Cardiac Phenotyping of Transgenic Mice Over expressing Sarcoplasmic Reticulum Calcium ATPase Pump

A Senior Honors Thesis

Presented in Partial Fulfillment of the Requirements for graduation *with research distinction* in the undergraduate colleges of The Ohio State University

by
Summer Hayes

The Ohio State University
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Project Advisor: Veronique Lacombe D.V.M., Ph.D., College of Pharmacy

Thesis Committee: Dr. Marymegan Daly and Dr. Andrea Doseff

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Common Abbreviations

Ca^{2+} : Calcium

CASQ2: Calsequestrin protein

CPVT: Catecholaminergic Polymorphic Ventricular Tachycardia

ECG: Electrocardiogram

Het: Heterozygote

Hom: Homozygote

M-mode: Motion mode

NSVT: Non-sustained ventricular tachycardia

Null: Calsequestrin null

PVC: Premature ventricular complex

SERCA: Sarcoplasmic reticulum ATPase pump

SR: Sarcoplasmic reticulum

TG: Transgenic

VT: Ventricular tachycardia

WT: Wild-type

2-D: Two dimension

ABSTRACT

Every year, approximately 40% of individuals in the United States die from heart failure. Pre-existing conditions such as diabetes and arrhythmia can lead to heart failure. Despite the number of individuals that develop heart failure, the underlying mechanisms are still not well understood. In order for cardiac contraction to occur, the myocardial cells uptake calcium from the cytosol into the sarcoplasmic reticulum (**SR**) via the sarcoplasmic reticulum ATPase pump **(SERCA**). With an increased SERCA pump, the cells can uptake and release more calcium, which may enhance cardiac fucntion. For this study, we hypothesized that (1) SERCA overexpression in the heart can be used to rescue the ventricular tachycardia caused by a depletion of calsquestrin, a buffer protein in the SR; (2) SERCA overexpression in the heart can be used to rescue cardiomyopathy that occurs during diabetes. Transgenic animal models were used for this study. In study 1, transgenic mice with SERCA overexpression were crossed with mice with calsquestrin depletion. In study 2, the SERCA overexpression mice along with their wild type littermates were made diabetic. Electrocardiographic recording was performed to detect arrhythmia in study 2. Cardiac function was measured by echocardiographic examination in both studies. Our data demonstrated that SERCA overexpression in the heart worsened ventricular arrhythmia and induced heart failure in mice with calsequestrin mutation. In addition, SERCA overexpression in the heart did not significantly improve cardiac function during diabetic cardiomyopathy. Overall this data suggests that SERCA overexpression in the heart does not rescue pre-existing conditions associated with arrhythmias and diabetic cardiomyopathy so individuals should be cautious when using SERCA gene therapy.

INTRODUCTION

The sarcoplasmic reticulum (**SR**) is an organelle that is present in many different cell types.[1] The function of the SR in muscle cells is to sequester and release intracellular calcium (**Ca^{2+}**). The SR contains the sarcoplasmic reticulum Ca-ATPase pump (**SERCA**). This SERCA pump has two functions. First, is to create muscle relaxation by up taking cytosolic calcium into the SR.[2] Second, is to release calcium from the SR into the cytosol, which is needed for muscle contraction. When the SERCA pump does not function properly the levels of cytosolic calcium will increase, which can lead to apoptosis. SERCA pump dysfunction along with increased levels of cytosolic calcium have been shown to contribute to heart failure and diabetic cardiomyopathy.[3] SERCA overexpression has been linked to enhanced contractile function so it is suggested that SERCA gene therapy could be used as a potential therapeutic option during cardiac failure.[4]

Heart failure is one of the leading causes of death in the United States. Heart failure can develop from a variety of conditions. Diabetes is one of the major conditions that predispose one to heart failure and ventricular arrhythmias have been associated with heart failure.[5] Although both of these conditions have been linked to heart failure, the underlying mechanisms are not well understood.

The purpose of this study is to determine if increased SERCA expression in the heart can be used as a means to rescue arrhythmia and diabetic cardiomyoapthy, in order to prevent heart failure. For the proposed study, a well-characterized transgenic mouse model that overexpresses SERCA protein in the heart was used.[2] The SERCA1a isoform in the heart was used in this study since there was a two fold increase in the total amount

of cardiac SERCA protein and SR Ca^{2+} uptake function.[2] The hypothesis was that increased SERCA pump expression in the heart will increase SR Ca^{2+} reuptake and decrease the incidence of ventricular arrhythmia and the severity of diabetic cardiomyopathy.

STUDY 1: SERCA AS A MEANS TO RESCUE ARRHYTHMIA

In the United States, cardiovascular disease is one the leading cause of death and many individuals die from ventricular arrhythmia. Although the molecular mechanisms underlying these arrhythmias are not well understood, altered intracellular calcium handling in the heart has been implicated as a common pathway predisposing to ventricular tachycardia in acquired or congenital heart disease. Especially, Catecholaminergic Polymorphic Ventricular Tachycardia (**CPVT**), which is a condition, that is inherited, can lead to sudden cardiac death that is triggered by physical or emotional stress.[1] Catecholaminergic Polymorphic Ventricular Tachycardia can be detected by a normal baseline electrocardiogram (**ECG**) and polymorphic ventricular tachycardia was induced by catecholaminergic challenge. When Catecholaminergic Polymorphic Ventricular Tachycardia remains untreated, the mortality rate is high, reaching 30-35% by the age of 30.

Catecholaminergic Polymorphic Ventricular Tachycardia has recently been linked to an autosomal recessive mutation in the calsequestrin (**CASQ2**) gene[6]. CASQ2 is located in SR and plays a prominent role in buffering free Ca^{2+} and allows an increase the amount of Ca^{2+} accumulated in the SR [7]. In addition, the SERCA pump uptakes excess Ca^{2+}, which was released during cardiac muscle contraction.[8] The calcium that is taken

up by the SERCA pump is then buffered by CASQ2. Furthermore, when there is a mutation in the CASQ2 gene there is an alteration in calcium buffering capacity, which can lead to apoptosis and/or ventricular arrhythmia. Other studies have created a CASQ2 null mouse model in which the CASQ2 gene is not expressed.[9] Previous studies have shown that after catecholaminergic challenge mice with the CASQ2 mutation display ventricular arrhythmia and Catecholaminergic Polymorphic Ventricular Tachycardia.[10]

The goal of the present study was to investigate if SERCA overexpression can be used as a means to rescue the effects of CASQ2 protein deficiency that causes ventricular arrhythmia leading to Catecholaminergic Polymorphic Ventricular Tachycardia. To test our hypothesis, we generated transgenic (**TG**) mice that have over-expression of the SERCA pump in the heart with TG mice that have CASQ2 depletion in the heart.

MATERIAL AND METHODS

Generating TG mice

Dr. Knollman created the CASQ2 null TG mice model in which these mice have an absence of the CASQ2 buffering protein .[9] The CASQ2 null mice were created by the deletion of the CASQ2 promoter and first exon. Dr. Periasamy developed a TG rodent model for SERCA overexpression in the heart. These two mice were then crossed in order to create the SERCA overexpression crossed with CASQ2 Null TG. When these two mice were crossed it resulted in offspring that were homozygote and heterozygote for the CASQ2 protein deficiency. In this study, five groups were used (**Fig. 1**). When the mice were 3-4 weeks old they were genotyped. The care of the mice conforms to the guide for Care and Use of laboratory animals published by the US national Institutes of

Health (NIH publication # 85-23, revised 1996)[10]. All procedures were approved by The Ohio State University Institutional Animal Care and Use Committee.

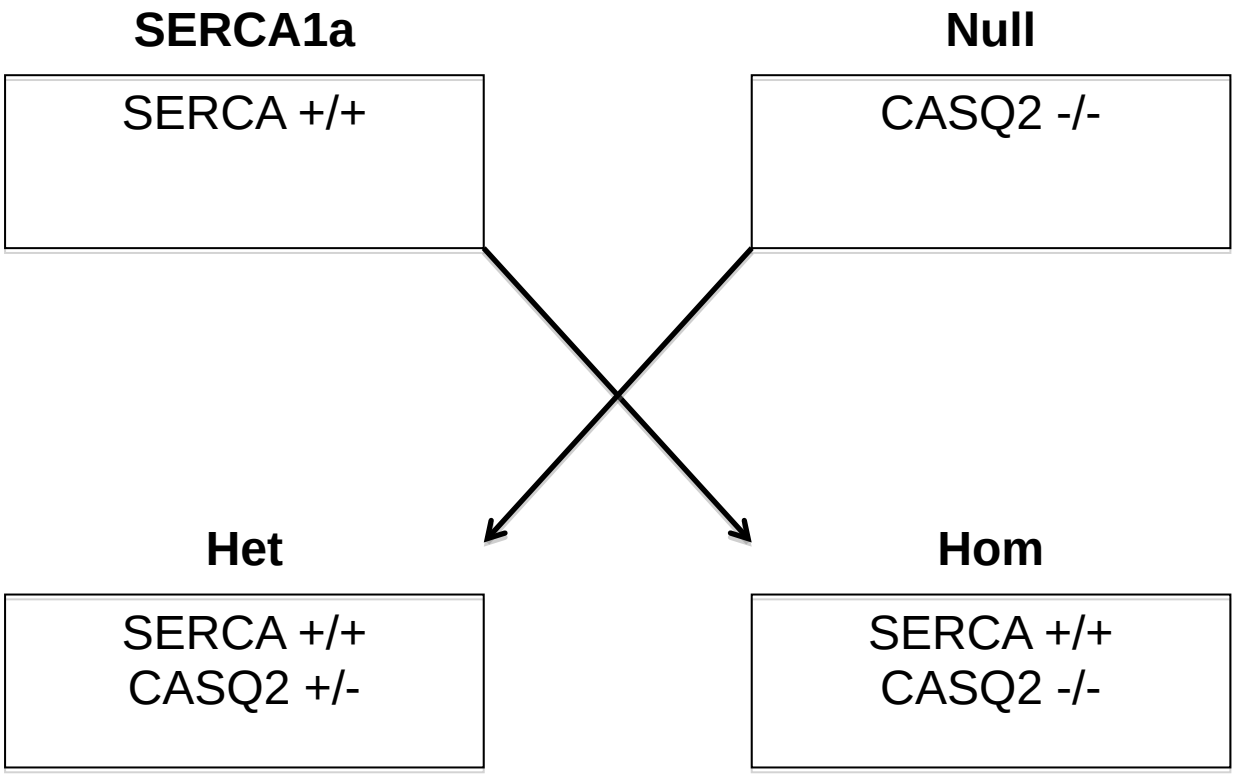

Fig 1. For this study five different groups were used. WT: wildtype, SERCA: SERCA overexpression, Null: CASQ2 null, Het: Heterozygote (a cross between the SERCA overexpression and CASQ2 null resulting in decreased CASQ2 protein expression), Hom: Homozygote (a cross between the SERCA overexpression and CASQ2 null resulting in depletion of CASQ2 protein).

Echocardiographic monitoring

The equipment for the echocardiographic monitoring was available at the Biomedical Research Tower. The equipment used included an anesthesia chamber, anesthesia machine, and an ultrasound (Vevo 2100, Visual Sonics) with ultra-high linear frequency probes (ranging 9-70 MHz) which was suited to performing echocardiographic examinations on mice. Echocardiographic examination was performed (n=6) under general anesthesia (**Fig 2**). The mouse was placed in the anesthesia chamber with 3%

isoflurane and 100% oxygen. Once the mouse was under light anesthesia the mouse was transferred to a warmed platform, where the mouse was placed on its dorsal side and the anesthesia was maintained at ~1.5% isoflurane (inhalation via a mask). Gel was then applied to the hands and feet of the mice and the appendages were taped down to the platform to monitor the heart rate. After clipping of the hair the conducting gel was applied.

Diastole (i.e., cardiac relaxation) and systole (i.e., contraction of the heart) was assessed. Images acquired included: the motion mode (**M-mode**) of the left ventricle, two-dimensional (**2-D**) of the left ventricle, E and A wave, Pulmonary Artery velocity, and Aortic velocity. The M-mode image of the left ventricle was obtained by a cross section of the short axis of the left ventricle over time. The measurements for the short axis M-mode included the interventricular sepal dimension (**IVS**), left ventricular internal diameter (**LVID**), left ventricular posterior wall (**LVPW**) for both diastole and systole, and the calculated ejection fraction (**EF**) (EF = [(LVID end-diastolic – LVID end-systolic)/ LVID end-diastolic]* 100%). The E and A wave measured the velocity of blood leaving the atrium and entering the left ventricle during the diastolic phase. The velocity of the E and A wave was measured along with timing of the contraction of the left ventricle, which includes aortic ejection time (**AET**), isovolumetric contraction time (**IVCT**) and isovolumetric relaxation time (**IVRT**). The pulmonary artery velocity was obtained by the velocity of the blood going through the pulmonary artery. The doppler aortic velocity was obtained by measuring the velocity of blood going through the aortic arch and the measurements include the aortic ejection time (**AET**) and the left ventricular outflow tract (**LVOT**). Systolic function was measured using the M-mode of the left

ventricle and doppler views of the aorta and pulmonary artery. Once all of the images had been acquired, the isoflurane was turned off and the mouse was given time to awake on its own while on the table. Then the mouse was warmed for a few minutes before it is returned to the cage.

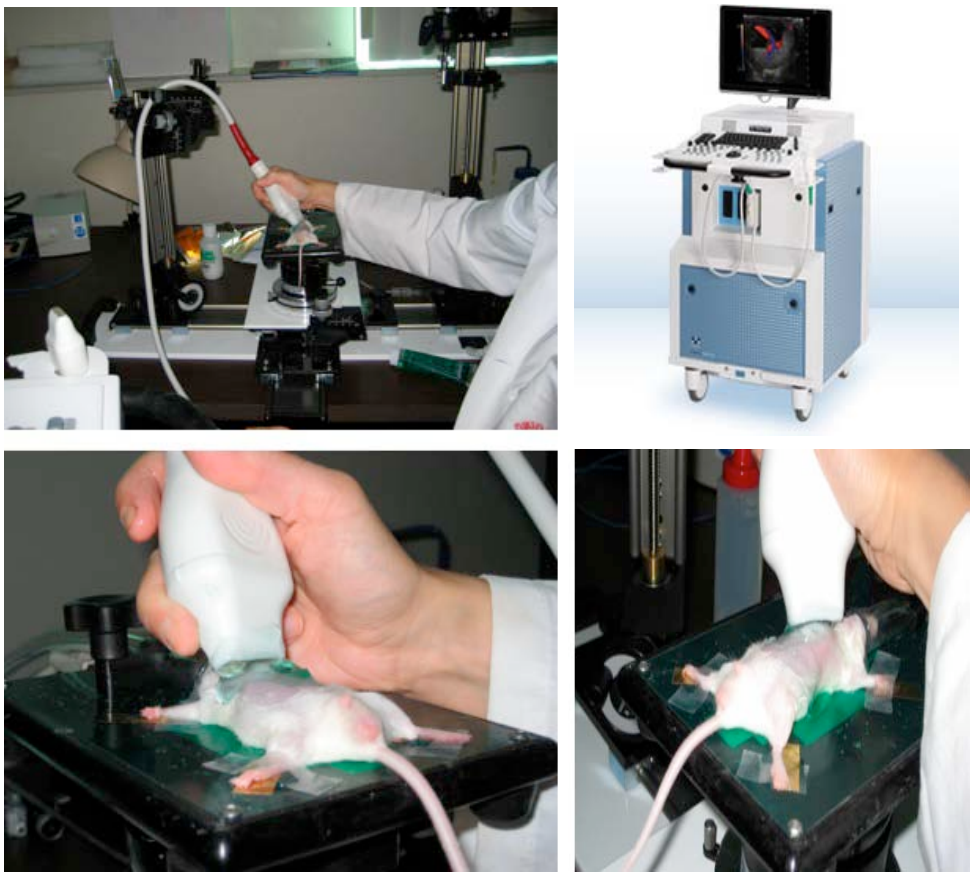

Fig 2. The setup of the echocardiograph equipment depicting the probe and the anesthetized mouse on the heated platform.

ECG monitoring and induction of arrhythmia

The equipment for the echocardiographic recording (**ECG**) was located at the Veterinary Medical Center. ECG recordings were performed for 30 min for all five-mouse groups (n=5 per group) before and after catecholaminergic challenge. All of the necessary equipment was located in the Veterinary Medical Center, which included anesthesia induction chamber, anesthetic machine for small mammals and a physiologic

data acquisition system (MP 100, Biopac Systems) with a sampling rate of 2 kHz [6] (**Fig 3**). The mouse was placed in the anesthesia chamber with 3% isoflurane in 100% oxygen. Once the mouse was anesthetized it was placed on a warmed heat pad with the dorsal side down. Then the leads were placed on the feet of the mouse with conducting gel. After a baseline recording of 10 minutes each mouse received one dose of isoproterenol (1.5 mg/kg intraperitoneal injection). The recording was performed for 30 minutes, and then the isoflurane was turned off and the mouse was given time to wake up on its own. Once the mouse was awake it was placed back into the cage under a heat lamp.

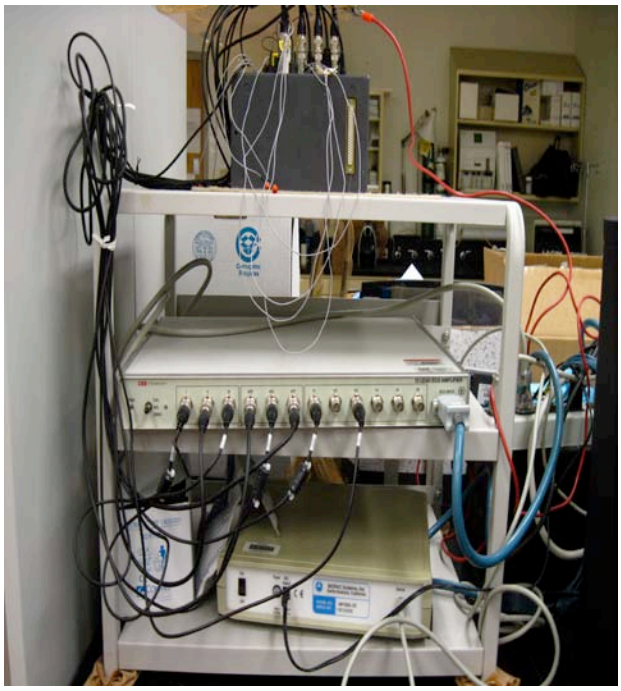

Fig 3. Electrocardiograph Biopac system used to obtain ECG recording in mice.

Data and Statistical analysis

Signal average was obtained with software (AcqKnowledge, Biopack). The echocardiographic recording was analyzed for the presence of arrhythmias. The incidence of arrhythmias was analyzed by using a chi-squared test since there was a comparison

between two groups at a time. A one-way analysis of variance was used to analyze the heart rate and QT corrected. Statistical significance was evaluated at $P<0.05$ and all results were reported as mean ± SE.

RESULTS

Baseline heart rate (before isoproterenol injection)

The heart rate of the mice groups were calculated from the baseline recording of the ECG and then compared to one another. There was not a significant difference in the heart rate between the 5 groups of mice (**Fig 4**).

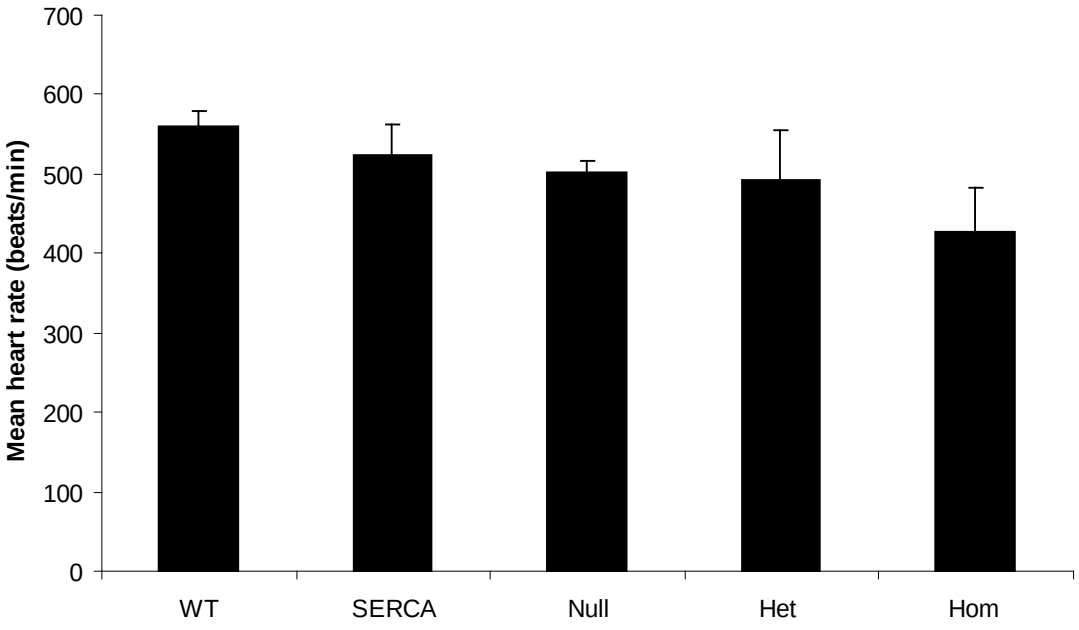

Fig 4. The mean (± SE) heart rate of the five groups showed that there was not a significant difference between groups. Hom: Homozygote, Het: Heterozygote, Null: CASQ2 null, SERCA: SERCA overexpression, WT: Wild-type mice.

Signal Average

The ECG machine recorded each heartbeat of the mice and the signal average was obtained with the use of AcqKnowledge software. The signal average was an average of each heart beat over 60 seconds. A beat consisted of a P wave, QRS complex and a T wave. The P wave indicated atrial depolarization, which precedes atrial contraction.[5] QRS represented ventricular depolarization, which precedes ventricular contraction. The T wave represented ventricular repolarization (**Fig 5**).

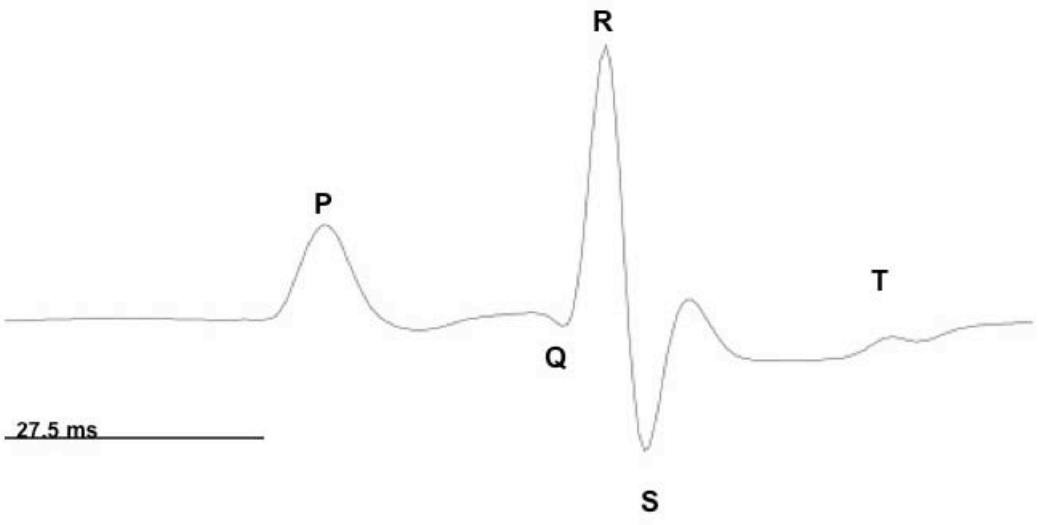

Fig 5. Signal average (lead 1) depecting a P wave, QRS complex and a T wave.

Baseline QT corrected

If the QT corrected (for heart rate, **QTc**) is prolonged then the mouse is pre-exposed to ventricular arrhythmia. The QTc is a value that was calculated using the measured QT interval (ms) divided by 1000 and then divided by the cube root of the R-R interval (s). There was a significant difference in QT corrected between the Het mice and WT mice (P =0.013). There was not a significant difference between the other groups of mice (**Fig 6**).

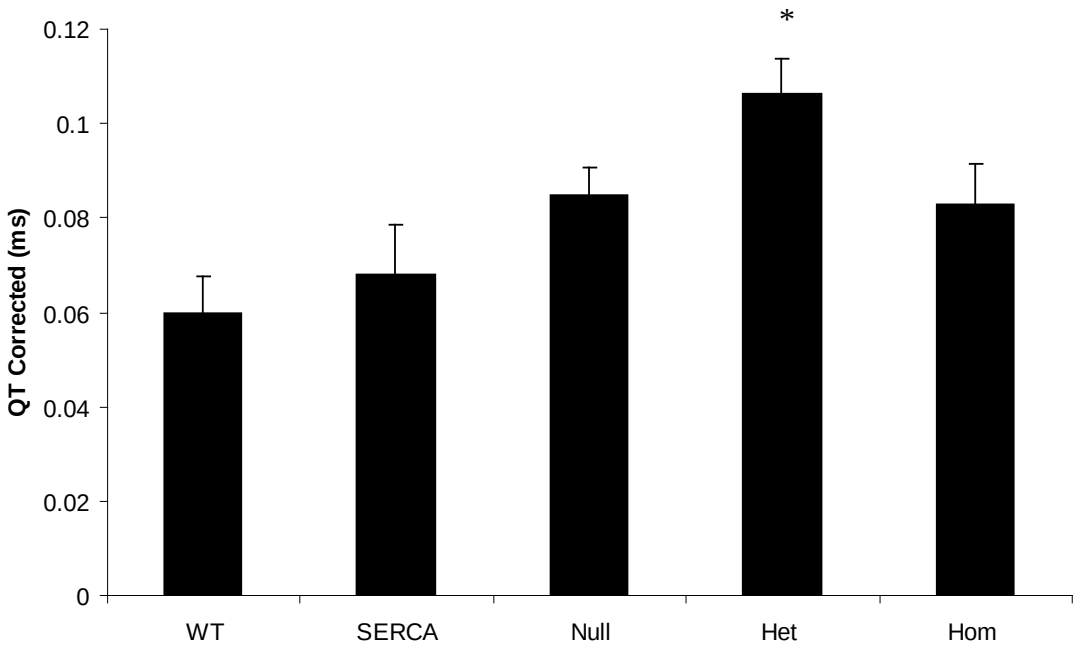

Fig 6. There was a significant difference in QT corrected between the Het mice and WT mice. However there was not a significant difference between the other groups. * indicates a statistical difference from WT mice; $P<0.05$. Hom: Homozygote, Het: Heterozygote, Null: CASQ2 null, SERCA: SERCA overexpression, WT: Wild-type.

Types of arrhythmia

Several types of arrhythmia were observed in the Hom mice and Het mice. There are two types of arrhythmia, simple and complex. Simple arrhythmia includes the premature ventricular complex (**PVC**) (**Fig 7A**) and fusion beats (**Fig 7B**). A PVC is a premature pulse beat.[5] A fusion beat occurs on the ECG when there is a blending of a normal QRS with a PVC. Complex arrhythmia includes couplet (**Fig 8A**), triplet (**Fig 8B**), bigeminey (**Fig 8C**), trigeminy, and non-sustained ventricular tachycardia (**NSVT**) (**Fig 8D**). A couplet is defined when there are two PVC's concurrently, so a triplet is when there are three PVC's concurrently. Bigeminey is defined as a repeated pattern of a normal sinus beat followed by a PVC. Non-sustained ventricular tachycardia is when

there are three or more PVCs in a row. Ventricular tachycardia (**VT**) is defined by consecutive PVC complexes where the heart beats faster, which last for more than 30 seconds.

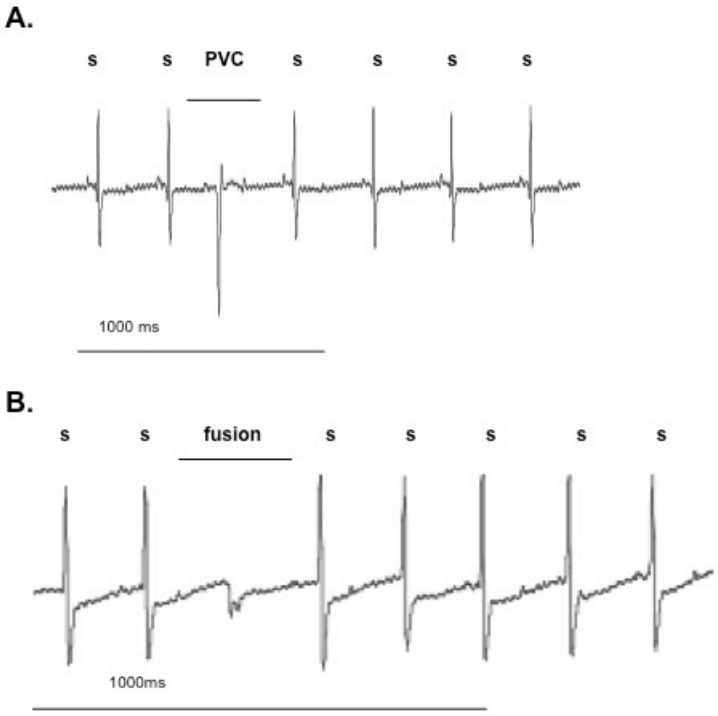

Fig 7. Representative figure of the types of simple ventricular arrhythmia found in the Hom and Het mice. A is a premature ventricular complex. B is a figure of a fusion beat.

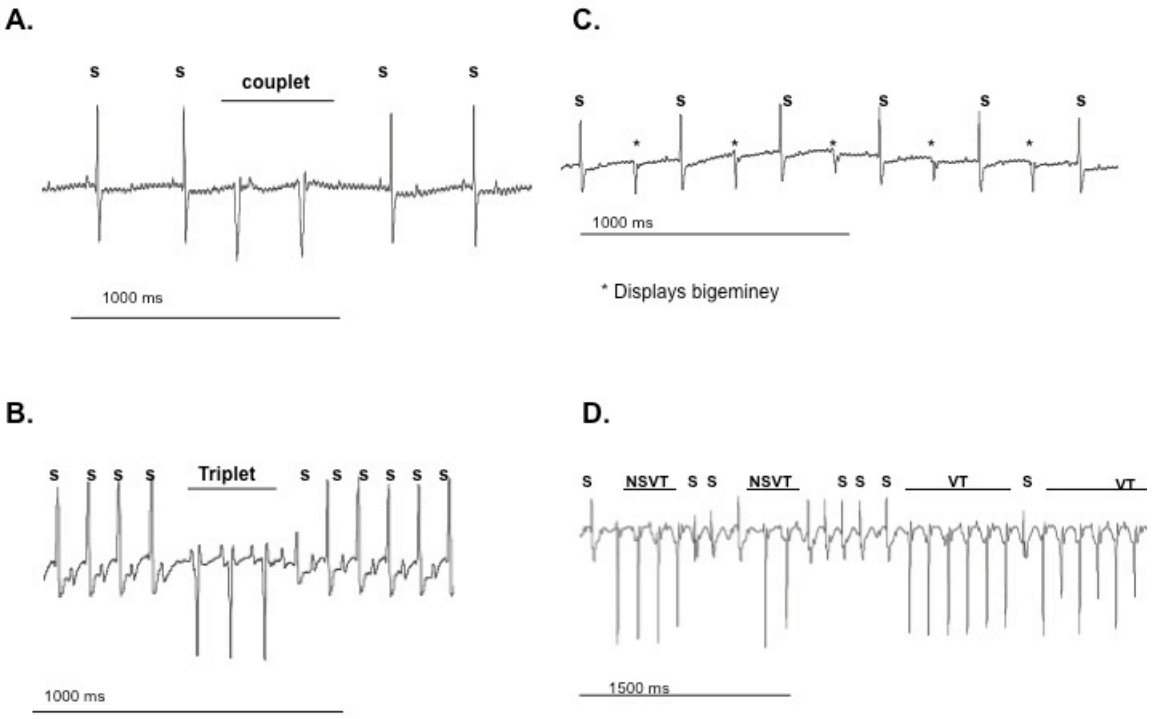

Fig 8. A representative figure of the types of complex ventricular arrhythmia displayed by Hom and Het mice. A is a couplet, B is a triplet, C is a bigeminey, D is non-sustained ventricular tachycardia.

Baseline ECG arrhythmia

After analysis of the ECG recording, it was found that the Het mice had significantly more ventricular arrhythmias during the baseline recording (**Fig 9**). Het mice displayed more simple ventricular arrhythmia in baseline than the other groups.

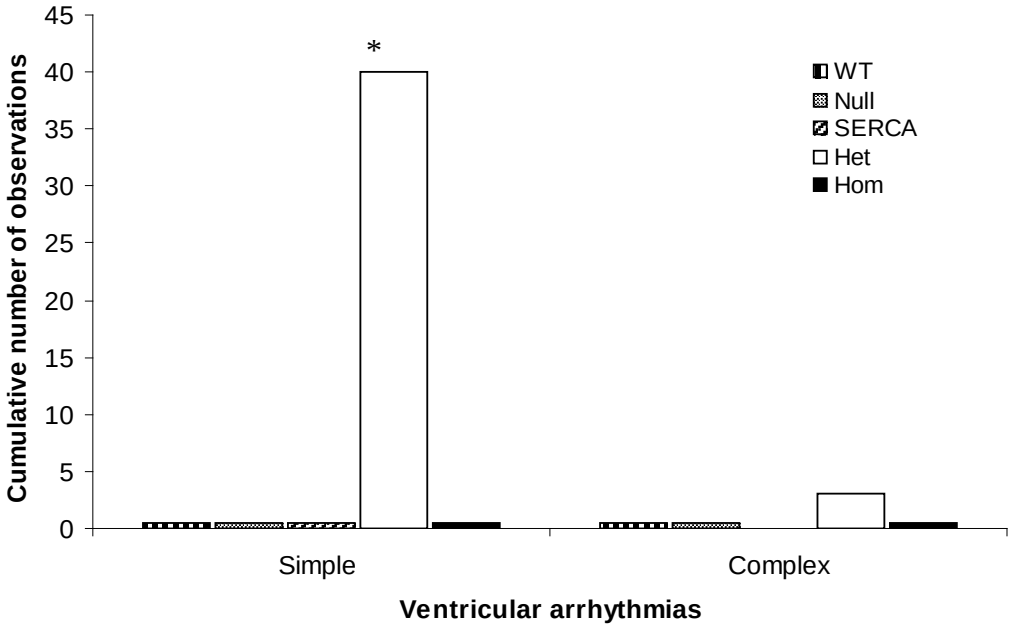

Fig 9. There was a significant increase in the cumulative number of simple ventricular arrhythmias in Het mice during the baseline ECG recordings. * represents statistical difference of $P<0.05$ from the other groups. N=5 per group

After catecholaminergic challenge

After injection of isoproterenol, all the mice had increased heart rate (i.e., sinus tachycardia) validating our catecholaminergic challenge (**Fig 10**).

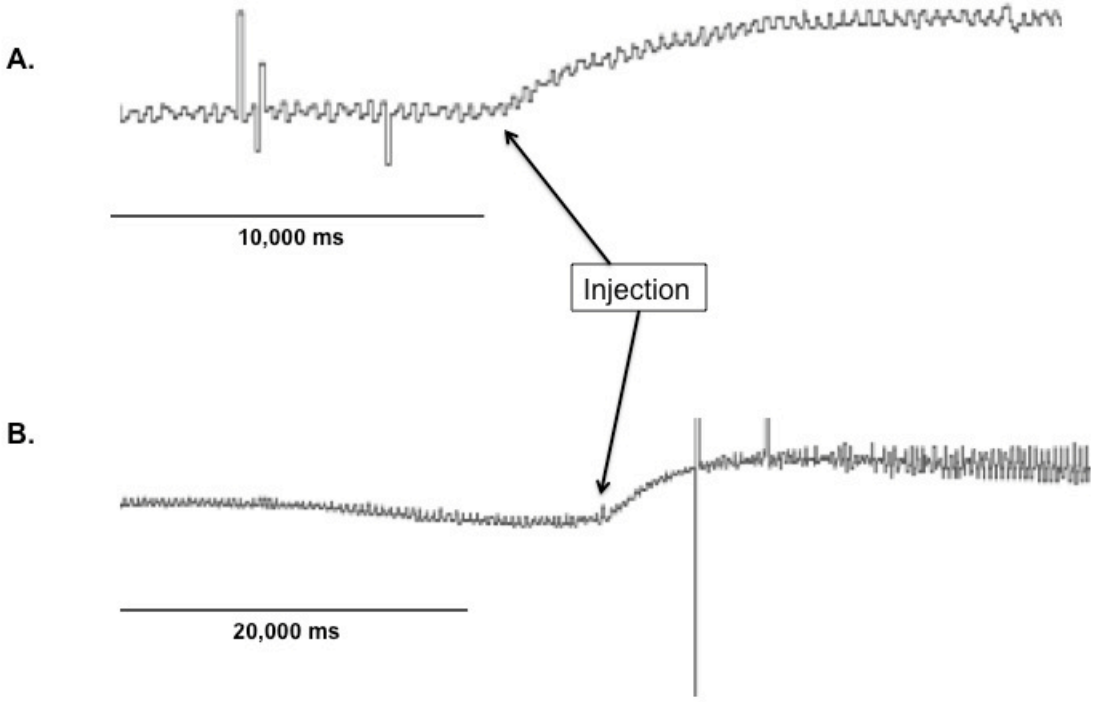

Fig 10. The heart rate increased after catecholaminergic challenge in WT mice (A) and Hom mice (B).

Arrhythmia after catecholaminergic challenge

After catecholaminergic challenge, the Hom and Het mice both displayed ventricular arrhythmia. The Hom and Het mice had significantly ($P<0.001$) more ventricular arrhythmia than the control groups (**Fig 11**). The Hom mice had significantly ($P<0.001$) more complex ventricular arrhythmia than the Het mice, including non-sustained ventricular tachycardia (**Fig 12**).

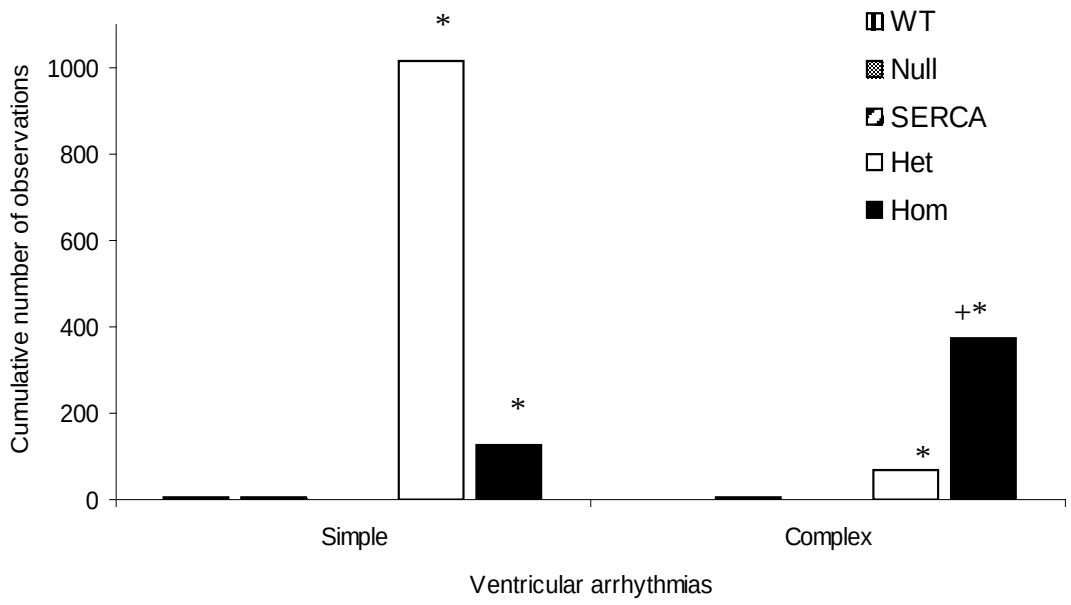

Fig 11. The Hom and Het mice display significantly more cumulative simple and complex ventricular arrhythmia after catecholaminergic challenge than the control groups (i.e., WT, SERCA, Null). * represents a statistical difference of $P<0.05$ from the control group, + indicates a statistical difference from the Het ($P<0.05$). WT: wildtype, SERCA: SERCA overexpression, Null: CASQ2 null, Het: Heterozygote, Hom: Homozygote.

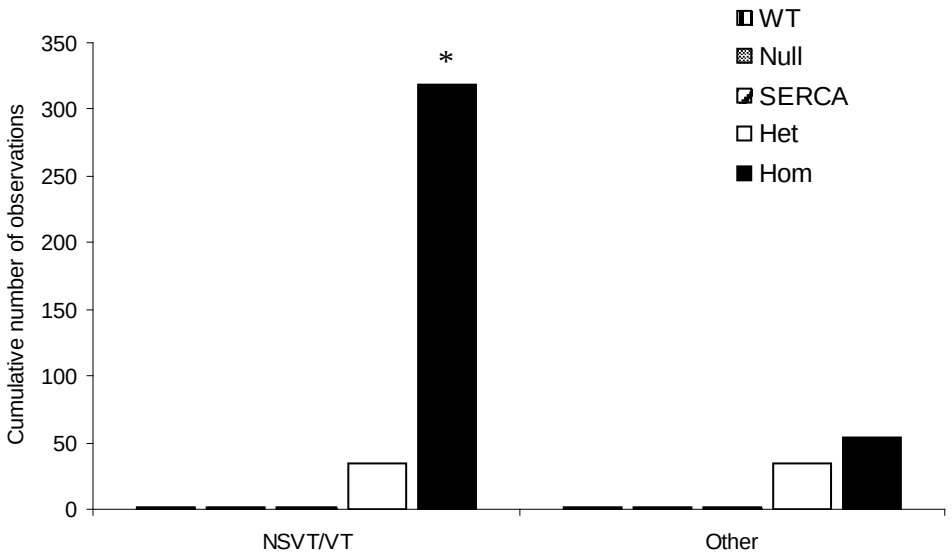

Fig 12. The Hom mice display significantly more cumulative complex ventricular arrhythmia after catecholaminergic challenge than the Het. * represents a statistical difference of $P<0.05$. WT: wildtype, SERCA: SERCA overexpression, Null: CASQ2 null, Het: Heterozygote, Hom: Homozygote.

Echocardiographic examination: systolic function

There were several mice that displayed heart failure in the Het and Hom groups: 26.7% of the Het mice, and 14.3% of Hom mice. Heart failure was defined when the ejection fraction, a measure of systolic function, was considerably lower than the healthy mice (**Fig 13**). Our data showed that cardiac function in Het mice with heart failure were significantly different from control groups (**Tables 1 and 2**).

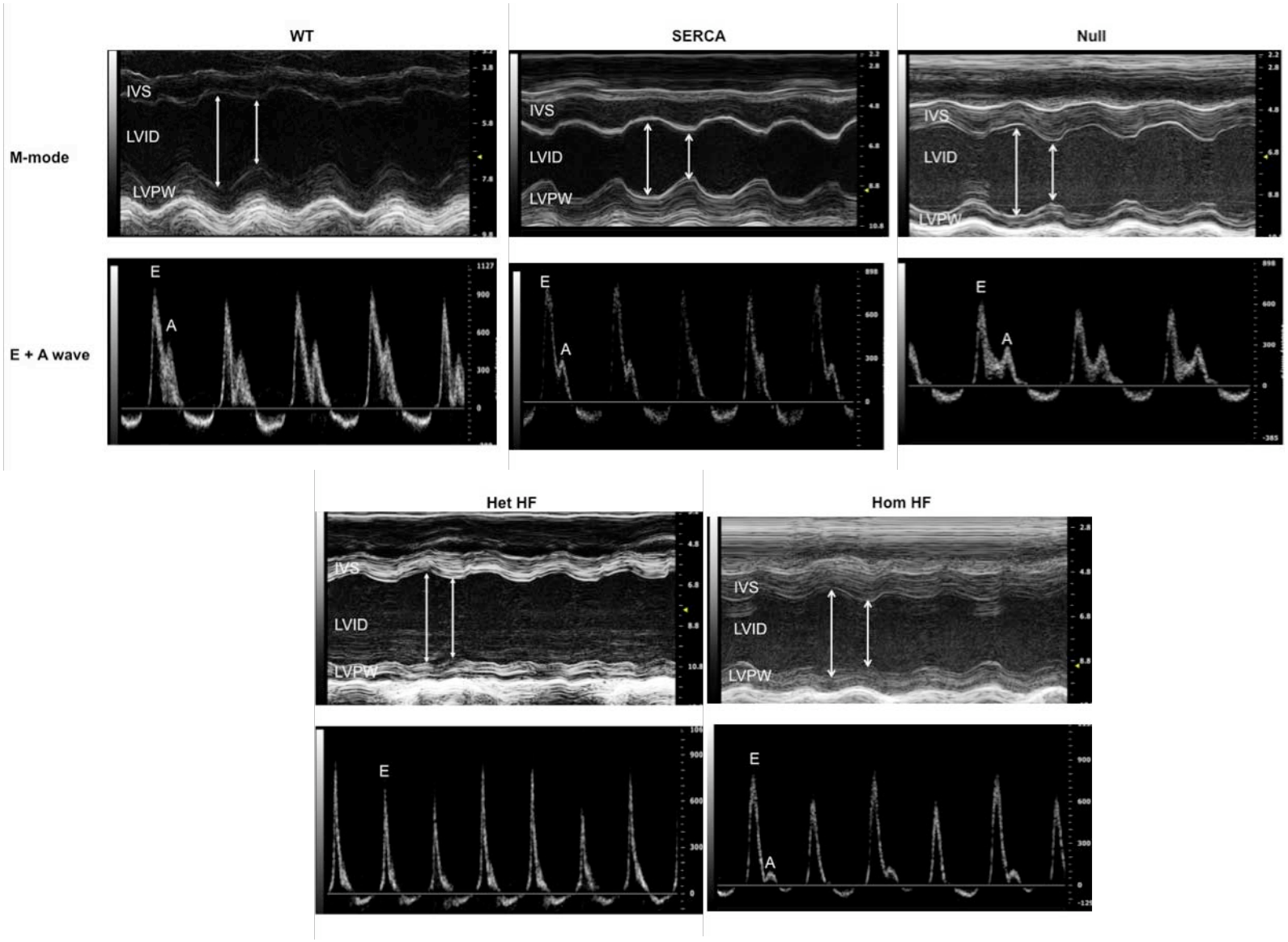

Fig 13. A: Representative short axis M-mode of the left ventricle. B: Representative E and A wave. IVS: Interventricular septal dimension, LVID: Left ventricular internal diameter, LVPW: left ventricular posterior wall dimension.

Table 1. Systolic function parameters of 2D m-mode of the mice groups.

	WT (n = 7)	SERCA (n = 7)	Null (n = 7)	Het (n = 11)	Het HF (n = 4)	Hom (n = 6)	Hom HF (n = 1)
IVS;s (mm)	1.76 ± 0.15^{abd}	1.76 ± 0.06^{b}	1.34 ± 0.08^{c}	1.42 ± 0.04^{cd}	1.42 ± 0.06^{abcd}	1.58 ± 0.06^{abcd}	1.19 ± 0.0
IVS;d (mm)	1.24 ± 0.08^{ac}	1.36 ± 0.07^{bc}	1.03 ± 0.07^{a}	1.12 ± 0.03^{a}	1.18 ± 0.08^{ab}	1.09 ± 0.05^{a}	0.99 ± 0.0
LVPW;s (mm)	1.30 ± 0.07	1.38 ± 0.15	1.02 ± 0.06	1.01 ± 0.05	0.88 ± 0.07	1.29 ± 0.08	0.99 ± 0.0
LVPW;d (mm)	0.95 ± 0.06^{ab}	1.06 ± 0.11^{b}	0.71 ± 0.02^{a}	0.72 ± 0.04^{a}	0.75 ± 0.06^{a}	0.89 ± 0.09^{ab}	0.91 ± 0.0
LVID;s (mm)	2.13 ± 0.12^{af}	2.74 ± 0.13^{b}	2.35 ± 0.14^{abcf}	2.09 ± 0.21^{bd}	4.20 ± 0.17^{e}	2.16 ± 0.14^{f}	3.01 ± 0.0
LVID;d (mm)	3.54 ± 0.13^{a}	3.97 ± 0.15^{a}	3.68 ± 0.17^{a}	4.14 ± 0.12^{a}	4.82 ± 0.28^{b}	3.60 ± 0.15^{a}	3.66 ± 0.0
LV Vol;s (ul)	15.4 ± 2.09^{a}	28.5 ± 3.30^{ac}	19.9 ± 2.97^{a}	33.2 ± 3.09^{c}	79.0 ± 7.58^{b}	16.0 ± 2.67^{a}	35.4 ± 0.0
LV Vol;d (ul)	52.8 ± 4.48^{a}	69.8 ± 5.99^{ab}	56.7 ± 6.91^{a}	76.9 ± 5.56^{ab}	110.1 ± 14.8^{b}	55.1 ± 5.60^{a}	56.5 ± 0.0
LV Mass (mg)	155.9 ± 19.3^{a}	211.1 ± 16.9^{b}	115.2 ± 11.4^{a}	152.5 ± 11.3^{a}	208.8 ± 24.3^{bc}	133.9 ± 10.0^{a}	128.9 ± 0.0
LV Mass;c (mg)	124.7 ± 15.5^{a}	168.9 ± 13.5^{b}	92.1 ± 9.10^{a}	122.0 ± 9.05^{a}	167.1 ± 19.4^{bc}	107.2 ± 8.03^{a}	103.1 ± 0.0
EF (%)	70.8 ± 3.58^{a}	59.5 ± 2.44^{b}	65.1 ± 2.82^{abc}	57.5 ± 2.47^{bc}	26.9 ± 3.90^{d}	71.5 ± 2.71^{a}	37.4 ± 0.0
HR (b/min)	462.9 ± 19.5	480.6 ± 7.34	429.9 ± 14.2	440.5 ± 30.6	523.3 ± 44.7	460.6 ± 15.2	542.3 ± 0.0

Data are mean ± SE. Different superscripts across rows indicate significant differences ($P<0.05$).
HF: Heart failure, IVSs: Interventricular septal dimension-systole, IVS;d: Interventricular septal dimension-diastole, LVPW;s: left ventricular posterior wall dimension-systole, LVPW;d: left ventricular posterior wall dimension-diastole, LVID;s: left ventricular internal diameter-systole, LVID;d: Left ventricular internal diameter-diastole; LV Vol;s: Left ventricular volume-systole, LV Vol;d: Left ventricular volume-diastole, LV Mass;c: Left ventricular mass corrected, EF: ejection fraction, HR: heart rate.

Table 2. Systolic function doppler parameters.

	WT (n = 7)	SERCA (n = 7)	Null (n = 7)	Het (n = 11)	Het HF (n = 4)	Hom (n = 6)	Hom HF (n = 1)
Pulmonary							
PET/ET	0.27 ± 0.06[ab]	0.25 ± 0.02[ab]	0.20 ± 0.03[b]	0.26 ± 0.02[ab]	0.36 ± 0.06[ab]	0.33 ± 0.02[a]	
Aorta							
LVOT VTI VTI (mm)	29.2 ± 1.96	25.5 ± 2.62	29.6 ± 2.34	26.1 ± 2.82	15.5 ± 2.32	24.8 ± 2.27	26.02 ± 0.00
LVOT VTI Peak Vel (mm/s)	-767.6 ± 47.9	-829.9 ± 77.6	-767.9 ± 71.9	-754.9 ± 80.3	-579.8 ± 67.9	-726.1 ± 71.6	-808.8 ± 0.00

Data are mean ± SE. Different superscripts across rows indicate significant differences ($P<0.05$).
HF: Heart failure, PET: Pulmonary ejection time, ET: Ejection time, LVOT VTI VTI: Left ventricular outflow tract length velocity time interval, LVOT VTI Peak Vel: Left ventricular outflow tract length peak velocity.

Echocardiographic examination: diastolic function

The diastolic parameters included the E and A wave, measured from the doppler image at the level of the mitral valve. The velocity of the blood entering the left ventricle during relaxation is measure by the E wave. The A wave was the velocity of the blood from the atrial kick, in which the atria contracts and blood flows into the left ventricle. There was a statistical difference between the Het HF group and the WT group for the E/A ratio (**Table 3**).

Table 3. Diastolic function Doppler parameters

	WT (n = 7)	SERCA (n = 7)	Null (n = 7)	Het (n = 11)	Het HF (n = 4)	Hom (n = 6)
MV E/A	2.01 ± 0.22[ac]	2.69 ± 0.20[bc]	1.60 ± 0.14[a]	1.73 ± 0.24[a]	1.01 ± 0.00[a]	2.15 ± 0.19[ac]
MV Dec Time (ms)	24.46 ± 2.15	21.7 ± 0.89	23.9 ± 1.76	22.8 ± 2.04	17.8 ± 0.00	16.3 ± 3.74
MV Dec Acc (mm/s^2)	-33746 ± 1833	-39619 ± 1525	-30205 ± 2969	-37145 ± 2351	-59051 ± 0.00	-57669 ± 18859

Data are mean ± SE. Different superscripts across rows indicate significant differences ($P<0.05$). HF: Heart failure, MV E: Maximum velocity of the E wave, MV A: Maximum velocity of the A wave, MV Dec Time: Maximum velocity deceleration time, MV Dec Acc: Maximum velocity slope of deceleration.

DISCUSSION

Our data demonstrated that: 1) QTc was prolonged in the Het mice compared to the WT mice. 2) More ventricular arrhythmias were present in the Het mice and Hom mice after catecholaminergic challenge, including non-sustained ventricular tachycardia. 3) Some of the Het and Hom mice developed heart failure.

QTc

The QTc was significantly prolonged in the Het group vs WT group. A prolonged QT indicated a delay in ventricular depolarization. A prolonged QT indicates that the mouse is more prone to develop ventricular arrhythmia. Since the Het group has a prolonged QT then it was pre-exposed to ventricular arrhythmia. Indeed, at baseline and after catecholaminergic challenge, the Het mice displayed ventricular arrhythmia, including ventricular tachycardia. In humans, many individuals that display an elongated QT do develop Catecholaminergic Polymorphic Ventricular Tachycardia.[11]

Arrhythmia in Het and Hom groups

There was a significant increase in the amount of ventricular arrhythmias present after catecholaminergic challenge in the Het mice and Hom mice than the control groups. The Het mice displayed more simple ventricular arrhythmia consisting of PVC and fusion beats where as the Hom mice had significantly more complex ventricular arrhythmias than the Het mice. These complex arrhythmias in the Hom mice mainly included non-sustained ventricular tachycardia or ventricular tachycardia. Since these mice have a deficiency in the CASQ2 protein, which buffers calcium in the SR, and they have overactive SERCA pump, which was up taking more calcium into the heart, they were more likely to display arrhythmia.[6] In conclusion, SERCA overexpression was unable to rescue Catecholaminergic Polymorphic Ventricular Tachycardia induced by CASQ2 depletion.

Heart failure in Het and Hom groups

A percentage of mice in the Hom group and Het group developed heart failure. The Het and Hom mice with heart failure had a significant decrease in the ejection fraction compared to the other groups. However, the Null mice do not develop heart failure. Only when the Null group is crossed with the SERCA overexpression group then we produced a heart failure genotype. Previous studies in humans have shown that the CASQ2 null gene mutation causes cardiac hypertrophy and heart failure along with an increased incidence of sudden death from stress-induced arrhythmia.[12]

We concluded that the SERCA overexpression did not rescue ventricular arrhythmias in mice with CASQ2 depletion, and instead it created a phenotype that displays ventricular arrhythmias (including ventricular tachycardia) and heart failure.

STUDY 2: SERCA AS A MEANS TO RESCUE DIABETIC CARDIOMYOPATHY

Diabetes is a major health problem that affects over 200 million individuals worldwide. Diabetes can lead to cardiomyopathy, independently of the presence of coronary artery disease. It has been shown that individuals with diabetes are more at risk for cardiac autonomic dysfunction.[13] Many individuals that have diabetes are then predisposed for heart failure. Several studies have shown that diabetic cardiomyopathy is associated with a depletion of SERCA pump.[3]

Previously, in our lab it was shown that SERCA pump was down regulated during cardiomyopathy.[3] The SERCA pump up takes cytosolic calcium and stores it for muscle

contraction. When the SERCA pump does not function properly this leads to a rise in cytosolic calcium levels, which is thought to occur during diabetes.

The purpose of this study is to determine if SERCA overexpression can decrease the effects of diabetes on cardiomyocytes and improve cardiomyopathy by reducing cytosolic calcium overload. For the proposed study, a well-characterized SERCA1a transgenic (**TG**) rodent model was used along with an insulin dependent rodent model. A few days after the streptozocin injection, the rodent had hyperglycemia that was sustained throughout the study and developed cardiac dysfunction, as shown previously in our laboratory. The hypothesis was that over expression of the SERCA pump will improve diabetic cardiomyopathy.

MATERIAL AND METHODS

Animal model of diabetic cardiomyopathy

A well-characterized insulin dependent rodent model was used. The TG mice over expressing SERCA1a pump and their WT littermates (n = 6) were made diabetic by streptozotocin injection. A few days after streptozotocin injection, rodents displayed hyperglycemia which was sustained throughout the study. Glucose, serum insulin concentrations and body weight were measured at baseline and every week for eight weeks. After the streptozotocin injection the blood glucose of both the WT and TG mice increased as expected, which indicated that all of the mice were diabetic. There were four groups of mice for this study 1) Wild-type diabetic (**WT**) 2) Wild-type control 3) SERCA over expression TG diabetic (**TG**) 4) SERCA overexpression TG control.

Generating the SERCA mouse model

A SERCA mouse model was established by Dr. Periasamy and was used for this study.[2] The SERCA1a isoform was used since they have a two-fold increase in the total amount of cardiac SERCA protein and in calcium uptake. It has been shown that the SERCA1a form can substitute for the SERCA2a in the heart for both structure and function along with enhancing calcium transport and cardiac contraction.[14, 15]

In vivo study

Echocardiographic examination was recorded at baseline and eight weeks after the induction of diabetes. Please refer to study 1 for a description of the methods.

Statistical analysis

A 2-way repeated measure ANOVA was performed. The reason for using this test was because the same individual was used for the baseline measurement and the eight week measurement in order to determine the effects of diabetes on cardiac function in WT and SERCA over expression TG mice. All results were reported as mean ± SE.

RESULTS

SERCA1a expression

The TG group did show expression of the SERCA1a (skeletal isoform) protein in cardiac tissue compared to the WT group (**Fig 14 A**). Also there was a prominent expression of the SERCA1a protein in both the healthy and diabetic TG mice (**Fig 14 B**).

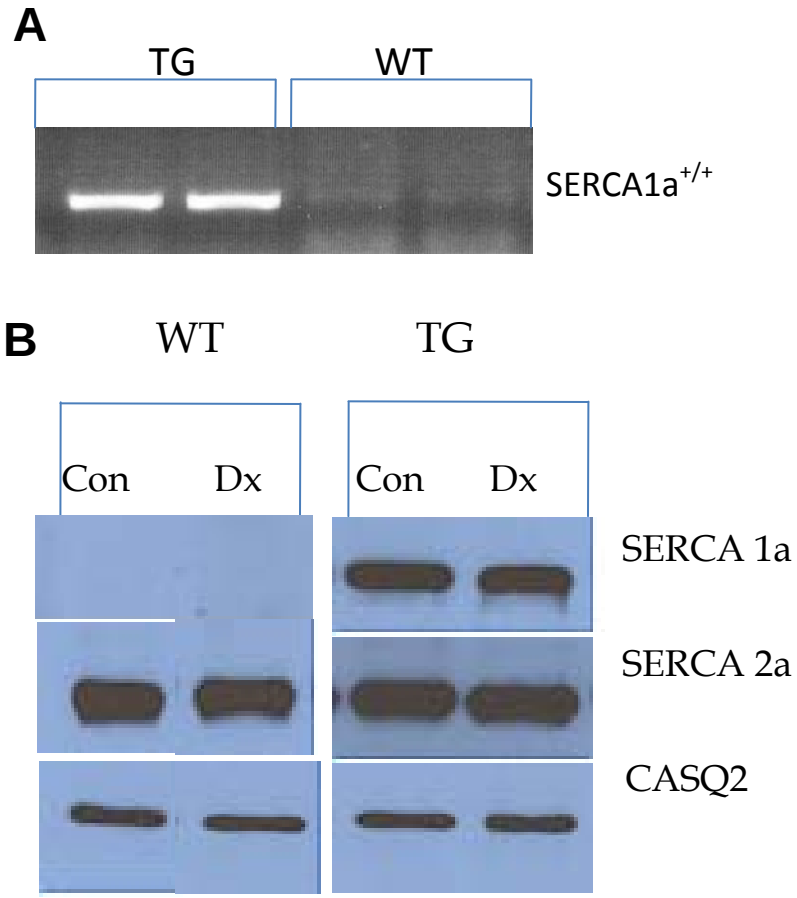

Fig 14. A) PCR analysis with tail samples from offspring of TG breeders, showing a distinct band of SERCA1a gene in the TG but not WT mice. B) Western blot of cardiac tissue from diabetic and littermate healthy WT and TG mice. CASQ2: Calsequestrin. Con: Control. Dx: Diabetic.

Echocardiographic examination

There was a statistical difference of the left ventricle mass corrected between the TG mice at eight weeks and the WT mice at eight weeks (**Fig 15**). The left ventricle mass was increased in the TG mice compared to the WT mice (**Table 4**). Heart rate of diabetic WT mice was significantly lower at eight weeks than baseline indicative of cardiomyopathy (**Fig 16**). There was not a statistical difference between the heart rate of the TG mice at baseline and at 8 weeks which could be due to a low sample volume or SERCA overexpression does decrease effects of cardiomyopathy. There was not a statistical difference regarding other systolic and diastolic parameters at baseline and 8 weeks in both groups (**Tables 5 and 6**).

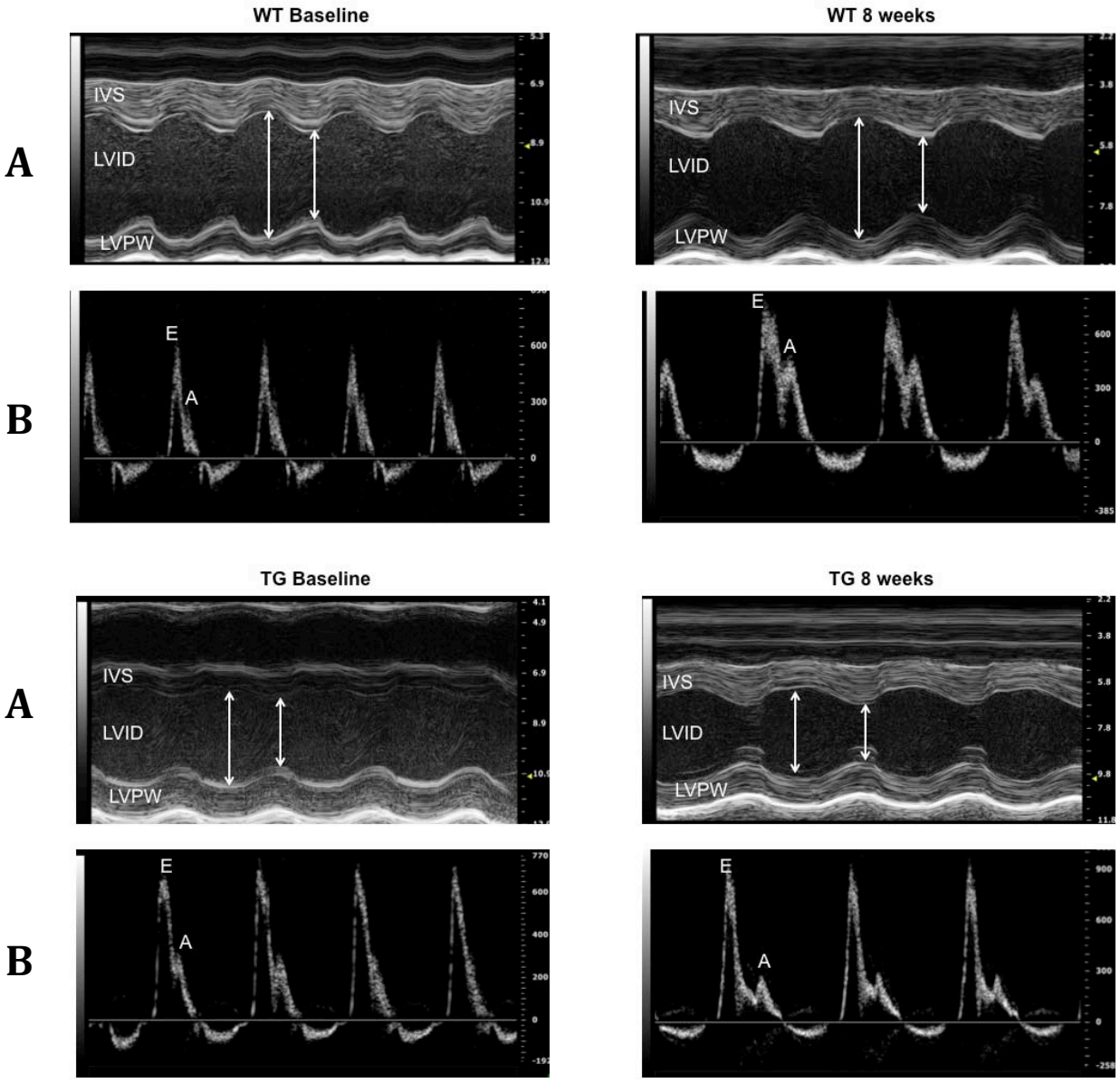

Fig. 15. A) Representative figure of the M-mode demonstrating systolic function. B) Representative figure of the E and A wave demonstrating diastolic function. IVS: Interventricular septal dimension, LVID: Left ventricular internal diameter, LVPW: left ventricular posterior wall dimension.

Table 4. Systolic function parameters in wildtype and transgenic mice at baseline and 8 weeks following induction of diabetes.

	Wildtype		**Transgenic**	
	Baseline	8 Weeks	Baseline	8 Weeks
IVS;s (mm)	1.62 ± 0.06	1.62 ± 0.09	1.56 ± 0.13	1.71 ± 0.09
IVS;d (mm)	1.16 ± 0.08	1.12 ± 0.08	1.19 ± 0.07	1.32 ± 0.09
LVPW;s (mm)	1.19 ± 0.04	1.43 ± 0.24	1.41 ± 0.14	1.22 ± 0.08
LVPW;d (mm)	0.78 ± 0.06	0.75 ± 0.06	1.22 ± 0.12*	0.98 ± 0.08*
LVID;s (mm)	2.71 ± 0.08	2.69 ± 0.11	2.61 ± 0.29	2.92 ± 0.08
LVID;d (mm)	4.07 ± 0.07	4.10 ± 0.13	3.74 ± 0.30	4.14 ± 0.09
LV Vol;s (ul)	27.5 ± 1.92	27.1 ± 2.77	27.5 ± 5.28	32.5 ± 2.06
LV Vol;d (ul)	73.1 ± 2.94	74.7 ± 5.44	62.4 ± 9.86	75.4 ± 3.97
LV Mass;d (mg)	148.0 ± 10.6	160.1 ± 7.12	149.5 ± 7.41	152.0 ± 6.92
LV Mass;c (mg)	127.4 ± 9.78	121.9 ± 7.29	151.5 ± 13.5	164.9 ± 7.89*
FS (%)	33.4 ± 0.96	34.5 ± 1.28	31.2 ± 4.22	29.1 ± 1.79
EF (%)	62.6 ± 1.38	64.0 ± 1.74	58.6 ± 5.75	56.4 ± 2.64

Data are mean ± SE for n= 6/group. $P<0.05$.
* denotes different from WT for same time point. † denotes different from baseline.
IVSs: Interventricular septal dimension-systole, IVS;d: Interventricular septal dimension-diastole, LVPW;s: left ventricular posterior wall dimension-systole, LVPW;d: left ventricular posterior wall dimension-diastole, LVID;s: left ventricular internal diameter-systole, LVID;d: Left ventricular internal diameter-diastole; LV Vol;s: Left ventricular volume-systole, LV Vol;d: Left ventricular volume-diastole, LV Mass;c: Left ventricular mass corrected, FS: fractional shortening, EF: ejection fraction, HR: heart rate, CO: cardiac output

Table 5. Diastolic function parameters in wildtype and transgenic mice at baseline and 8 weeks following induction of diabetes.

	Wildtype		**Transgenic**	
	Baseline	8 Weeks	Baseline	8 Weeks
MV E/A	1.61 ± 0.29	1.44 ± 0.28	2.17 ± 0.19	2.22 ± 0.40
MV Dec Time (ms)	19.4 ± 2.77	25.0 ± 2.12	19.2 ± 1.89	20.9 ±0.72
MV Dec Acc (mm/s^2)	-41351 ± 4415	-37094 ± 8119	-39583 ± 2191	-35360 ± 4092
LV MPI	0.49 ± 0.06	0.42 ±0.03	0.62 ± 0.06	0.59 ± 0.07

Data are mean ± SE for n= 6/group. P<0.05. * denotes different from WT for same time point. MV E: Maximum velocity of the E wave, MV A: Maximum velocity of the A wave, MV Dec Time: Maximum velocity deceleration time, MV Dec Acc: Maximum velocity slope of deceleration, LV MPI: Left ventricle myocardial performance index.

Table 6. Systolic function doppler parameters in wildtype and transgenic mice at baseline and 8 weeks following induction of diabetes.

	Wildtype		**Transgenic**	
	Baseline	8 Weeks	Baseline	8 Weeks
Pulmonary				
PET/ET	0.23 ± 0.70	0.26 ± 0.43	0.25 ± 1.03	0.26 ± 0.47
Aorta				
AET (ms)	48.6 ± 0.90	56.5 ± 3.16†	44.3 ± 2.14	52.0 ± 1.99†
LVOT VTI VTI (mm)	29.8 ± 1.87	28.6 ± 2.18	27.6 ± 2.18	26.5 ± 1.74
LVOT VTI Peak Vel (mm/s)	-887.7 ± 77.1	-776.5 ± 67.4	-899.8 ± 73.9	-733.8 ± 65.5

Data are mean ± SE for n= 6/group. P<0.05, † denotes different from baseline.
PET: Pulmonary ejection time, ET: Ejection time, AET: aortic ejection time, LVOT VTI VTI: Left ventricular outflow tract length velocity time interval, LVOT VTI Peak Vel: Left ventricular outflow tract length peak velocity.

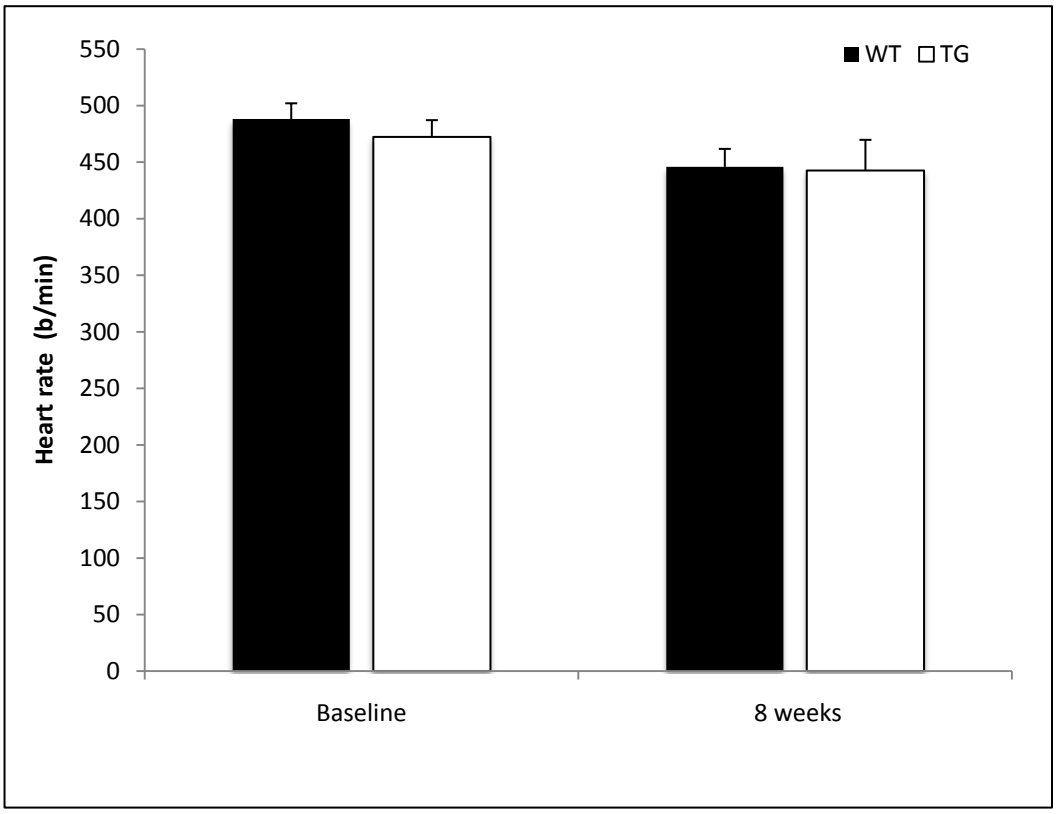

Fig 16. There was a significant difference between the baseline and at 8 weeks in the WT mice heart rates. * denotes a significant difference from baseline ($P<0.05$).

DISCUSSION

Our data demonstrated that: 1) There was not a difference in cardiac function between SERCA overexpression TG and WT healthy mice. 2) The WT mice had diabetic cardiomyopathy as evident by the decrease in heart rate of the WT mice eight weeks after diabetes. Diabetic TG mice did not display an improvement in cardiac function.

SERCA pump

Other studies have found that the SERCA overexpression increases the amount of SR calcium intake, which increases the left ventricle contraction and relaxation.[16, 4] However; our study found that there was not a significant difference between the healthy

SERCA overexpression TG mice and the healthy WT mice. This could be due to the fact that the SERCA overexpression was only a 2 fold increase compared to other studies that use up to a 10 fold increase in SERCA overexpression.[16] Previous studies have shown that the SERCA2a overexpression in mouse hearts did increase energetic costs of contraction and was detrimental to individuals with decreased energetics such as diabetes.[17] Furthermore, in our animal model the mice genetically have SERCA overexpression whereas other studies have delivered SERCA gene via a catheter. All of these factors could account for the lack of an improvement in cardiac function of healthy and diabetic SERCA overexpression TG mice.

Diabetic cardiomyopathy

WT mice displayed diabetic cardiomyopathy, as evident by a decrease in heart rate after 8 weeks of diabetes. Similarly, cardiac autonomic dysfunction has been reported during diabetes in humans.[13] Autonomic dysfunction is a malfunction of the autonomic nervous system, specifically cardiac autonomic dysfunction. Autonomic dysfunction is created by the effects of diabetes that leads to weight loss and hypotension. The effects of autonomic dysfunction complicate underlying cardiovascular disease.[18] In TG mice, there was not a statistical difference in the heart rate at 8 weeks compared to baseline which could be due to our relative small sample size or due to the fact that SERCA-overexpression improved diabetic cardiomyopathy.

Previous studies have demonstrated that diabetes leads to altered calcium handling in the heart which can lead to cardiomyopathy and hypotension due to weight loss.[13, 18] In addition, diabetes results in cardiomyopathy due to reduced contractility from

decreased Ca^{2+} handling by the SERCA pump.[19] However, there was no major systolic or diastolic dysfunction in the WT, which could be due to the low dose of streptozotocin used to induce a mild form of diabetes. Other studies have shown that conditional increase in the SERCA2a protein to rodents with pre-existing cardiomyopathy had improved cardiac function.[20, 21] Therefore, in our study; the lack of improvement in cardiac function in TG mice may be due to the difference in TG animal model. In addition, a low dose of streptozotocin was given to our mice so they have a mild case of diabetes compared to the other studies where the mice had a more severe form of cardiomyopathy.[20, 21, 22] Finally, pressure volume loop measurement may offer a more sensitive tool to detect systolic and diastolic dysfunction compared to echocardiographic examination. Therefore, further in vitro studies are required to determine subtle changes in cardiac function in our TG mice with SERCA1a overexpression and diabetic cardiomyopathy.

CONCLUSION

This study demonstrated that the increased expression of SERCA pump does not attenuate the incidence of arrhythmias or diabetic cardiomyopathy. SERCA overexpression did not rescue ventricular tachycardia in individuals with the CASQ2 mutation and rather it induced heart failure. In regards to diabetes, SERCA only slightly improved diabetic cardiomyopathy that occurs during diabetes. These findings do not support the theory that SERCA gene therapy rescues heart failure and individuals should be cautious when promoting the use of this novel type of therapeutic intervention.

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