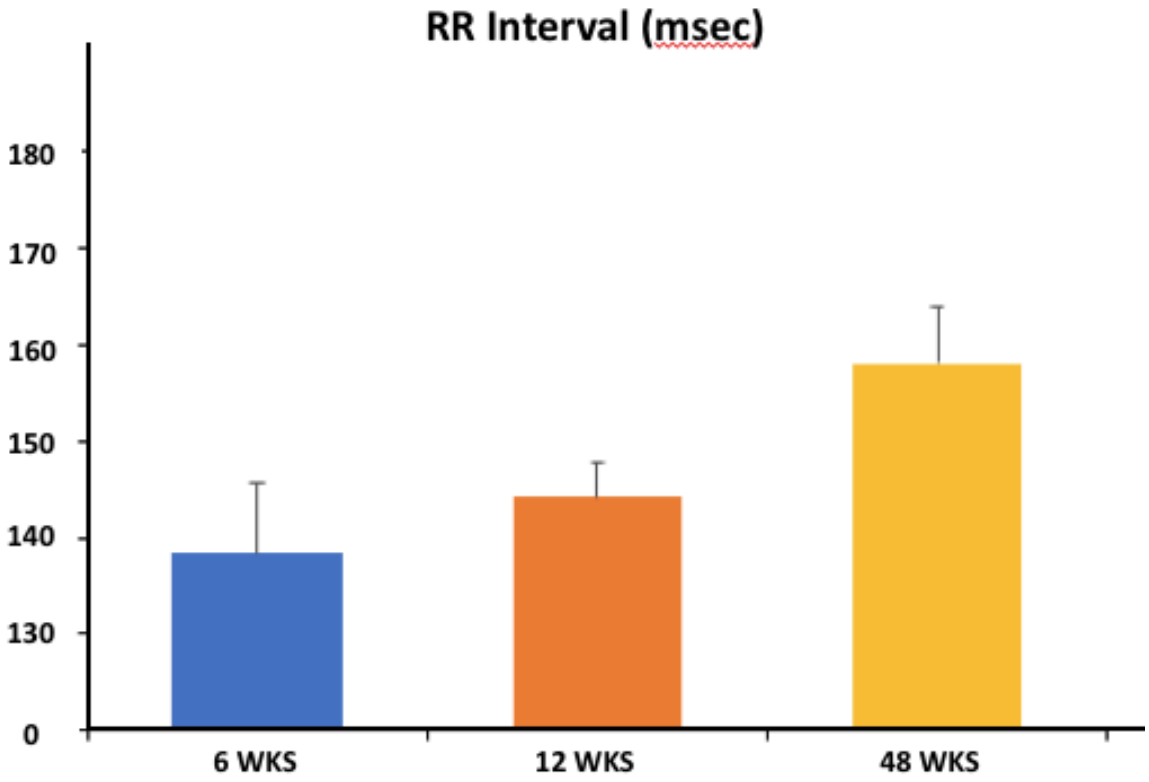

Figure 9.

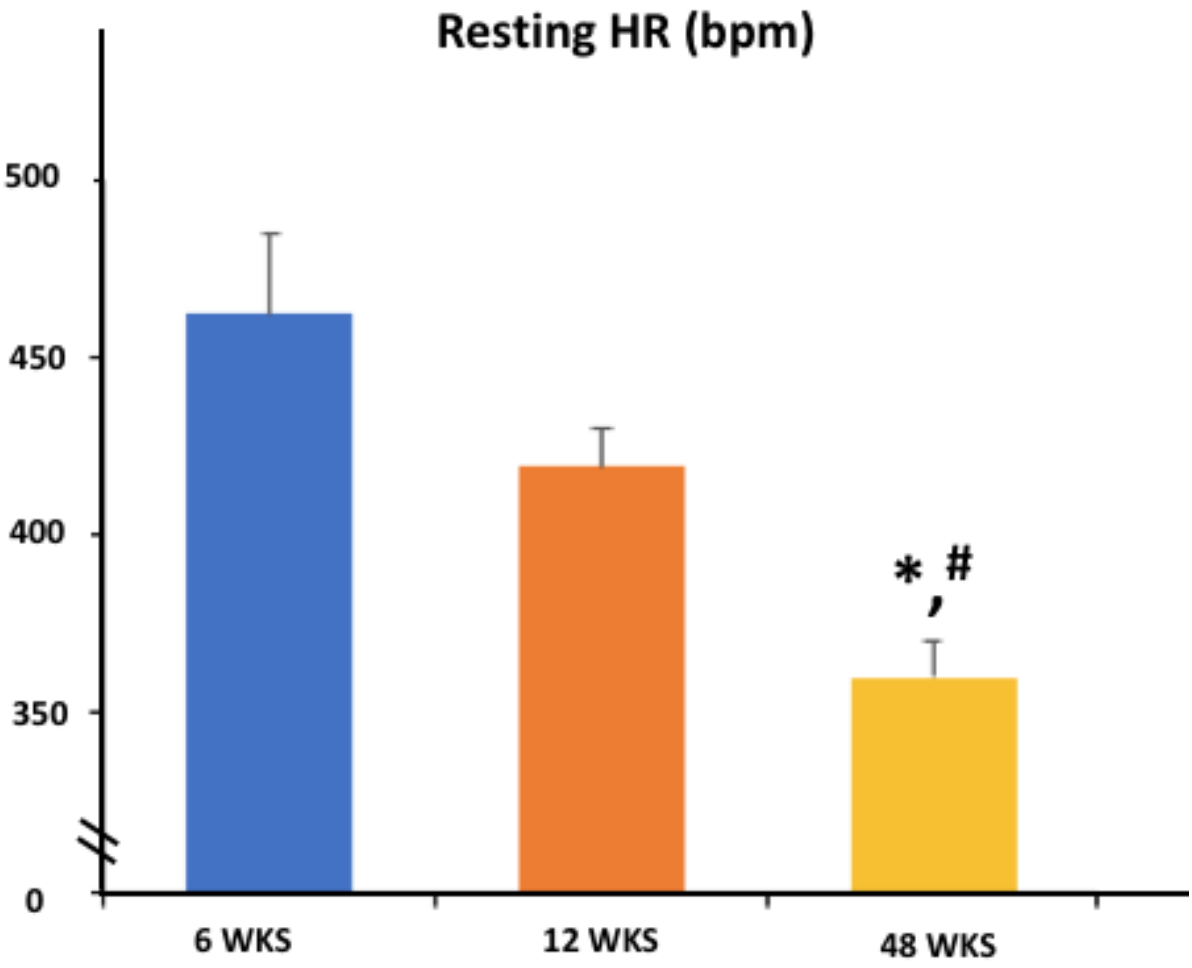

Figure 10:

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