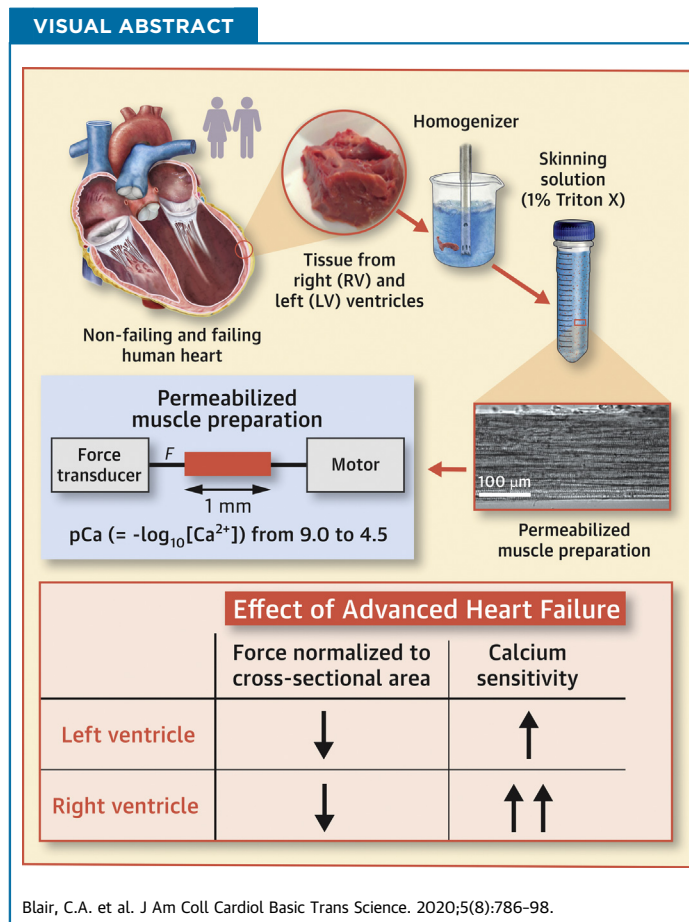


CLINICAL RESEARCH

# Heart Failure in Humans Reduces Contractile Force in Myocardium From Both Ventricles



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## HIGHLIGHTS

- Contractile assays were performed using multicellular preparations isolated from the left and right ventricles of organ donors and patients with heart failure.
- Heart failure reduced maximum force and power by approximately 30% in the myocardium from both ventricles.
- Heart failure increased the  $\text{Ca}^{2+}$  sensitivity of contraction, but the effect was bigger in right ventricular tissue than in left ventricular samples.
- The changes in  $\text{Ca}^{2+}$  sensitivity may reflect ventricle-specific post-translational modifications to sarcomeric proteins.

## SUMMARY

This study measured how heart failure affects the contractile properties of the human myocardium from the left and right ventricles. The data showed that maximum force and maximum power were reduced by approximately 30% in multicellular preparations from both ventricles, possibly because of ventricular remodeling (e.g., cellular disarray and/or excess fibrosis). Heart failure increased the calcium ( $\text{Ca}^{2+}$ ) sensitivity of contraction in both ventricles, but the effect was bigger in right ventricular samples. The changes in  $\text{Ca}^{2+}$  sensitivity were associated with ventricle-specific changes in the phosphorylation of troponin I, which indicated that adrenergic stimulation might induce different effects in the left and right ventricles. (J Am Coll Cardiol Basic Trans Science 2020;5:786-98) © 2020 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## ABBREVIATIONS AND ACRONYMS

$\text{Ca}^{2+}$	= calcium
$F_{\text{pas}}$	= passive force
$F_{\text{act}}$	= maximum $\text{Ca}^{2+}$ -activated force
$k_{\text{tr}}$	= rate of force recovery
LV	= left ventricle
MyBP-C	= myosin binding protein-C
$n_{\text{H}}$	= Hill coefficient
$P_{\text{max}}$	= maximum power output
PKA	= protein kinase A
RLC	= regulatory light chain
RV	= right ventricle
TnI	= troponin I
$V_{\text{max}}$	= maximum shortening velocity

In healthy humans, the left ventricle (LV) is a thick-walled, bullet-shaped chamber that generates approximately 120 mm Hg during systole. Conversely, the right ventricle (RV) is a thinner crescent-shaped structure that produces approximately 25 mm Hg (1,2). Laplace's law (3) shows that wall thickness will contribute to the systolic pressure difference, but it is not known whether interventricular differences in contractile function augment the structural effect.

The impact of heart failure on cells from the LV and RV is also unclear. Although numerous studies have shown that heart failure reduces maximum force and increases the calcium ( $\text{Ca}^{2+}$ ) sensitivity of contraction of the permeabilized LV myocardium (4–10), few experiments have been performed using human RV samples. One recent study by Hsu et al. (11) demonstrated that the  $\text{Ca}^{2+}$  sensitivity of contraction was increased in the RV of patients who had systemic sclerosis-associated pulmonary arterial hypertension. However, these investigators were not able to study LV tissue. Previous experiments using

rat models of heart failure have yielded apparently conflicting results. Belin et al. (12) demonstrated that heart failure reduced maximum force in myocytes from both ventricles. Conversely, Perreault et al. (13) showed that heart failure increased maximum force in RV tissue but had no effect on LV samples. Belin et al. (12) found that heart failure reduced  $\text{Ca}^{2+}$  sensitivity in the LV without affecting the RV, whereas Perreault et al. (13) observed no change in LV samples but did observe an increase in the sensitivity of RV tissue.

This paper extends these studies to the human myocardium. The data showed that heart failure reduced the maximum force and power generated by multicellular preparations from both ventricles without changing contractile kinetics.  $\text{Ca}^{2+}$  sensitivity was increased in failing myocardium from both ventricles, but the effect was greater for RV samples than that for LV tissue. Biochemical assays suggested that the sensitivity effects might reflect ventricle-specific modulation of the phosphorylation of cardiac troponin I (TnI).

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the JACC: Basic to Translational Science [author instructions page](#).

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**TABLE 1** Summary of Clinical Characteristics and Medication Use for Patients and Organ Donors

	Heart Transplant	Organ Donor	p Value
Age (yrs)	48.0 ± 17.3	41.7 ± 15.7	0.494
Male	58.3	60.0	1.000
Ejection fraction (%)	24.3 ± 10.8	47.6 ± 15.6	0.006
Percentage of patients/donors taking medication			
β-blocker	83.3	0.0	0.008
ACE inhibitor	83.3	0.0	0.008
Statin	66.7	25.0	0.262
Aldosterone antagonist	50.0	0.0	0.234
Inotrope	83.3	0.0	0.008
Digitalis	8.3	0.0	1.000
Vasopressor	0.0	50.0	0.050
Aspirin	75	25.0	0.118

Values are mean ± SD or %. p Values for age and ejection fraction were calculated using unpaired Student's *t*-tests. All other p-values were calculated using Fisher's exact tests. Data values that were unknown for a patient or organ donor were excluded from the analysis.

ACE = angiotensin-converting enzyme.

## METHODS

**HUMAN SAMPLES.** Myocardial samples were acquired at the University of Kentucky using a published protocol (14). Briefly, hearts from 12 patients who underwent cardiac transplantation (Supplemental Table S1) and from 5 organ donors who did not have heart failure (Supplemental Table S2) were handed to a researcher in the operating room, who then dissected samples from through-wall sections of the distal region of the LV and RV. LV samples were further dissected transmurally into sub-epicardial, mid-myocardial, and sub-endocardial specimens. RV samples were not separated transmurally because the RV wall is typically much thinner. Specimens were snap frozen in liquid nitrogen within approximately 30 min and stored in the vapor phase of liquid nitrogen until use. The University of Kentucky Institutional Review Board approved all procedures, and subjects or their legally authorized representatives gave informed consent.

Previous data from the laboratory (15) showed that heart failure had a greater impact on the contractile properties of the middle transmural region of the LV than on samples from the sub-epicardium or sub-endocardium. This study compared samples from the RV and the LV mid-myocardium to optimize the probability of detecting important effects.

Table 1 summarizes clinical characteristics and the use of medications for the patients and organ donors.

**PREPARATIONS AND EXPERIMENTAL SETUP.** Multi-cellular preparations were obtained by mechanical disruption of approximately 100 mg of tissue followed by chemical permeabilization (1% v/v Triton detergent). Preparations (860 ± 248 μm) from 17 patients (5 nonfailing and 12 failing hearts) were attached between a force transducer (resonant frequency, 600 Hz; Model 403, Aurora Scientific, Aurora, Ontario, Canada) and a motor (step time 0.6 ms; model 312B, Aurora Scientific) and stretched to a sarcomere length of 2.24 ± 0.01 μm in pCa (= -log<sub>10</sub>[Ca<sup>2+</sup>]) 9.0 solution. The cross-sectional area (4.05 ± 2.08 × 10<sup>-8</sup> m<sup>2</sup>) was estimated from the width of the preparation, assuming a circular profile. Measurements were performed at 15 °C using SLControl software (16).

**FORCE AND Ca<sup>2+</sup> SENSITIVITY MEASUREMENTS.** As described by Haynes et al. (15), preparations were initially activated in a solution with a saturating Ca<sup>2+</sup> concentration of pCa 4.5. Maximum force was defined as the steady-state force measured during this trial. Subsequent trials measured force in solutions with higher pCa values. Force values were corrected for potential preparation-dependent run down using reference contractions measured in pCa 9.0 and pCa 4.5 solutions throughout the protocol.

Tension pCa curves were calculated by fitting a function of the form:

$$F = F_{pas} + F_{act} \frac{[Ca^{2+}]^{n_H}}{[Ca^{2+}]^{n_H} + [Ca_{50}^{2+}]^{n_H}} \quad (\text{Eq. 1})$$

to the data, where  $F_{pas}$  is the passive force,  $F_{act}$  is maximum Ca<sup>2+</sup>-activated force,  $n_H$  is the Hill coefficient, and is the free Ca<sup>2+</sup> concentration needed to develop half-maximum Ca<sup>2+</sup>-dependent force. Thirty-one of the 98 preparations studied were discarded from the analysis because the force measured in pCa 4.5 solution dropped by >30% during the protocol (n = 19), or the r<sup>2</sup> value for the fit to the Hill curve was <0.95 (n = 12).

**POWER MEASUREMENTS.** Myocardial power output was measured by allowing each preparation to shorten against pre-set loads for 80 ms in pCa 4.5 solution. Shortening velocity was calculated as the rate of change of fiber length during the final 50 ms of each force clamp. A hyperbolic equation of the form (F+a)(V+b) = (F<sub>max</sub>+a)b was then fitted to the dataset, where F is force, F<sub>max</sub> is the isometric force, and a and b are constants. V<sub>max</sub>, the preparation's maximum shortening velocity, was estimated by extrapolating the equation to zero force.

Because power is the product of force and velocity, power-force curves were calculated by fitting the following function:

$$P = F b \left( \frac{F_{\max} + a}{F + a} - 1 \right) \quad (\text{Eq. 2})$$

to the experimental data. Maximum power,  $P_{\max}$ , was calculated as the greatest value of Equation 2. Data from the 43 preparations, in which the  $r^2$  value for the power-force fit was  $<0.9$ , were analyzed.

**BIOCHEMICAL ASSAYS.** The phosphorylation statuses of TnI, myosin binding protein-C (MyBP-C), and myosin regulatory light chain (RLC) were analyzed using gel electrophoresis as described by Haynes et al. (15). The samples used in these gels were saved from the homogenate after the mechanical disruption and chemical permeabilization procedures were used to prepare samples for contractile assays. Phosphorylated proteins were assessed using Pro-Q Diamond (Invitrogen, Carlsbad, California). Total protein was measured using SYPRO Ruby (Invitrogen). Data from multiple gels were collated and normalized to values from a single reference control that was included in every experiment.

TnI Ser23/24 specific phosphorylation was determined by Western blot as described by Nixon et al. (17) and Salhi et al. (18) using the following antibodies: rabbit anti-phosphorylated TnI Ser-23/24 (Cell Signaling Technology, Inc., Danvers, Massachusetts), anti-rabbit Cy5-labeled secondary antibody (Jackson ImmunoResearch Laboratories, Inc, West Grove, Pennsylvania), mouse anti-cardiac TnI antibody (clone 5; Fitzgerald, Acton, Massachusetts), and anti-mouse Cy2 labeled fluorescent secondary antibody (Jackson ImmunoResearch Laboratories).

**STATISTICAL ANALYSIS.** Data were analyzed in SAS version 9.4 (SAS Institute, Inc., Cary, North Carolina) using linear mixed effects models that incorporated 2 main effects (heart failure status and ventricular region) and their interaction. Testing for the interaction determined whether the effect of 1 independent variable depended on the other. An example of an interaction is a value that decreases with heart failure in samples from the LV but increases with heart failure in samples from the RV, so that the effect of heart failure depends on the ventricle that is being studied.

As previously described (15), the linear mixed model accounted for the hierarchical nested structure of the data (values obtained from multiple preparations from different regions of the same hearts) and had greater statistical power than a standard 2-way analysis of variance test for this type of experimental design. Post hoc analyses were performed using

Tukey-Kramer corrections that took into account the number of hearts and the number of samples from each heart. The  $p$  values  $<0.05$  were considered significant. Data are reported as mean  $\pm$  SEM, unless otherwise specified. Data could be clinically significant although statistical significance was not achieved, especially with smaller sample sizes.

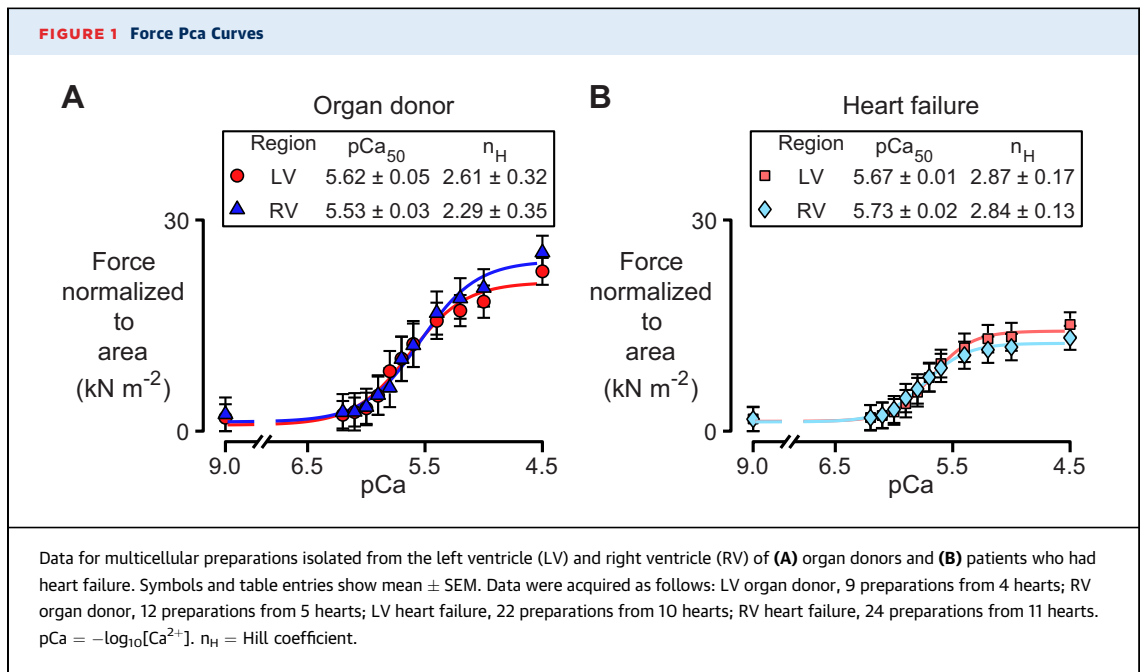
## RESULTS

**HEART FAILURE REDUCES  $\text{Ca}^{2+}$ -ACTIVATED FORCE IN MULTICELLULAR PREPARATIONS FROM BOTH VENTRICLES.** Figure 1 and Supplemental Figure 1 show force normalized to the cross-sectional area as a function of the activating  $\text{Ca}^{2+}$  concentration (pCa) for multicellular preparations from the LVs and RVs of organ donors and patients with heart failure. The solid lines are the best-fits of Equation 1 to the mean data values measured at each  $\text{Ca}^{2+}$  concentration for each type of preparation.

Figure 2 shows passive forces ( $F_{\text{pas}}$ ) and  $\text{Ca}^{2+}$ -activated forces ( $F_{\text{act}}$ ) calculated by fitting Equation 1 to the data for each experimental preparation. The statistical analysis showed that  $F_{\text{pas}}$  did not depend on heart failure status or ventricular region. In contrast,  $F_{\text{act}}$  was reduced by  $>30\%$  in samples from failing hearts ( $p < 0.001$ ). Similar reductions were observed in the samples from the LVs and RVs (i.e., there was no effect of cardiac region).

**HEART FAILURE HAS A GREATER EFFECT ON THE  $\text{Ca}^{2+}$  SENSITIVITY OF THE RV.** Figure 3 shows the  $\text{Ca}^{2+}$  sensitivity ( $\text{pCa}_{50}$ ) and the cooperativity ( $n_H$ ) calculated for each multicellular preparation. The  $\text{pCa}_{50}$  values were higher for the failing samples than that for preparations from the organ donors ( $p = 0.005$ ) but there was a statistical interaction between disease status and cardiac region ( $p = 0.029$ ). The interaction meant that the increase in calcium sensitivity induced by heart failure was statistically greater in samples from the RV than in samples from the LV. In addition, the post hoc tests showed that in failing hearts, samples from the RV were more sensitive to  $\text{Ca}^{2+}$  than the samples from the LV ( $p = 0.039$ ). No statistically significant effects were observed for the  $n_H$  values.

**HEART FAILURE REDUCES THE POWER OUTPUT OF MYOCARDIUM FROM BOTH VENTRICLES.** Power quantified the rate at which the myocardial preparations performed mechanical work and was calculated as the product of force and velocity. Figure 4A to 4C illustrates the experimental technique that was used to calculate the maximum shortening velocity ( $V_{\max}$ ) and the maximum power output ( $P_{\max}$ ) of a single representative preparation. Data from multiple



preparations are shown in **Figure 4D and 4E**. As in the case of  $F_{\text{act}}$  (**Figure 2B**),  $P_{\text{max}}$  was reduced by approximately 30% in samples from both the LVs and RVs of failing hearts. In contrast, no statistically significant effects were observed for  $V_{\text{max}}$ .

$V_{\text{max}}$  is believed to depend on cross bridge cycling kinetics (19), which can be estimated by measuring the rate of force recovery ( $k_{\text{tr}}$ ) after a quick shortening and/or re-stretch perturbation. **Figure 5A** shows representative records measured for a LV preparation isolated from an organ donor. Consistent with previous results from human myocardium (15), force overshoot the steady-state isometric value during the recovery phase (20,21). The  $k_{\text{tr}}$  values increased approximately linearly with steady-state isometric force in every experimental group (**Figure 5B**). The rates measured in maximally activating pCa 4.5 solution did not depend on heart failure status or ventricular region.

Data for  $k_{\text{tr}}$  values measured at sub-maximal activation levels ranging from pCa 6.0 to pCa 5.2 are shown in **Supplemental Figure S2**. The kinetics were faster in the samples from patients with heart failure at some activation levels, with the difference being more pronounced in the RV. The physiological significance of these results is not yet clear.

**HEART FAILURE MODULATES THE PHOSPHORYLATION OF MYOFILAMENT PROTEINS.** **Figure 6A** shows

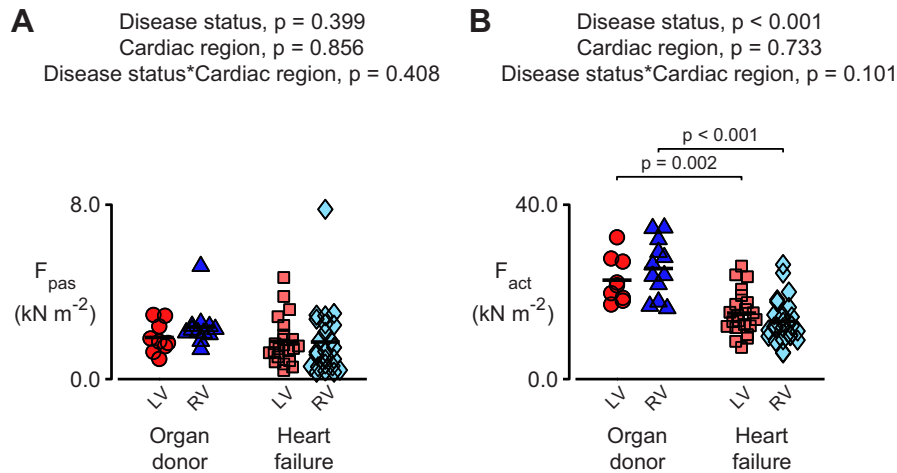
images from a representative gel that that was stained with Pro-Q Diamond to show phosphorylated proteins and then SYPRO Ruby to show total protein. **Figure 6B to 6D** shows data obtained from similar images quantifying the phosphorylation levels of TnI, MyBP-C, and RLC. Both TnI and RLC exhibited a statistical interaction ( $p = 0.037$ ) between heart failure status and the cardiac region with the phosphorylation levels trending higher in RV samples from organ donors and lower in RV samples from patients with heart failure.

The data for MyBP-C showed a different pattern. No interaction was observed, but there was a main effect of heart failure status ( $p = 0.010$ ). This implied that the phosphorylation of MyBP-C was lower in samples from both ventricles in failing hearts.

**Supplemental Figures S3 to S7** show the relative phosphorylation of TnI, MyBP-C, and RLC plotted against pCa<sub>50</sub>, n<sub>H</sub>,  $F_{\text{pas}}$ ,  $F_{\text{act}}$ , and the maximum value of  $k_{\text{tr}}$ . Deming regression (which takes into account experimental uncertainty in both the x and y coordinates) showed that pCa<sub>50</sub> was negatively correlated with the relative phosphorylation of both MyBP-C and RLC, and that  $F_{\text{act}}$  was positively correlated with the relative phosphorylation of MyBP-C.

Pro-Q Diamond is commonly used to study sarcomeric proteins (22–24) but the stain is not completely specific to phosphorylated proteins and

**FIGURE 2 Heart Failure Reduces  $Ca^{2+}$ -Activated Force in Multicellular Preparations From Both Ventricles**

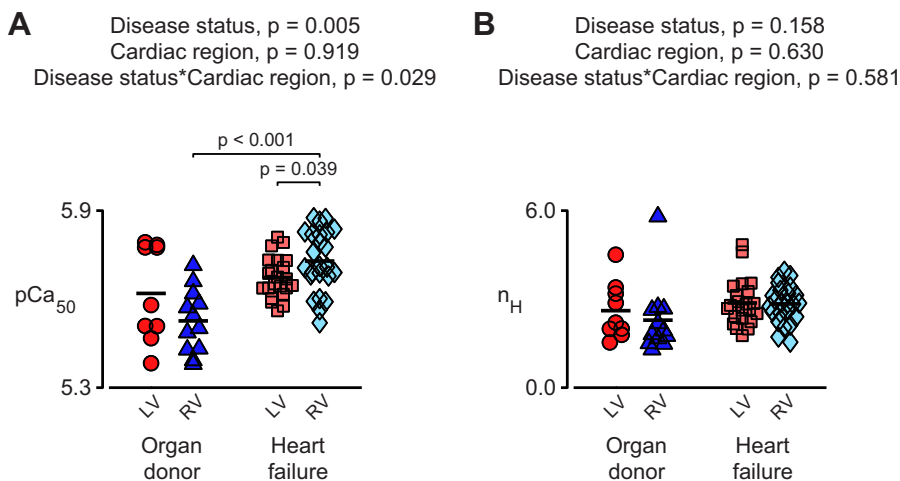


(A) Passive ( $F_{pas}$ ) and (B) active ( $F_{act}$ ) force values deduced by fitting Equation 1 to data from the individual preparations summarized in Figure 1. The total force in pCa 4.5 solution ( $F$ ) is equal to  $F_{pas} + F_{act}$ . The mean  $r^2$  value for these fits was  $0.979 \pm 0.002$ . In this, and all similar Figures, data were analyzed using a linear mixed model that tested for 2 main effects (heart failure status and ventricular region) and their statistical interaction. Post hoc tests were performed using Tukey-Kramer corrections that took into account the number of hearts as well as the number of samples from each heart.  $Ca^{2+}$  = calcium; other abbreviations as in Figure 1.

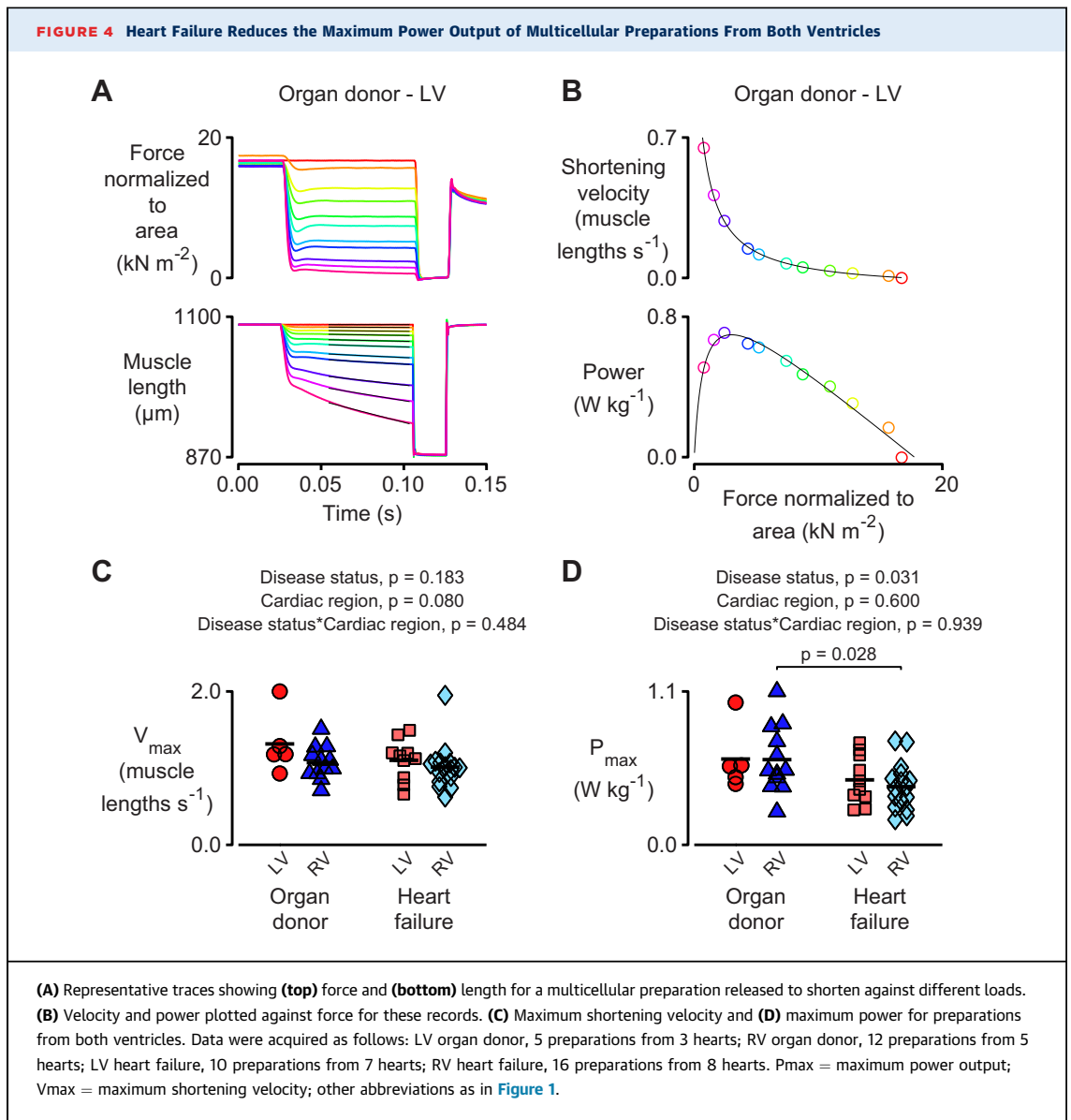
does not provide information about post-translational modifications to identified residues. Western blotting (Figure 7) was performed to address these potential limitations. As for the experiments

performed using Pro-Q Diamond (Figure 6B), the phosphorylation status of TnI 23/24 exhibited a statistical interaction ( $p = 0.039$ ) between heart failure status and cardiac region, which indicated that the

**FIGURE 3 Heart Failure Has a Greater Effect on the  $Ca^{2+}$  Sensitivity of Myocardium From the RV**



Values of (A)  $Ca^{2+}$  sensitivity ( $pCa_{50}$ ) and (B) cooperativity ( $n_H$ ) deduced by fitting Equation 1 to data from the individual preparations summarized in Figure 1. Abbreviations as in Figure 1.



effect of heart failure on TnI phosphorylation depends on the ventricle.

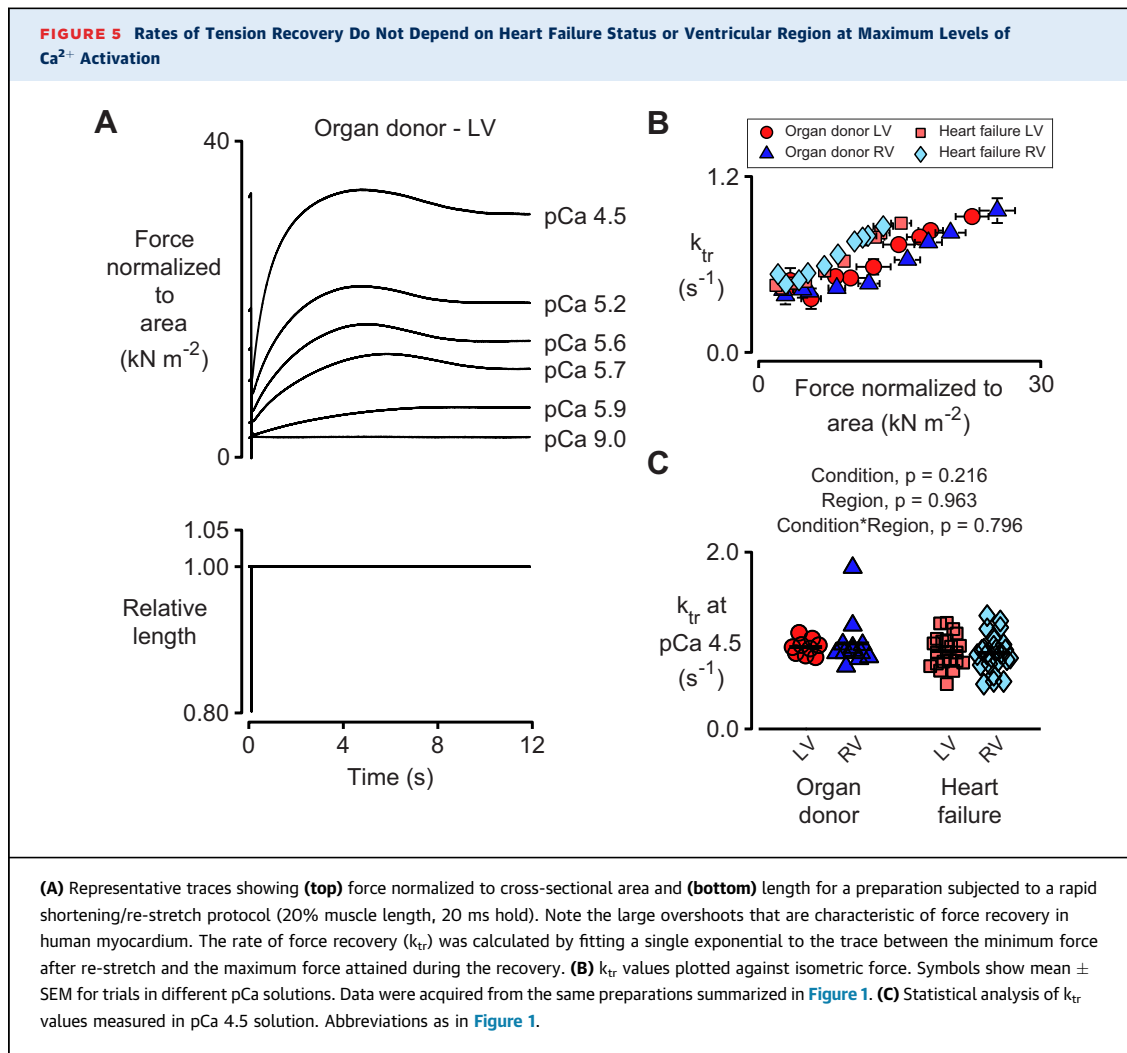
## DISCUSSION

To our knowledge, this is the first study that systematically investigated how heart failure in humans affects the contractile properties of chemically permeabilized myocardial samples from LVs and RVs. The main findings are that heart failure: 1) reduces isometric force and maximum power output in preparations from both ventricles; and 2) increases the calcium sensitivity ( $p\text{Ca}_{50}$ ) of RV tissue more than that of LV tissue. Biochemical data suggested that the changes in calcium sensitivity

might reflect ventricle-specific modulation of TnI phosphorylation.

**REDUCED ISOMETRIC FORCE AND POWER IN FAILING TISSUE MAY REFLECT VENTRICULAR REMODELING.** Figures 1 and 2B show that heart failure reduced maximum  $\text{Ca}^{2+}$ -activated force by at least 30% in multicellular preparations from both ventricles. Maximum power was also reduced by a similar amount in both ventricles (Figure 4D). In principle, these effects could reflect a change in the fractional volume occupied by sarcomeres and/or alterations to cross-bridge kinetics that reduce the force-generating capacity of myosin heads in failing ventricles.



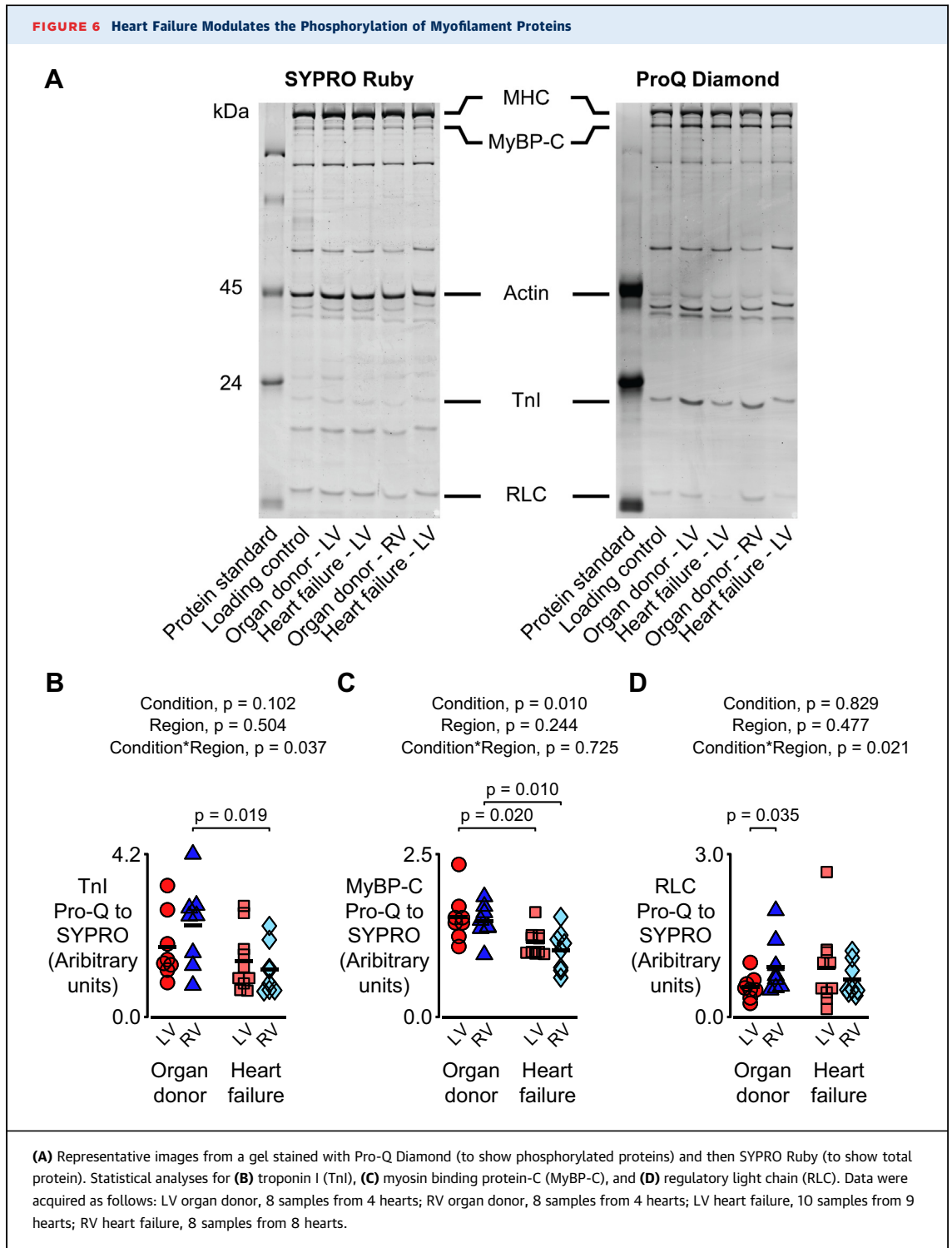


Figures 4E, 5B, and 5C show that neither  $V_{max}$  nor  $k_{tr}$  were affected by heart failure status or cardiac region at maximal levels of  $Ca^{2+}$  activation. Both parameters are indicative of cross-bridge kinetics and would be expected to change if the attachment and/or detachment rates of myosin were altered. Thus, the present data did not support a kinetic basis for the reduction in force. Instead, the mostly likely explanation for the approximate 30% reduction in force and power in failing samples was a decrease in the fractional volume occupied by contractile machinery.

This conclusion was consistent with a previous study by Haynes et al. (15) that compared the contractile properties of multicellular preparations from the sub-endocardial, mid-myocardial, and sub-epicardial regions of the LV free wall of organ donors and patients with heart failure. Histological data

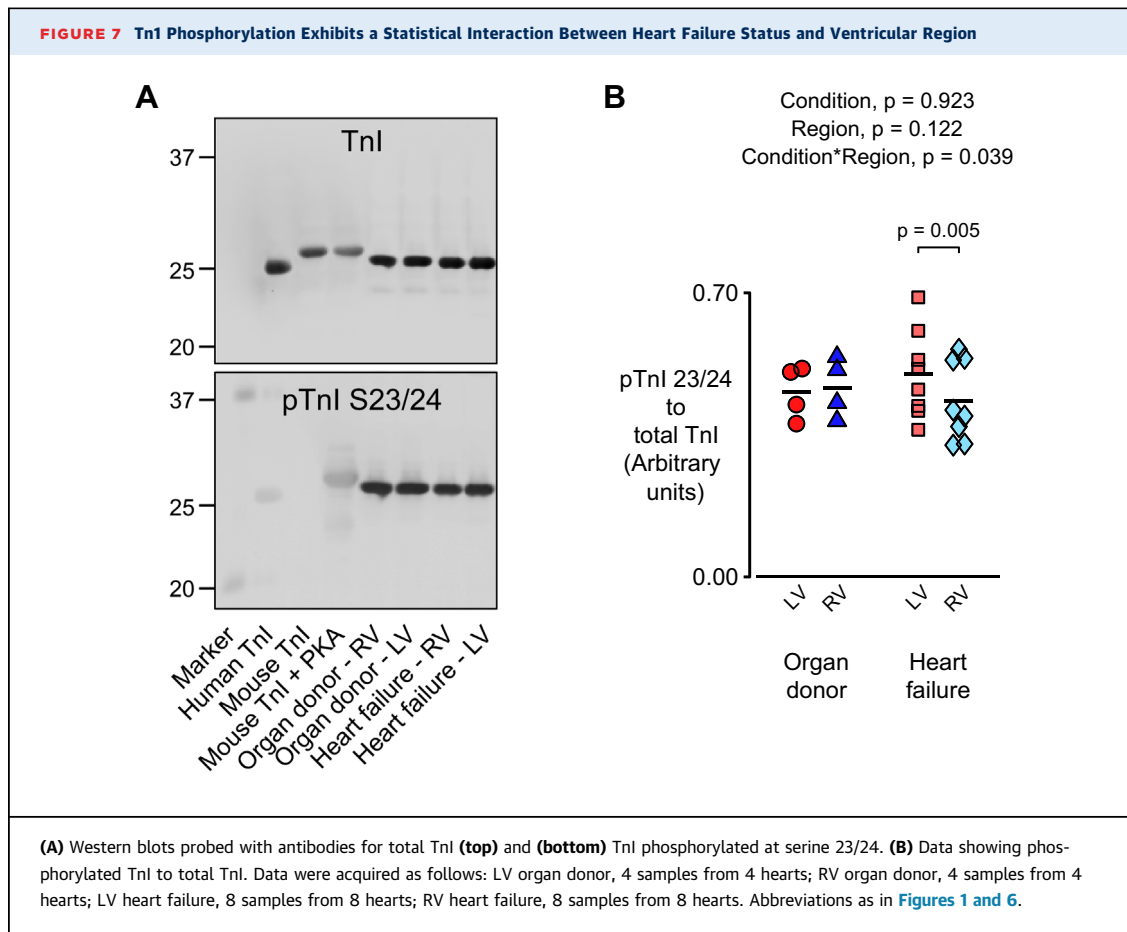
(staining with picrosirius red) included in that study showed that the collagen to tissue ratio fell from approximately 27% in mid-myocardial tissue from patients with heart failure to approximately 15% in corresponding samples from organ donors. Unfortunately, histological data from RV samples were not available for the present study. However, if the preparations had exhibited similar trends to those reported by Haynes et al., the increased fibrosis in the failing samples could account for approximately 50% of the reductions shown in Figures 1, 2B, and 4E. Other potential mechanisms that could reduce maximum contractile force in failing myocardium include disarray of myocytes and/or myofibrils (25) and changes in the fractional volume occupied by mitochondria (26). These possibilities could be tested in future work: 1) measuring the contractile function of single myocytes that do not have extracellular





collagen (27); and 2) analyzing tissue samples with immunohistochemistry and electron microscopy (28). **INTERVENTRICULAR DIFFERENCES IN  $Ca^{2+}$  SENSITIVITY ARE ASSOCIATED WITH PHOSPHORYLATION OF TnI.** Because myocardium is only

transiently activated during each heart beat, its sensitivity to  $Ca^{2+}$  (quantified as a  $pCa_{50}$  value) is critically important for function. For example, all other things being equal, myocytes with higher  $pCa_{50}$  values will develop more contractile force in response



to a given intracellular  $Ca^{2+}$  transient than myocytes with low  $Ca^{2+}$  sensitivities. [Figure 3A](#) confirmed previous work (7,29) that showed that myocardium from failing hearts is more sensitive to  $Ca^{2+}$  than tissue from organ donors ( $p < 0.005$ ). One possibility is that the failing heart enhances  $Ca^{2+}$  sensitivity to try and compensate for its reduced force-generating capacity. [Supplemental Figure S1](#) superposes force pCa curves measured with samples from organ donors and patients with heart failure. The curves nearly overlap at the sub-maximal activation levels where the heart normally operates.

The present data also revealed a new statistical interaction between heart failure status and cardiac ventricles. More specifically, heart failure increased the  $Ca^{2+}$  sensitivity of RV myocardium more than that of LV tissue. [Figures 6 and 7](#) show data that provide insights into the molecular mechanisms that drive these effects. Two independent measures of TnI phosphorylation (Pro-Q Diamond staining [[Figure 6B](#)] and Western blotting with an antibody specific to Ser-

23/24 phosphorylation [[Figure 7B](#)]) showed a statistical interaction between disease status and the cardiac ventricle. It is well known that phosphorylation of TnI (most notably at serine 23/24) reduces  $Ca^{2+}$  sensitivity (4,6,10,30-35); therefore, these statistical interactions are consistent with post-translational modifications to TnI contributing to the ventricle-specific effects.

Phosphorylation of RLC also exhibited a significant interaction ([Figure 6C](#)) with higher phosphorylation levels again being associated with reduced  $Ca^{2+}$  sensitivity. This effect is also evident in [Supplemental Figure S3C](#), which shows the relative phosphorylation of RLC plotted against pCa50. This was a more complex relationship because increased phosphorylation of RLC was shown to enhance  $Ca^{2+}$  sensitivity (36,37). One possibility was that the phosphorylation of RLC changes during human heart failure partially compensated for the post-translational modifications to TnI and mitigated diastolic dysfunction. A similar conclusion was

reached by van der Velden et al. (5), who observed changes in the phosphorylation of TnI and RLC in human heart failure that were similar to those shown here.

**Figure 6D** shows that MyBP-C was dephosphorylated in failing samples from both ventricles. This modification increased myofilament  $\text{Ca}^{2+}$  sensitivity (38-41) and could therefore be driving the global increase in  $\text{Ca}^{2+}$  sensitivity observed in the failing samples (**Figure 3A** and **Supplemental Figure S3B**).

The physiological basis of the interventricular difference in  $\text{Ca}^{2+}$ -sensitivity remains unclear. One possibility is that the ventricles are tuned to different mechanical loads. Another possibility is that they respond differently to  $\beta$ -adrenergic stimulation. Recent work that used Förster Resonance Energy Transfer (FRET) biosensors suggested that maximal inotropy required compartmentalized signaling responses (42). In addition, Surdo et al. (43) reported an enrichment in the expression of genes involved in adrenergic signaling in cells from the RV of healthy human donors in comparison to cells from the LV using single cell RNA-Seq. Building on this theme, Molina et al. (44) showed that in healthy dogs, the RV was more sensitive to  $\beta$ -adrenergic stimulation and exhibited increased activity of protein kinase A (PKA). This enzyme phosphorylates TnI (45) so differential responses to  $\beta$ -adrenergic stimulation could contribute to the effects shown in **Figures 6B and 7B**. Interestingly, Hsu et al. (11) showed that treatment with PKA equalized the  $\text{Ca}^{2+}$  sensitivity of RV myocardium from organ donors and in patients with systemic sclerosis-associated pulmonary hypertension, a finding that further reinforced the importance of PKA signaling in disease. However, the present data suggested that PKA was unlikely to be the only mechanism that contributed to interventricular differences because enhanced activity of the kinase in the RV should also have increased the phosphorylation of MyBP-C. This was not observed (**Figure 6C**).

**STUDY LIMITATIONS.** Although experiments using human biospecimens sometimes have greater translational significance than studies that only use animals, they also have limitations. For example, it was exceedingly difficult to control for all of the potential confounders (including age, sex, genetic background, and comorbidities) that could have influenced the data (46). Some clinical data were also unavailable. This reduced the statistical power of tests that investigated potential relationships between cellular

and organ-level function and was a particular concern for parameters related to the RV (**Supplemental Table S3**).

Sample processing was another concern. All of the hearts used were procured by the same personnel and cooled in ice cold saline within moments of being removed from the patient or donor (14). Nevertheless, the mean time from procurement to the sample being frozen in liquid nitrogen was approximately 30 min. As demonstrated by Walker et al. (47), this is clearly enough time to alter the post-translational status of sarcomeric proteins, which sometimes changes on a beat-to-beat basis (48).

Another issue was that the patients and organ donors were treated with drugs that were intended to alter contractile properties. Catecholamine levels also rose during tissue procurement (46), which might have added additional complications. Again, it was difficult to know how to overcome these limitations in a translational research setting without having a negative impact on patient care.

Finally, although this study investigated the phosphorylation of several key myofilament proteins, the experiments could have missed other critical modifications. This could be addressed in future work using an unbiased approach (e.g., phosphoproteomics).

## CONCLUSIONS

This study presented 3 important results. First, heart failure in humans depressed maximum contractile force and maximum power by similar amounts in myocardium from both ventricles. Second, human heart failure increased the  $\text{Ca}^{2+}$  sensitivity of RV myocardium more than the  $\text{Ca}^{2+}$  sensitivity of LV myocardium. Third, the differences in  $\text{Ca}^{2+}$  sensitivity were likely to involve interventricular differences in post-translational modifications to sarcomeric proteins, including TnI.

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## PERSPECTIVES

**COMPETENCY IN MEDICAL KNOWLEDGE:** This study showed that heart failure in humans reduced the force and power generated by myocardium from the LVs and RVs. The kinetics of the contraction (assessed as shortening velocity and the rate of force generation) were not markedly affected, so the contractile dysfunction was most likely to reflect remodeling (e.g., excess fibrosis and/or cellular disarray). In addition, heart failure increased the Ca<sup>2+</sup> sensitivity of contraction, with the effect being greater in RV samples. These changes were

associated with altered phosphorylation of TnI and MYP-C, which indicated that adrenergic signaling might induce different consequences in the LVs and RVs.

**TRANSLATIONAL OUTLOOK:** Although technically challenging, more studies are required to determine how the Ca<sup>2+</sup> sensitivity of contraction varies in different regions of failing hearts. Abnormalities in these patterns might contribute to regional hypokinesia and/or hyperkinesia.

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- KEY WORDS**  $Ca^{2+}$  sensitivity, heart failure, human myocardium, myofilament proteins, ventricular function
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- APPENDIX** For supplemental figures and tables, please see the online version of this paper.