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Increased cross-bridge recruitment contributes to transient increase in force generation beyond maximal capacity in human myocardium

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Abstract

Cross-bridge attachment allows force generation to occur, and rate of tension redevelopment (k_{tr}) is a commonly used index of cross-bridge cycling rate. Tension overshoots have been observed briefly after a slack-restretch k_{tr} maneuver in various species of animal models and humans. In this study, we set out to determine the properties of these overshoots and their possible underlying mechanism. Utilizing human cardiac trabeculae, we have found that tension overshoots are temperature-dependent and that they do not occur at resting states. In addition, we have found that myosin cross-bridge cycle is vital to these overshoots as inhibition of the cycle results in the blunting of the overshoots and the magnitude of the overshoots are dependent on the level of myofilament activation. Lastly, we show that the number of cross-bridges transiently increase during tension overshoots. These findings lead us to conclude that tension overshoots are likely due to a transient enhancement of the recruitment of myosin heads into the cross-bridge cycling, regulated by the myocardium, and with potential physiological significance in determining cardiac output.

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Disclosures

None

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Keywords

cross-bridge cycling rate; cardiac contraction; contractile kinetics; cardiac regulation

Introduction

Cardiac stroke volume is determined by several factors, most notably the amount of pressure the heart can generate during each cardiac cycle (1). One of the most important factors determining this pressure is myocardial contractility (1). Increasing calcium concentration results in increased thin filament activation and enhanced cross-bridge recruitment which ultimately leads to increased tension generation (2). This relationship reaches a maximal steady state where a further increase in calcium concentration has no effect on tension. At this point, the number of attached cross-bridges is at a relative steady state, and cardiomyocytes have reached their maximal tension development capacity (3).

Rate of tension redevelopment (k_{tr}), a kinetic parameter commonly used as an index of cross-bridge cycling rate, utilizes a rapid slack-restretch maneuver (4). The tension that cardiomyocytes generate following the rapid slack-restretch maneuver can briefly exceed the tension developed during maximal myofilament activation preceding the maneuver. Such tension “overshoots” have been reported in intact human cardiac trabeculae (5, 6), permeabilized human cardiac preparations (7), permeabilized mouse cardiac preparations (8), intact rat cardiac trabeculae, and permeabilized rat cardiomyocytes upon Protein Kinase A (PKA) treatment (9) and unpublished experiments in permeabilized porcine, canine, and rat cardiac preparations (10). Our laboratory has been working with intact human ventricular trabeculae and have observed tension overshoots in vast majority of the non-failing as well as failing trabeculae with similar amplitudes of overshoot.

It is incompletely understood whether tension overshoots are simply a passive experimental artifact or they occur due to an active intrinsic mechanism of the myocardium itself. We hypothesized that tension overshoots are an active physiological intrinsic property. In the present study, we show that tension overshoots are impacted by factors such as temperature, myofilament activation level, electrical activation of myocytes, and myosin activity. Finally, we show that a transient increase in cross-bridge recruitment is the underlying mechanism for transient tension overshoots suggesting an additional mechanism whereby the myocardium can enhance its force generation capacity.

Materials and Methods

Human Heart Procurement

All experiments were performed with approval of the Institutional Review Board (IRB) at The Ohio State University Wexner Medical Center and The Declaration of Helsinki. Donor non-failing human hearts ($n = 14$) were acquired in collaboration with the LifeLine of Ohio Organ Procurement. Failing hearts ($n = 13$) were acquired with informed consent from patients undergoing cardiac transplantation at The Ohio State University Wexner Medical Center. Table 1 provides characteristics of the human hearts which include a subset of hearts

that have been reported in our previous studies (5, 6). Following explantation, human hearts were immediately flushed with a cold cardioplegic solution containing (in mM): 110 NaCl, 16 KCl, 16 MgCl₂, 10 NaHCO₃, and 0.5 CaCl₂ and transported promptly to the laboratory in this solution.

Isolation of Intact Cardiac Trabeculae

The right ventricles were dissected from the remainder of the hearts and transferred to a cold (0–4 °C) modified Krebs-Henseleit solution (K-H) containing (in mM): 137 NaCl, 5 KCl, 20 NaHCO₃, 1.2 NaH₂PO₄, 1.2 MgSO₄, 10 dextrose, 0.25 CaCl₂, and 20 BDM (2,3-butanedione monoxime). The solution was in equilibrium with 95% O₂-5% CO₂ resulting in pH of 7.4. Right ventricular cardiac trabeculae were isolated and stored in K-H solution at 0–4 °C until the time of the experiment.

Trabeculae were placed in a custom-made setup to a force transducer and a linear motor via two pairs of hooks on each side as previously described (5, 11). Preparations were continuously superfused with a modified K-H solution without BDM at 37 °C. Trabeculae were stimulated to contract at 1 Hz and the CaCl₂ concentration was incrementally and gradually increased to 2 mM. Afterwards, trabeculae were stretched to optimal length, close to *in vivo* end-diastolic sarcomere length of 2.2 μm (12), as previously described. The K⁺ contractures and k_{tr} experimental protocol were performed as previously detailed (3, 5). All experiments, unless otherwise specified, were performed at optimal length, 1 Hz, and 37 °C.

Temperature Dependency of Tension Overshoots

Four trabeculae (n = 4 donor hearts, n = 1 failing heart) were used to assess the amplitude and the decline phase of the tension overshoots at different temperatures. k_{tr} experiments were performed at optimal length, 1 Hz, and temperatures of 37 °C, 32 °C, and 27 °C in each of the muscles.

Tension Overshoots in Resting Conditions

Four trabeculae (n = 2 donor hearts and n = 2 failing hearts) were used to determine whether tension overshoots occur in non-contracting trabeculae. The electrical stimulation was paused and 3–4 successive k_{tr} maneuvers were performed without inducing a K⁺ contracture.

Two additional trabeculae were initially stabilized at L_{opt} and 1 Hz for 15 minutes. K⁺ contractures were induced and the k_{tr} maneuvers were performed at maximal contracture tension. Afterwards, the trabecula was re-stabilized at L_{opt} and 1 Hz for 15 minutes. The electrical stimulation was paused and the trabeculae were stretched until the resting tension was approximately equal to the total tension of the preceding K⁺ contracture. A k_{tr} maneuver was performed at this high resting tension level. The trabecular length was returned to L_{opt} and re-stabilized at 1 Hz for 15 minutes. Subsequently, K⁺ contracture and k_{tr} , at maximal contracture tension, were performed.

Effects of BDM on Tension Overshoots

All experiments were performed at L_{opt} , 1 Hz, and 37 °C. Trabeculae ($n = 2$ donor hearts, $n = 2$ failing hearts) were initially stabilized at these conditions for 15 minutes. K^+ contractures and k_{tr} experiments were performed without BDM, a myosin inhibitor. Trabeculae were then re-stabilized in the K-H solution without BDM for 15 minutes. The high K^+ /high Ca^{2+} solution (i.e. the K^+ contracture solution) was changed to one which contained 10 mM of pre-dissolved BDM, and the K^+ contracture and k_{tr} experiments were performed. Trabeculae were re-stabilized in K-H solution without BDM for 15 minutes while the high K^+ /high Ca^{2+} solution was changed to another solution with 20 mM of BDM. The K^+ contracture and k_{tr} experiments were performed in the presence of 20 mM BDM. Subsequently, trabeculae and the setup were thoroughly superfused with K-H and high K^+ /high Ca^{2+} solutions that did not contain BDM in order to washout any remaining BDM. Trabeculae were re-stabilized for 15 minutes, and the K^+ contracture and k_{tr} experiments were again performed in the absence of BDM. Finally, trabeculae were re-stabilized in the K-H solution for 15 minutes. The high K^+ /high Ca^{2+} solution was changed to one that contained 50 mM of BDM, and the K^+ contracture and k_{tr} experiments were performed. The overall order of the experiments was Control $\rightarrow$ 10 mM BDM $\rightarrow$ 20 mM BDM $\rightarrow$ Washout $\rightarrow$ 50 mM BDM. In 2 trabeculae, 50 mM BDM solution was washed out to determine whether the overshoot phenomenon would return. All k_{tr} experiments were performed at the maximal tension of the K^+ contracture.

Tension Overshoots at Sub-Maximal Contracture Tensions

We analyzed all the data that were previously presented in our recent reports (5, 6) in order to assess the effects of relative K^+ contracture tension levels on the amplitude of tension overshoots. In brief, data from 29 separate experiments on non-failing and 27 separate experiment on failing human myocardium were combined. Multiple experimental conditions were included: L_{opt} (optimal length), L_{95} (95% of optimal length), L_{90} (90% of optimal length), and L_{opt} and L_{90} in the presence of 20 μ M H-89 and 7.5 nM PKC β II peptide inhibitor I.

Sinusoidal Length Perturbations

The relative number of attached cross-bridges was assessed by performing a modified sinusoidal length perturbations in the intact cardiac trabeculae ($n = 4$). For these experiments, a K^+ contracture was induced as described above. At the plateau of the contracture, trabeculae were slacked and re-stretched by 1% of the trabecular length at a frequency of 50 Hz for approximately 4 seconds. Afterwards, a k_{tr} maneuver as described above was performed and immediately the trabeculae underwent the modified sinusoidal length perturbation maneuver. As the trabeculae was stabilizing after the k_{tr} maneuver, it underwent sinusoidal length perturbation for 1 second. The amplitude of the sinusoidal length perturbations was calculated at baseline (10 consecutive oscillations) before the k_{tr} , at the peak of the tension overshoot, and baseline (10 consecutive oscillations) after the k_{tr} .

Data and Statistical Analysis

All data were collected and analyzed using custom-made programs in LabView (National Instruments). Tensions were normalized to the cross-sectional areas (mm^2) of the trabeculae. All k_{tr} tracings were fit to the equation $F = F_{\max} \cdot (1 - e^{-k_{tr}(t)}) + F_{\text{initial}}$ using Origin 7 (OriginLab Corp). Differences between tension overshoot kinetics at different temperatures were determined with Statistical analysis was performed via one-way ANOVA for repeated measures with Bonferroni post-hoc test and paired t-tests where appropriate. Statistical significance was set as $P < 0.05$ for all tests. All data is shown as mean $\pm$ S.E.M.

Results

Effects of Temperature on Tension Overshoots

A schematic presentation of the terms used for characterizing tension overshoots is provided in Figure 1. Note that the motor is stationary at its original length when the overshoot occurs. We previously showed, as expected, that decreasing temperature results in slowing of k_{tr} in human myocardium (5). Furthermore, decreasing temperature also slowed down the kinetics of the Tension Decline Phase with a Q_{10} of ~ 1.9 (range: ~ 1.7 – 2.2) (Figure 2A). There was a close relationship between k_{tr} (during the force redevelopment phase) and $t_{1/2}$ of the Tension Decline Phase (inverse of this parameter shown for clarity purposes) (Figure 2B). We also used a $\ln(1/2)/t_{1/2}$ function to calculate the decay rate and found that relaxation kinetics accelerate approximately 1.8 times and 2.2 times at 32°C and 37°C , respectively. Also, we fit k_{tr} tracings with a negative $\ln(1/2)/(t_{1/2})$ function and found that k_{tr} accelerates approximately 2 times and 2.9 times at 32°C and 37°C , respectively, which shows that force redevelopment rates and decay rates increase due to increased temperature. Temperature did not affect the amplitudes of tension overshoots suggesting that while temperature regulates kinetics potentially without affecting the average number of attached cross-bridges (Figure 2C). However, this assumes that temperature does not alter force per attached cross-bridge. Whether temperature affects the number of cross-bridges or force per cross-bridge is an important and unresolved topic in muscle physiology. At this point, we cannot conclusively determine if either theory is supported by our study, as we did not incorporate experiments to answer this particular questions into the study design.

No Tension Overshoots in Quiescent Trabeculae

In a subset of trabeculae ($n = 4$, 2 non-failing and 2 failing), we determined whether tension overshoots occur when the trabeculae are resting. In these trabeculae we initially observed tension overshoots during the K^+ contracture (Figure 3A). However, there were no tension overshoots in any of the k_{tr} maneuvers performed while the trabeculae were quiescent (Figure 3B, 3C, and 3D). The tension tracings shown in Figures 3B, 3C and 3D do not show tension overshoots because tension overshoots typically take ~ 150 ms to develop after the motor movement is completed, which is much slower than the time scale of the tension tracings in these figures.

We also investigated whether tension overshoots occur if the resting tension of quiescent trabecula is matched to the tension during maximal contracture ($n = 1$ donor, $n = 1$ failing). There was initially a tension overshoot when the trabecula was under maximal contracture at

L_{opt} (Figure 4A). However, there was no tension overshoot if the trabecula was quiescent even though the trabecula was stretched to match its resting tension to the total tension of the previous contracture (Figure 4B). Performing the k_{tr} maneuver during the maximal tension of the K^+ contracture at L_{opt} for a second time resulted in the reappearance of the tension overshoot (Figure 4C).

BDM Decreases Tension Overshoot Amplitudes

The effects of BDM on tension overshoots were assessed in another group of trabeculae ($n = 2$ donor hearts, $n = 2$ failing hearts). Under baseline conditions, tension overshoots (amplitude of $\sim 20.7 \pm 4.1\%$) were present in both trabeculae (Figure 5A). In the presence of 10 mM BDM, the tension developed during the K^+ contracture decreased by $\sim 55.1 \pm 3.5\%$. The tension overshoots were still present though with a lower amplitude ($\sim 15.2 \pm 6.0\%$) (Figure 5B). Further titrating the BDM concentration to 20 mM resulted in absence of tension overshoot (Figure 5C). This concentration of BDM decreased the tension generated during the K^+ contracture by $\sim 75.8 \pm 3.1\%$. Washing out BDM resulted in the reappearance of tension overshoots (amplitude of $\sim 24.6 \pm 8.7\%$) (Figure 5D). Finally, there were no tension overshoots with the introduction of 50 mM BDM, which decreased the tension developed during K^+ contracture by $\sim 88.9 \pm 3.8\%$ (Figure 5E). Washing out 50 mM BDM also resulted in the return of tension overshoots with the similar amplitudes as before adding BDM (amplitude of $\sim 19.4 \pm 1.8\%$).

Tension Overshoot Amplitudes Depend on the Relative K^+ Contracture Tension Levels

There was a relationship between relative K^+ contracture tension and the amplitude of the tension overshoots in non-failing and failing myocardium (Figure 6). In both groups, this amplitude increased as the relative K^+ contracture increased up to half maximal contracture tension (~ 0.6 and ~ 0.4 – 0.6 in non-failing and failing myocardium, respectively). Tension overshoot amplitudes then decreased as the relative K^+ contracture tensions increased from half maximal to maximal.

The Number of Cross-bridges Increase during Tension Overshoot

We measured the amplitudes of sinusoidal oscillations at pre-overshoot, tension overshoot, and post-overshoot phases to assess the number of cross-bridges in these three phases ($n = 5$, 1 non-failing and 4 failing). The amplitudes of sinusoidal oscillations, an index of the number of cross-bridges, during the tension overshoot was significantly increased by approximately 11% during tension overshoots as compared to the steady-state maximal activation (amplitude before $k_{tr} = 2.2 \pm 0.24$ mN/mm² and amplitude during overshoot = 2.44 ± 0.19 mN/mm²; $P < 0.05$) (Figure 7).

Discussion

We show that 1) tension overshoots occur consistently after slack-restretch maneuver in intact human myocardium 2) kinetics of tension overshoot are temperature-dependent, 3) activation of the cardiomyocytes is necessary for tension overshoots, 4) tension overshoots are prevented by inhibiting actin-myosin interactions, 5) the amplitude of overshoot is dependent on the relative contracture tension level, and 6) the number of cross-bridges

increase during tension overshoots. These results collectively show that tension overshoots in human k_{tr} tracings are mediated by the intrinsic properties of myocardium.

If tension overshoots are simply an artifact of the experimental protocol, it would be highly unlikely that both k_{tr} and Tension Decline Phase $t_{1/2}$ parameters would be temperature dependent. Several studies show that kinetics of cardiac contraction and cross-bridge cycle speed up with increasing temperatures (11, 13–15). Our finding of the temperature dependency of the kinetics of tension overshoot suggest that these overshoots are mediated by some cardiomyocyte process, most likely the cross-bridges. Cross-bridge involvement was further supported by the lack of tension overshoots in the k_{tr} maneuvers performed when trabeculae were resting (i.e. neither electrically stimulated nor under contracture). Under these conditions, the myofilaments should be inactive due to low intracellular calcium concentration. Conversely, tensions overshoots were observed under the K^+ contracture where the myofilaments are activated due to an increase in intracellular calcium concentration (3). This suggests that it is necessary to have activated myofilaments in order to induce these overshoots. Additionally, this also disposes of the possibility that overshoots are due to a transient increase in intracellular calcium, such as calcium entry through stretch-activated channels (16), following the slack-restretch maneuver. The resting tension of cardiac trabecula is very low as compared to the maximal K^+ contracture tension. It can be argued that overshoots are an artifact of the experimental setup and that a sufficiently high amount of tension, regardless of its source, is required to reveal these artifacts. In order to dispose of this possibility, we performed a k_{tr} maneuver in a resting trabecula while its resting tension was matched to that of the K^+ contracture tension. Despite the matched total tensions, there was no tension overshoot present.

The role of cross-bridges in tension overshoots was further investigated with the use of BDM. Increasing BDM concentration progressively decreased both developed tension during the K^+ contracture and the amplitude of the overshoots. There were no overshoots observed when the majority of actin-myosin interactions was inhibited. A final piece of information is that the magnitude of tension overshoots varies with relative contracture tension and that the number of attached cross-bridges is increased during these transient tension overshoots.

Only a few studies have investigated the properties of overshoots following a k_{tr} maneuver. Campbell K.S. suggested that cross-bridges are the underlying mechanism, while discounting the role of titin (10). Additionally, calcium affected the magnitude of tension overshoots with maximal amplitudes occurring near pCa_{50} in this study. This is very similar to our observation in intact preparations where the magnitude of tension overshoots was greatest during half maximal K^+ contracture tension levels. As argued by Campbell, a temporary increase in the number of bound cross-bridges results in these overshoots (10). We have noted that, at a given submaximal tension, the overshoot phenomenon does not occur when our cardiac trabeculae are exposed to BDM but that it does occur without BDM. From this data, we conclude that overshoot phenomenon requires actively cycling cross-bridges and BDM would inhibit myosin and prevent actin-myosin interaction. The cross-bridge requirement for occurrence of tension overshoots in our study is in accordance with this model. The dependency of the amplitude of the overshoots on the relative tension levels

might also implicate cross-bridge feedback on the thin filament as a potential mechanism. Only a portion of the thin filament regulatory units are presumably activated by calcium during the half maximal point of the K^+ contracture. Therefore, during the force redevelopment, as more and more myosin heads bind to the actin, they can spread the activation to the neighboring thin filament regulatory units (17, 18). This will potentially allow the myosin heads in the vicinity to be transiently recruited into the cross-bridge cycle resulting in tension overshoots. However, during higher levels of activation, the majority of the thin filament regulatory units will be already activated due to the higher calcium concentrations. This will limit the importance of cross-bridge feedback mechanism in recruiting the neighboring myosin heads into the cross-bridge cycle. This will explain why the amplitude of tension overshoots decreases as relative K^+ contracture tension is increased from half-maximal to maximal levels. However, this putative mechanism does not provide explanation as to why the amplitude increases from the lowest tensions to the half-maximal point. It is possible that substantial amount of myosin heads are required in order for the cross-bridge feedback mechanism to have its greatest effect.

Modeling studies suggest that compliant filaments are a necessary requirement for such transient increase in attached cross-bridges and tension overshoots (19). Some have proposed that titin also plays a role in these overshoots (9) since PKA treatment of permeabilized rat cardiomyocytes resulted in tension overshoots, and the magnitudes were reduced after trypsin digestion of titin. It should be noted that PKA can cause a decrease in stiffness of titin (20, 21) which agrees with the requirement for a more compliant myofilament apparatus put forth by Campbell (19). While tension overshoots sometimes occurred in our studies on rat myocardium, they were infrequent and had lower amplitudes (11). Rat hearts predominately express the stiff N2B titin isoform. Human hearts, on the other hand, express both the stiff N2B and compliant N2BA isoforms (22), and it is possible that a compliant titin along with cross-bridges are both necessary requirements for these overshoots to occur in humans.

We showed that tension overshoots following the k_{tr} maneuvers are mediated by the myocardium; however, their significance to *in vivo* cardiac contraction is currently unclear. Since tension overshoots are mediated by cross-bridges, as shown by our study, then it can be speculated that overshoots also occur in isolated trabeculae during contraction and even in the intact heart during systole. The question then becomes, why are these overshoots not observed during cardiac contraction? One possibility is that there is no reference point for comparison purposes which results in these overshoots being masked by the contraction of the myocardium. Overshoots allow the myocardium to recruit more myosin heads to the cross-bridge cycle than would normally be achievable for the same amount of calcium. This will allow the heart to develop more force and pressure during the cardiac cycle. Additionally, the heart will enter the ejection phase of the cardiac cycle sooner and maintain this phase longer which will increase the amount of blood pumped. Moreover, once these cross-bridges go through their force-generating cycle, the synchronizing action of the overshoot may also provide an additional “cooperative impact” on relaxation (i.e. a faster pressure fall because a larger fraction of cross-bridges all detach near simultaneously). We hypothesize that the rapid recruitment of the cross-bridges cause a temporary overshoot and that due to this synchronizing event, the breaking also occurs with an “overshoot”

(technically more of an “undershoot”) to allow the heart to relax faster if normally distributed duty cycle would be maintained. This means that the magnitude of negative dP/dt , a relaxation parameter, would be increased. Alternatively, or perhaps in addition, the “undershoot” phenomenon resembles spontaneous oscillatory contractions (SPOCs), which have been shown to occur when intermediately activated by calcium (Ca-SPOCs) and when the concentration of ADP is higher than ATP (ADP-SPOCs) (23, 24). The period of ADP-SPOCs is about 20 times longer than that of resting heart beat and also much longer than the period of the undershoot phenomenon we have observed (23). However, the period of Ca-SPOCs, at least in porcine myocardium, have been observed to be very fast (in the order of ms) at 39 °C (23). The potential effect of potassium contracture on the myocardium also cannot be ignored, as the concentration of free calcium in our muscle preparation reaches about 2 mM at maximal contracture (25). We have not observed oscillatory contractions during potassium contractures, but the increase in intracellular calcium could potentially result in transient spontaneous repetitive release to lead to the undershoot phenomenon.

Our current results collectively show that overshoots in human trabeculae are not an artifact of our experimental protocol and that the cross-bridges are the likely candidate as the underlying mechanism. Further investigations to determine whether such transient increase in force occurs during the systolic phase of the cardiac cycle *in vivo* and its contribution to maintaining cardiac output are required.

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News and Noteworthy

We show that isolated human myocardium is capable of transiently increasing its maximal force generation capability by increasing cross-bridge recruitment following slack-restretch maneuver. This process can potentially have important implications and significance in cardiac contraction in vivo.

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Highlights

- Human myocardium can transiently increase its force generating capacity
- This process involves a transient enhancement in cross-bridge recruitment
- This physiological regulatory mechanism has potentially essential role in maintaining cardiac output

Figure 1.

Schematic definitions of the terms used for tension overshoot. The movement of the motor is shown on top. Note that the overshoot occurred when the motor was stationary. The overshoot amplitude (%) was calculated as the difference between overshoot tension and developed tension during K^+ contracture tension (pre- k_{tr}) and normalized to the latter. The Tension Decline Phase $t_{1/2}$ was calculated as the time it takes for the overshoot amplitude to decrease by 50% from the peak of the overshoot to the lowest point after the overshoot.

Figure 2.

Temperature affects kinetics of tension overshoot. A) Tracings of the Tension Decline Phase at different temperatures. Decreasing temperature resulted in an increase in $t_{1/2}$ of the Tension Decline Phase, B) Close relationship between k_{tr} and $t_{1/2}$ of Tension Decline Phase, C) Temperature had no effect on the amplitude of the tension overshoot. $n = 4$ trabeculae from four non-failing hearts. * indicates $P < 0.05$ vs. 27 °C. Data is shown as mean $\pm$ S.E.M. ** indicates $P < 0.05$ between 32 and 37 °C.

Figure 3.

No tension overshoots without contracture or stimulation. A) k_{tr} shown is at the plateau of the contracture. B) Stimulation was paused and three consecutive k_{tr} s were performed without a contracture. C) Expansion of the first k_{tr} maneuver from Panel B shows lack of tension overshoot without a contracture or stimulation. D) Zoom-in of Panel C on a shorter time-scale

Figure 4.

High resting tension is not sufficient to cause tension overshoot in a resting trabecula. A) k_{tr} maneuver during the maximal K^+ contracture, B) stretching resting trabecula, no stimulation and no contracture, in order to match its resting tension to that of the K^+ contracture tension in Panel A, C) The trabecular length was reset to that of Panel A and k_{tr} protocol was performed during the maximal K^+ contracture.

Figure 5. BDM decreases magnitude of tension overshoot. A) Control (no BDM), B) 10 mM BDM, C) 20 mM BDM, D) Washout (no BDM), E) 50 mM BDM.

Figure 6. Relationship between tension overshoot amplitude and relative K^+ contracture tension. 1–4 data points per experiment per point shown.

Figure 7.

Number of cross-bridges increase during overshoot. A) A representative tracing where we performed a slack-retretch k_{tr} maneuver as the trabeculae was sinusoidally oscillating. B) Oscillation amplitude increases during overshoot compared to before slack-restretch k_{tr} maneuver (Prek_{tr}). $n = 4$, and * indicates $P < 0.05$ between Prek_{tr} and overshoot via paired t-test.

Table 1

Characteristics of human hearts.

Heart	Type	Age	Gender	Race	Temperature	Resting	BDM	Sub-Maximal	Sinusoidal
442404 *	Donor	69	Male	Caucasian	X				
118258 *	Donor	38	Male	Caucasian	X				
768159 *	Donor	44	Male	African-American	X		X		
600245 *	Donor	51	Female	Caucasian	X				
156910	Donor	62	Female	Caucasian		X			
600245	Donor	51	Female	Caucasian			X		
685884 *	Donor	36	Male	Caucasian				X	
474083 *	Donor	41	Female	African-American				X	
481043 *	Donor	65	Female	Caucasian				X	
947200 *	Donor	63	Female	Caucasian				X	
476074 *	Donor	29	Female	Caucasian		X		X	
240603 *	Donor	51	Female	Caucasian		X		X	
452192 *	Donor	55	Female	African-American				X	
987692	Donor	34	Female	Caucasian					X
479062 *	Failing	50	Male	Caucasian				X	
323104 *	Failing	63	Male	Caucasian				X	
537263 *	Failing	62	Male	African-American		X		X	
522421 *	Failing	56	Female	Caucasian				X	
214010 *	Failing	64	Male	Caucasian				X	
597750 *	Failing	67	Male	Caucasian		X		X	
806554	Failing	39	Female	African-American		X			X
378549	Failing	47	Male	Caucasian					X
554147	Failing	56	Male	Caucasian					X
897154	Failing	53	Female	African-American					X
437918	Failing	40	Male	Caucasian					X

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Heart	Type	Age	Gender	Race	Temperature	Resting	BDM	Sub-Maximal	Sinusoidal
187899	Failing	32	Female	Caucasian			X		
388426	Failing	58	Female	Caucasian	X		X		

* indicate hearts reported in previous studies (5, 6)