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Pathogenic Troponin T Mutants with Opposing Effects on Myofilament Ca^{2+} Sensitivity Attenuate Cardiomyopathy Phenotypes in Mice

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Abstract

Mutations in cardiac troponin T (TnT) associated with hypertrophic cardiomyopathy generally lead to an increase in the Ca^{2+} sensitivity of contraction and susceptibility to arrhythmias. In contrast, TnT mutations linked to dilated cardiomyopathy decrease the Ca^{2+} sensitivity of contraction. Here we tested the hypothesis that two TnT disease mutations with opposite effects on myofilament Ca^{2+} sensitivity can attenuate each other's phenotype. We crossed transgenic mice expressing the HCM TnT-I79N mutation (I79N) with a DCM knock-in mouse model carrying the heterozygous TnT-R141W mutation (HET). The results of the Ca^{2+} sensitivity in skinned cardiac muscle preparations ranked from highest to lowest were as follow: I79N > I79N/HET > NTg > HET. Echocardiographic measurements revealed an improvement in hemodynamic parameters in I79N/HET compared to I79N and normalization of left ventricular dimensions and volumes compared to both I79N and HET. *Ex vivo* testing showed that the I79N/HET mouse hearts had reduced arrhythmia susceptibility compared to I79N mice. These results suggest that two disease mutations in TnT that have opposite effects on the myofilament Ca^{2+} sensitivity can paradoxically ameliorate each other's disease phenotype. Normalizing myofilament Ca^{2+} sensitivity may be a promising new treatment approach for a variety of diseases.

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Keywords

Hypertrophic cardiomyopathy; Dilated cardiomyopathy; myofilament Ca^{2+} sensitivity; Troponin T mutations; Arrhythmias

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is the leading cause of sudden cardiac death in young adults. HCM affects approximately 1 in 500 individuals who exhibit a wide range of symptoms including diastolic dysfunction, fibrotic tissue formation and impaired contractile properties [1, 2]. Heart failure and arrhythmias are clinical staples of this disease [3], with the pathology of HCM including left ventricular (LV) hypertrophy as a tell-tale sign [2, 4]. In contrast, primary dilated cardiomyopathy (DCM) exhibits systolic dysfunction and LV chamber dilation. Interstitial fibrosis and abnormal contractile properties are also present [5]. Currently, there are no effective pharmacological treatments for either disease.

The contractile properties of the heart are primarily dictated by the myofilament proteins in conjunction with the sarcoplasmic reticulum [6]. The turnover and recruitment of actomyosin cross-bridges as well as Ca^{2+} binding to, and dissociation from the thin filament can determine the kinetics and force of contraction [6]. The troponin complex, a crucial Ca^{2+} regulatory nexus for cardiac muscle contraction, is thought to play a role in both diseases. Located on the actin filament, the troponin complex consists of the subunits Troponin T (TnT), Troponin I (TnI), and Troponin C (TnC). Together with tropomyosin (Tm), the complex acts as a “molecular switch” to expose myosin-to-actin interaction sites upon Ca^{2+} binding to TnC [7]. This allows thick and thin filaments to slide past each other causing the sarcomere to shorten [6]. Mutations in the Tn subunits can cause either primary HCM or primary DCM. Evidence for the deleterious effect of Tn mutations comes from *in-vitro* and animal models that show abnormalities in Ca^{2+} sensitivity of contraction, maximal force development, regulation of actomyosin ATPase activity, Ca^{2+} handling proteins, contractile transients, among others [8].

Among the Tn subunits, human disease mutations occur most commonly in TnT [8]. Interestingly, mutations in TnT can result in either HCM or DCM, depending on whether the mutation increases (HCM) or decreases (DCM) myofilament Ca^{2+} sensitivity. Prior studies have looked specifically at the TnT I79N mutant, previously established as a human cardiac mutation found in HCM patients. Tested in mouse, the mutation leads to an increase in myofilament Ca^{2+} sensitivity without causing hypertrophy [9]. Our group has suggested that the Ca^{2+} sensitized myofilament is the primary reason for the high risk of arrhythmia caused by the TnT-I79N mutation [10]. Ventricular arrhythmia and sudden death are also a primary disease manifestation in HCM patients with TnT mutations [11]. Conversely, the missense TnT mutation R141W is associated with DCM in humans and mice [12–14]. This DCM associated mutation has been shown to induce Ca^{2+} desensitization of the myofilament [15–18].

To test the hypothesis that abnormal myofilament Ca^{2+} sensitivity is a primary cause for the pathogenicity of TnT mutations, here we investigated whether two pathogenic TnT

mutations that cause opposite effects on myofilament Ca^{2+} sensitivity can rescue each other's cardiomyopathy phenotype. Transgenic mice expressing the HCM TnT-I79N mutation (I79N) were crossed with a knock-in (KI) mouse expressing the DCM TnT-R141W mutation (HET). The new double-transgenic mice (I79N/HET) were subsequently studied for cardiac myofilament function as well as heart morphology and physiology. The I79N/HET mice exhibited an overall normalization of myofilament Ca^{2+} sensitivity, left ventricular dimensions and reduction in susceptibility to arrhythmias. These results suggest an independent role of myofilament Ca^{2+} sensitivity in the development of morphological and functional changes within the diseased heart.

MATERIALS AND METHODS

Mice –

All protocols and experimental procedures followed NIH guidelines and were approved by both Vanderbilt University and Florida State University Animal Care and Use Committee (ACUC). Transgenic mice expressing the HCM TnT-I79N mutation (replacing ~ 50% of the endogenous cTnT-WT, line 8) were crossed with a DCM knock-in TnT-R141W (~ 60% of total is the mutant mouse cardiac TnT transcripts). The founders were heterozygous for the TnT-R141W mutation and overexpressed the TnT-I79N mutation in the heart. Although we have not measured relative TnT expression levels in the double crossed mice (I79N/HET), functional findings indicate that both mutant TnT proteins should be present in the heart in addition to one allele that expresses the mouse cardiac TnT-WT. Non-transgenic (NTg), TnT-I79N transgenic (I79N) and Knock-in TnT-R141W heterozygote mice (HET) were used as controls. The I79N mice were maintained in B6/SJLFl background strain, while the HET mice were originally generated in 129/SvEv background strain. The I79N/HET is therefore a hybrid background strain. The generation and characterization of the I79N and HET mouse models have been previously published [9, 12].

Cardiac Skinned Preparations –

Skinned cardiac preparations were prepared according to established protocols [10, 19]. Papillary muscle preparations were isolated from the left ventricles of 15–36 week old male and female mice. The membranes were skinned using 1% Triton X-100, rinsed and then stored in a relaxing solution ($\text{pCa } 8.0 = 10^{-8} \text{ M free } \text{Ca}^{2+}$, 150 mM ionic strength, 2.5 mM MgATP^{2-}) containing 50% glycerol (v/v) at -20°C . Skinned cardiac preparations were mounted onto a force transducer and immersed in $\text{pCa } 8.0$ solution. Muscle preparations were stretched at $\text{pCa } 8.0$ by 20% over slack length. The Ca^{2+} dependence of tension was measured in a series of solutions with increasing Ca^{2+} concentrations at room temperature ($\sim 20^{\circ}\text{C}$). Methods for solving the free and bound metal ion equilibria in our pCa solutions were provided by the computer program, *pCa Calculator* [20].

Echocardiography (ECHO) Measurements –

ECHO was performed using a Vevo 2100 high-resolution *in vivo* imaging system. Male and female mice (14–61 weeks old) were put under light anesthesia with isoflurane ($\sim 2\%$) during the procedure. M-mode imaging of the parasternal short axis view allowed evaluation of left ventricular systolic and diastolic function and morphology. The mitral valve flow

parameters were acquired through a four-chamber view using the pulsed wave spectral Doppler to be used as an index of diastolic function [21, 22].

Isolated heart perfusion and arrhythmia measurements –

Hearts were removed from 15–61 week old male and female mice after anesthesia (3% Isoflurane with 100% O₂). Then the aorta was cannulated for retrograde perfusion as previously described [23]. The tyrode solution used for perfusion contained in mmol/l: NaCl 139, KCl 4, NaHCO₃ 14, NaH₂PO₄ 1.2, MgCl₂ 1, CaCl₂ 1.5, Glucose 10, S-propranolol 0.0002. Tyrode solution was filtered before use and oxygenated with carbon dioxide (95% O₂/5% CO₂) to achieve pH 7.4. Hearts were lowered into a warm bath and volume conducted ECGs were recorded using two platinum wire electrodes. Two-prong platinum pacing wires contacted the right ventricles for recording. ECGs were recorded using a custom built pre-amplifier and amplifiers connected to a PowerLab (ADI instruments). Hearts were stabilized for 15 min with sinus rhythm, then paced 12 Hz, 15 Hz, and 18 Hz every 5 min. Arrhythmias (premature ventricular contraction and ventricular tachycardia) observed during 18 Hz pacing and thereafter as previously reported [23].

Histopathology –

Mouse hearts were excised and perfused with a 10% buffered formaldehyde solution. The hearts were sliced, stained with Masson's trichrome (MTX) or periodic acid-Schiff (PAS) and examined using a Nikon AZ 100M. Analysis was blinded and performed as previously described using NIH ImageJ program [24].

Gross morphology –

Body weight was recorded at time of sacrifice and mouse hearts excised and weighed.

Statistical Analysis –

All values are presented as mean ± S.E. Error bars represent S.E. Statistical significance was determined by one-way ANOVA using Tukey or Student-Newman-Keuls post- hoc analysis for the myofilament Ca²⁺ sensitivity, maximal steady-state force developed and echocardiography data, respectively. Histopathology was tested by independent T-test. The statistical analysis of the prior experiments were completed using the program Sigma Plot 12. The incidence of arrhythmias was determined by non-parametric Mann-Whitney test. $p < 0.05$ was considered to be significant. The arrhythmia data statistical analysis was performed using Origin Lab Pro 2018.

RESULTS

Myofilament Ca²⁺ sensitivity –

We first tested the Ca²⁺ sensitivity of contraction in NTg, I79N, HET and I79N/HET mice skinned cardiac muscle preparations. The I79N cardiac muscle preparations showed increased Ca²⁺ sensitivity of contraction compared to NTg (Figure 1A), while the HET cardiac preparations displayed decreased Ca²⁺ sensitivity of contraction (Figure 1B). The I79N/HET cardiac preparations showed a promising Ca²⁺ sensitivity closer to NTg than the

I79N mutation alone (Figure 1C). Furthermore, the HET cardiac skinned preparations displayed increased cooperativity of thin filament activation (n_{Hill}) compared to NTg (Table 1). Interestingly, the I79N/HET cardiac skinned preparations did not show a significant change in n_{Hill} compared to NTg, implying that the I79N mutation rescued the increased n_{Hill} imposed by the R141W (HET) mutation (Table 1). The maximal steady-state forces developed among the four groups were not statistically significant. The $p\text{Ca}_{50}$, n_{Hill} , and F_{max} values for all groups are reported in Table 1.

Heart Size and Histopathology –

There was no significant difference in heart weight (HW) between NTg vs HET (Figure 2A), however mice expressing the TnT I79N mutation (I79N and I79N/HET) had smaller hearts compared to NTg and HET group mice. The HW normalized to body weight (BW) ratio followed the same trend as observed in HW alone (Figure 2B). Thus, the I79N/HET mice show a heart and body weight phenotype similar to I79N. MTX histopathological staining of the I79N/HET mice hearts showed a trend in the reduction of fibrosis when compared to the I79N mice depicted in Figure 2C and corresponding Figure 2D.

Echocardiography –

Echocardiography of each mouse group showed that the ejection fraction was not significantly different in any of the groups (I79N, HET and I79N/HET) compared to NTg mice (Figure 3A). The I79N mice had a statistically significant lower stroke volume compared to NTg, which was normalized in the I79N/HET mice (Figure 3B). I79N and HET mice displayed statistically significant lower and higher volumes, respectively, compared to NTg mice (Figure 3C and 3D). Interestingly, the I79N/HET mice showed a normalization of both systolic and diastolic volumes (Figure 3C and 3D). The diameter of the left ventricle (LV) during diastole and systole followed the same trend as observed for the volumes, e.g., while I79N and HET mice have small and large hearts, respectively, the I79N/HET mice showed similar dimensions to NTg (Figure 3E and 3F). These data are further exemplified with the LV mass shown in Table 2. While HET mice display increased LV mass, the I79N/HET showed further normalization. Additional echocardiography parameters are presented in Table 2.

Ventricular Arrhythmias –

I79N mice displayed an increased susceptibility to ventricular tachycardia compared to NTg mice (Figure 4A and 4B) during rapid pacing protocol, consistent with previous findings reported by our group [23]. Ventricular arrhythmias occurred in 5 out of 6 (83% incidences) I79N mouse hearts, but only in 1 out of 4 (25% incidences) I79N/HET mouse hearts (Figure 4A and 4B). Therefore, the presence of the Ca^{2+} desensitizing R141W mutation in I79N hearts attenuated the incidence of ventricular tachycardia during rapid pacing protocol.

QRS Complex –

In I79N mice, the arrhythmia susceptibility induced by rapid heart rates is in part caused by regional conduction slowing, which manifests itself as QRS widening on the ECG [23]. Hence, we next analyzed the QRS duration during the rapid pacing protocol that elicited the

ventricular arrhythmia in I79N mice. Importantly, only I79N mice exhibited QRS widening (30 % compared to sinus rhythms) after fast pacing (Figure 5). QRS widening was blunted in I79N/HET mice (Figure 5), suggesting that expression of the R141W mutation prevents the intraventricular conduction delay caused by the I79N mutation.

Discussion

Our study shows that two pathogenic TnT mutations — which by themselves cause cardiomyopathy in mice but manifest as opposite effects on myofilament Ca^{2+} sensitivity — can ameliorate each other's “disease” phenotypes. When transgenic I79N mice that exhibit increased myofilament Ca^{2+} sensitivity are crossed with knock-in R141W mice that exhibit a decrease Ca^{2+} myofilament Ca sensitivity and increased n_{Hill} , the resulting double-transgenic I79N/HET mice show an overall decrease in the severity of the cardiomyopathy phenotype caused by either mutation alone: Co-expression of the pathogenic R141W mutation prevented the arrhythmia phenotype caused by the TnT-I79N mutation (Figure. 4). Conversely, the I79N mutation prevented the chamber dilation and reduced contractile function caused by the R141W mutation (Figure. 3). Since the two pathogenic mutations have opposite effects on myofilament Ca^{2+} sensitivity, our results suggest that regardless of the mutation, there exists an independent role of myofilament Ca^{2+} sensitivity in the development of morphological and functional changes within the diseased heart.

Over 1000 mutations that cause cardiomyopathies in patients have been found constituting the cardiac sarcomere [25, 26]. Mutations that can affect normal myofilament mechanism of activation and relaxation include multiple TnT domains. The TnT I79N transgenic mouse mutation used in this study is a mutation found in HCM patients. Although the I79N related patients and murine model do not exhibit left ventricle hypertrophy, it does show an increased Ca^{2+} sensitivity of contraction and an increase in the arrhythmic profile—the latter being a hallmark of HCM. The reduction of cardiac output without increased LV thickness suggest that this mutation may also be implicated in the development of a phenotype that resembles restrictive cardiomyopathy (RCM). This mixed HCM and RCM phenotype has been acknowledged in the contemporary classification of inherited cardiomyopathy and highlights the clinical overlap of these two diseases [27]. The KI TnT R141W mouse mutation, used as a “counter effecting” sarcomeric mutation in this study, is also found in humans, and manifests in decreased myofilament Ca^{2+} sensitivity.

Both I79N and R141W mutations are located in TnT1, the tail domain of TnT, known to interact with Tm. Tropomyosin is known to control cooperativity of thin filament activation. Previous studies report on the TnT1 domain's crucial flexibility and its role in regulating Tm positioning for contraction [28–30]. Further evidence for the importance of this domain in our study of TnT1 mutations lies in their ability to alter the affinity of troponin complex for Tm [31]. Therefore, we suggest that the most targeted approach to “rescuing” either HCM or DCM phenotype would be to have a mutation within that same domain to counteract manifestation of the disease, probably through TnT1's flexibility and eventually altered contraction. The increased binding affinity between R141W troponin and Tm [31] possibly underlies the increased cooperativity of thin filament activation displayed by the HET mice within this study (Table 1). Interestingly, I79N/HET mice displayed a normalization of

cooperativity of thin filament activation (Table 1). These data support a role for R141W (HET)'s rescuing potential towards the HCM phenotype displayed in the I79N mice.

Ultrasound cardiac imaging allowed for several cardiac parameters to be measured and compared between the groups. There was an increase in overall hemodynamic parameters for I79N/HET compared to I79N mice, i.e., cardiac output and stroke volume (Figure 3B and Table 2), suggesting a betterment in heart function. The I79N/HET also showed a normalization of left ventricular volume and dimension during both diastole and systole compared to I79N and HET. Furthermore, I79N/HET mice exhibited reduced susceptibility to arrhythmia during *ex-vivo* testing, thus lending evidence to the attenuation of ventricular tachycardia in the double mutant. Taken together, these measurements of improved cardiac blood flow, morphology and arrhythmia incidences in I79N/HET demonstrate positive support for the balance between the two studied TnT mutations with opposing effects on the myofilament Ca^{2+} sensitivity of contraction.

Myofilament Ca^{2+} sensitivity has been previously shown to be essential in the manifestation of DCM pathophysiology [32–35]. To note, the TnT R141W mutation showed significantly Ca^{2+} desensitization and also displayed an increase in Ca^{2+} transient amplitudes as a consequence [12]. Our data are consistent with prior studies that corrected myofilament Ca^{2+} sensitivity in mice rescued the cardiomyopathy phenotype. Li et al have shown that a TnI RCM mutation crossed with an N-terminal truncated TnI reversed Ca^{2+} hypersensitivity and improved cardiac function [36]. In addition, an HCM thin filament mutation crossed with a pseudophosphorylated TnI displayed myofilament Ca^{2+} desensitization and “associated correction” of systolic dysfunction [37]. Our understanding that manifestations, specifically within the thin filament that affect Ca^{2+} sensitivity, could have the potential to be corrected mechanistically was supported. This fueled the idea of the potential to “reverse” or at least dampen the effect of opposing thin filament mutations to allow for an improvement in disease presentation. Although studies on the breeding of mice with opposing myofilament Ca^{2+} sensitivity responses have been previously completed, this study would be the first to test the idea of breeding HCM and DCM mouse models for normalization of cardiac pathophysiology. This study is also novel in its achievement of a genetically engineered mouse model that mitigates the arrhythmias displayed in an HCM model through the desensitization of the myofilament. This had been previously shown with the I79N mice, however this was attained using the unspecific drug Blebbistatin to accomplish that myofilament desensitization *ex vivo* [10].

The experimental design used here is based on the hypothesis that thin filament changes will impact the disease phenotype directly. However, there is also the hypothesis that thick filament changes affecting myofilament Ca^{2+} sensitivity can alter the manifestation of thin filament associated cardiomyopathies as well. For example, permeabilized muscle preparations isolated from a DCM Tm mouse model treated with a small molecule cardiac myosin activator showed Ca^{2+} an increased myofilament Ca^{2+} sensitivity that was restored to physiological levels compared to control [38]. This small molecule cardiac myosin activator has also been shown to increase Ca^{2+} sensitivity of force production in healthy permeabilized rat cardiomyocytes [39]. More recently, human induced pluripotent stem cells-derived cardiomyocytes containing a mutant TnT, associated with DCM, showed

improved sarcomere function when treated with the cardiac myosin activator [40]. This modulation of myofilament Ca^{2+} sensitivity through the thick filament may also be accomplished by enhancing myosin regulatory light chain phosphorylation. Interestingly, a DCM Tm mouse model treated with angiotensin II type I receptor biased ligand showed an improved cardiac contractility that was correlated with an increased ventricular myosin light chain-2 phosphorylation [41, 42]. It is also possible that the mutual rescue observed in I79N/HET mice can involve the remodeling of Z-disk-related proteins. A recent study reported that the alterations in desmin and α -actinin which are pivotal sensor molecules of the sarcomere are directly related to cardiac dysfunction [43].

Our study revealed that pathogenic TnT mutations (I79N and R141W) that by themselves cause cardiomyopathy but have opposite effect on myofilament Ca^{2+} responsiveness can cancel out each other's effects during development of diseases. The experiments provide evidence in support of the double transgenic mouse as a mutually "rescued" cardiac phenotype. Our results further emphasizes the function of TnT having an imperative role in the manifestation of the disease. Finally, our study points to the importance of TnT as a cardiomyopathy susceptibility protein and its role in regulating heart function, specially arrhythmias—a major hallmark of HCM disease.

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Figure 1. Ca^{2+} -activated tension in skinned cardiac preparations.

A, Calcium dependent tension of I79N mice compared to NTg. **B,** Calcium dependent tension of R141W HET mice compared to NTg. **C,** I79N/HET mice mutually normalized the Ca^{2+} sensitivity towards WT. Data are shown as mean $\pm$ S.E. (n=8–10).

Figure 2. Cardiac morphology and histology.

A, Heart weight and **B**, Heart weight over body weight (HW/BW) ratios of the NTg, I79N, HET, and I79N HET mice groups (n=9–19). **C**, (Top) Cardiac histological Masson's Trichrome stain of I79N mouse compared to (Bottom) Masson's Trichrome stain of I79N/HET mouse. **D**, Comparative quantified fibrosis content of NTg, I79N, HET, and I79N/HET mice from histopathology stains. Data are shown as mean±S.E. (n=3–6). *p<0.05 vs NTg.

Figure 3. Echocardiography measurements of LV dimensions and function.
Data are shown as mean±S.E. (n=4-8). *p<0.05 vs NTg.

Figure 4. Incidence of ventricular tachycardia in isolated heart perfusion.

A, Rapid pacing protocol and representative examples for electrocardiogram in *ex vivo* heart preparations for I79N and HET/I79N mice. Note the ventricular arrhythmias observed during 18 Hz pacing and thereafter. **B**, Percent incidence of ventricular tachycardia calculated within each group (n=4–7). VT; ventricular tachycardia. *p<0.05 vs NTg.

Figure 5. Measurement of QRS complex duration before (PRE) and after (POST) rapid pacing in isolated perfused mouse hearts.

A, Representative QRS complexes. **B**, Average values in QRS duration. For each heart, the QRS duration was measured from a volume- conducted ECG record. The QRS duration values are expressed as a percent of PRE values. Data are shown as mean $\pm$ S.E. (n=4–7).

*p<0.05 vs NTg.

Table 1:

Summary of Ca^{2+} sensitivity of contraction in mouse cardiac skinned preparations.

	NTg (n=9)	I79N (n=8)	HET (n=9)	I79N/HET (n=10)
pCa_{50}	5.65 ± 0.04	$6.12 \pm 0.03^*$	$5.49 \pm 0.05^*$	$5.92 \pm 0.03^{*,\ddagger,\S}$
n_{Hill}	2.05 ± 0.18	2.29 ± 0.08	$2.88 \pm 0.28^*$	2.28 ± 0.13
F_{max} (mN/mm ²)	55.63 ± 3.00	57.14 ± 5.97	42.57 ± 5.37	41.60 ± 2.81

n = number of experiments Data are shown as mean $\pm$ S.E.M.

F_{max} = maximum steady-state force developed

^{*}
p < 0.05 vs NTg

[‡]
p < 0.05 I79N/HET vs I79N

[§]
p < 0.05 I79N/HET vs HET

Table 2:

Summary of echocardiography parameters.

	NTg (n=8)	I79N (n=7)	HET (n=6)	I79N/HET (n=4)
Heart Rate (BPM)	486 ± 13	483 ± 11	489 ± 8	435 ± 23
LVPW;d (mm)	1.02 ± 0.07	0.99 ± 0.05	1.09 ± 0.11	1.19 ± 0.18
LVPW;s (mm)	1.44 ± 0.08	1.32 ± 0.04	1.41 ± 0.10	1.38 ± 0.16
CO (mL/min)	19.41 ± 1.46	13.78 ± 1.23 *	22.00 ± 1.22	18.78 ± 3.19
SV (μl)	39.74 ± 2.55	28.40 ± 2.21 *	44.95 ± 2.18	42.72 ± 6.17 †
Diameter;d (mm)	3.75 ± 0.11	3.14 ± 0.12 *	4.15 ± 0.09 *	3.64 ± 0.18 †,‡
Diameter;s (mm)	2.39 ± 0.14	1.87 ± 0.12 *	2.87 ± 0.09 *	2.06 ± 0.05 ‡
EF (%)	66.54 ± 3.56	72.35 ± 2.53	58.73 ± 1.84	74.81 ± 2.32 ‡
FS (%)	36.65 ± 2.66	40.63 ± 2.15	30.92 ± 1.22	43.04 ± 2.20 ‡
V;d (μL)	60.48 ± 3.78	39.71 ± 3.81 *	76.76 ± 3.71 *	56.59 ± 6.66 †,‡
V;s (μL)	20.75 ± 2.85	11.31 ± 1.86 *	31.81 ± 2.50 *	13.87 ± 0.91 ‡
LV mass (mg)	118.19 ± 8.76	97.40 ± 5.58	168.47 ± 8.10 *	116.34 ± 4.87 ‡
MV E/A	1.34 ± 0.05	1.49 ± 0.18	1.47 ± 0.10	1.52 ± 0.12

AET, aortic ejection time; IVCT, isovolumetric contraction time; LVPW;d/s, left ventricle posterior wall thickness, diastole/systole; CO, cardiac output; SV, stroke volume; EF, ejection fraction; FS, fractional shortening; V;d/s, volume, diastole/systole; LV mass, left ventricle mass (corrected); MV E/A, mitral valve E/A ratio. n = number of experiments Data are shown as mean ± S.E.M.

* p < 0.05 vs NTg

† p < 0.05 I79N/HET vs I79N

‡ p < 0.05 I79N/HET vs HET