# Danicamtiv affected isometric force and cross-bridge kinetics similarly in skinned myocardial strips from male and female rats

Peter O. Awinda[1], Blake J. Vander Top[1], Kyrah L. Turner[2], and Bertrand C.W. Tanner[1*]

[1]Department of Integrative Physiology and Neuroscience, and [2]School of Molecular Biosciences
Washington State University Pullman, WA 99164

[*]Author addresses for correspondence:
Bertrand C. W. Tanner
Dept. of Integrative Physiology and Neuroscience
Room 255 Vet/Biomed Research Building
1815 Ferdinand's Lane
Pullman, WA. 99164-7620
email: bertrand.tanner@wsu.edu
ORCID: https://orcid.org/0000-0003-1711-852
phone: (509)-335-7785
Fax: 509-335-4650

**Abstract**

Myotropes are pharmaceuticals that have recently been developed or are under investigation for the treatment of heart diseases. Myotropes have had varied success in clinical trials. Initial research into myotropes have widely focused on animal models of cardiac dysfunction in comparison with normal animal cardiac physiology—primarily using males. In this study we examined the effect of danicamtiv, which is one type of myotrope within the class of myosin activators, on contractile function in permeabilized (skinned) myocardial strips from male and female Sprague-Dawley rats. We found that danicamtiv increased steady-state isometric force production at sub-maximal calcium levels, leading to greater $Ca^{2+}$-sensitivity to contraction for both sexes. Danicamtiv did not affect maximal $Ca^{2+}$-activated force for either sex. Sinusoidal length-perturbation analysis was used to assess viscoelastic myocardial stiffness and cross-bridge cycling kinetics. Data from these measurements did not vary with sex, and the data suggest that danicamtiv slows cross-bridge cycling kinetics. These findings imply that danicamtiv increases force production via increasing cross-bridge contributions to activation of contraction, especially at sub-maximal $Ca^{2+}$-activation. The inclusion of both sexes in animal models during the formative stages of drug development could be helpful for understanding the efficacy or limitation of a drug's therapeutic impact on cardiac function.

**Keywords**

cardiac muscle, contraction, myotrope, cross-bridge kinetics

## Introduction

Danicamtiv is a pharmaceutical under development for the treatment of heart disease. Danicamtiv binds to the head of myosin and acts as a 'myosin activator' because it is designed to increase contractility of cardiac muscle, potentially improving the pumping ability of the heart (Voors et al. 2020; Grillo et al. 2021). We recently contributed to a detailed study investigating the mechanism of action for danicamtiv in porcine cardiac muscle, and using a transgenic mouse model of dilated cardiomyopathy (Kooiker et al. 2023). We found that danicamtiv increased force and calcium sensitivity due to slowed ADP release (Kooiker et al. 2023), which prolongs cross-bridge attachment duration and slows relaxation (Voors et al. 2020; Shen et al. 2021; Ráduly et al. 2023; Kooiker et al. 2023) A portion of this increased cardiac contractility also followed from danicamtiv increasing the OFF-to-ON population of myosin to augment cross-bridge binding.

Previous studies of danicamtiv have not systematically investigated any potential effects of sex on cardiac muscle function (Voors et al. 2020; Shen et al. 2021; Ráduly et al. 2023; Kooiker et al. 2023; Choi et al. 2023). Where female subjects were included in a study, their representation was limited (Voors et al. 2020; Kooiker et al. 2023; Choi et al. 2023). With the current study we tested whether danicamtiv affected myocardial contractility differently between male and female rats. We find that danicamtiv increases $Ca^{2+}$-sensitivity of contraction, sub-maximal $Ca^{2+}$-activated isometric force production, and slowed cross-bridge cycling similarly in myocardial strips from male and female rats, with no observable sex difference.

## Materials and Methods

### *Animal Models*

All procedures were approved by the Institutional Animal Care and Use Committee at Washington State University and complied with the U.S. National Institute of Health's guidelines for animal use. Sprague-Dawley rats (4 male and 4 female, 15-30 weeks old) were acquired from Envigo (Indianapolis, IN). Rats were anesthetized by isoflurane inhalation (3% volume in 95% $O_2$-5% $CO_2$ flowing at 2 L/min), following which hearts were immediately excised and placed in dissecting solution on ice.

### *Papillary muscle preparation*

Papillary muscles from the left ventricle wall were dissected from the hearts and trimmed to ~180 μm in diameter and 700 μm in length with iridectomy scissors and skinned overnight at 4°C. Skinned papillary muscle strips were transferred to storage solution (which contained 50% glycerol), and if not used immediately for mechanics experiments, strips were stored at -20°C for 1-3 days until experiments were performed. Three to four strips from each heart were used for muscle mechanics experiments under each condition, with the specific number of strips listed in Table 1.

*Solutions*

Solutions were adapted from Pulcastro *et al*. (Pulcastro et al. 2016a) and Tanner *et al*. (Tanner et al. 2011), with solution formulations calculated via solving ionic equilibria according to Godt and Lindley (Godt and Lindley 1982). All concentrations are listed in mM unless otherwise noted. Dissecting solution: 50 N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (=BES), 30.83 K propionate, 10 Na azide, 20 Ethylene glycol- bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid (=EGTA), 6.29 $MgCl_2$, 6.09 ATP, 1 1,4-dithiothreitol (=DTT), 20 2,3-butanedione- monoxime (=BDM), 50 μM leupeptin, 275 μM pefabloc, and 1 μM trans-epoxysuccinyl-L-leucylamido(4-guanidino)butane (=E-64). Skinning solution: dissecting solution with 1% Triton-XT vol/vol and 50% glycerol vol/vol. Storage solution: dissecting solution with 50% glycerol vol/vol. Relaxing solution: pCa 8.0 (pCa = $-\log_{10}[Ca^{2+}]$), 20 BES, 5 EGTA, 5 MgATP, 1 $Mg^{2+}$, 0.3 $P_i$, 35 phosphocreatine, 300 U/mL creatine kinase, 3% dextran T-500 wt/vol, 200 ionic strength, and adjusted to pH 7.0 with Na methanesulfonate. Maximal $Ca^{2+}$-activating solution: same as relaxing solution with pCa 4.8.

Danicamtiv was purchased from MedChemExpress (Monmouth Junction, NJ) and dissolved in dimethylsulfoxide (DMSO) to make a 1 mM stock solution (Kooiker et al. 2023). This stock was then diluted in relaxing (pCa 8.0) and activating (pCa 4.8) solutions to yield a final danicamtiv concentration of 3 μM (with 0.3% DMSO). This 3 μM danicamtiv concentration was the $EC_{50}$ of actomyosin ATPase measurements from Voors *et al* (Voors et al. 2020) and the same concentration as they used in their mechanics measurements.

*Muscle mechanics*

Aluminum t-clips were attached to both ends of skinned papillary muscle strips and mounted between a piezoelectric motor (P841.40, Physik Instrumente, Auburn, MA) and a strain gauge (AE801, Kronex, Walnut Creek, CA) in relaxing solution that contained either no danicamtiv or 3 μM danicamtiv. These danicamtiv concentrations were maintained for each individual strip at a temperature of 28°C throughout the entire experiment. Sarcomere length was set to 2.2 μm using microscopy, imaging with a CCD camera, and a real-time Fourier transfer algorithm (Tanner et al. 2015; Pulcastro et al. 2016b; Kieu et al. 2019). Then the strip was shortened until slack, and the force gauge was balanced to 0 mV to set the reference voltage for the force-pCa curve. Next the strip was re-stretched, sarcomere length was confirmed, and the absolute, isometric tension value (=force normalized to cross-sectional area of each strip) at pCa 8.0 was recorded (=$T_{min}$, Table 1). From here, strips were $Ca^{2+}$-activated to assess steady-state isometric stress across a range of pCa values.

Sinusoidal length perturbations at an amplitude of 0.125% muscle length were applied at discrete frequencies (0.125-200 Hz) (Tanner et al. 2011). Elastic and viscous moduli were measured as a function of angular frequency from the in-phase and out-of-phase portions of the stress-strain response to the length perturbations. The resulting frequency-dependent viscoelastic response reflects characteristics of cross-bridge binding and cross-bridge cycling, as further discussed in the results.

*Statistical analysis*

All data are listed or plotted as mean ± SE. Constrained non-linear least squares fitting of data to a three-parameter Hill equation (Table 1) was performed using sequential quadratic programming methods in Matlab (v.9.13.0, The Mathworks, Natick MA). Statistical analyses applied nested linear mixed models with main effects of danicamtiv, sex, and their interaction in SPSS (IBM Statistical, Chicago, IL). An additional main effect of pCa or frequency was used for statistical analysis of the force-pCa relationships (Fig. 1A-B), or the elastic and viscous moduli vs. frequency relationships (Figs. 2-3). Nested linear mixed model analyses link data from the same heart, with hearts being a random effect, to optimize statistical power.

## Results

*Steady-state isometric stress*

Developed stress (=$Ca^{2+}$-activated stress minus stress at pCa 8.0, $T_{min}$, for each strip) increased as activating [$Ca^{2+}$] increased in skinned papillary muscle strips from all groups (Fig. 1A-B, Table 1). There were no differences in maximal force among all four groups, nor between male and female force measurements in the absence or presence of danicamtiv (main effects of pCa, treatment, and a pCa*treatment interaction at $p<0.001$). Danicamtiv produced greater stress at sub-maximal pCa values between 6.25-5.8, leading to greater $Ca^{2+}$-sensitivity (Fig. 1E) and decreased cooperativity (Fig. 1D) of the force-pCa relationship for both sexes (main effect of treatment at $p<0.001$ for $pCa_{50}$ and $n_H$).

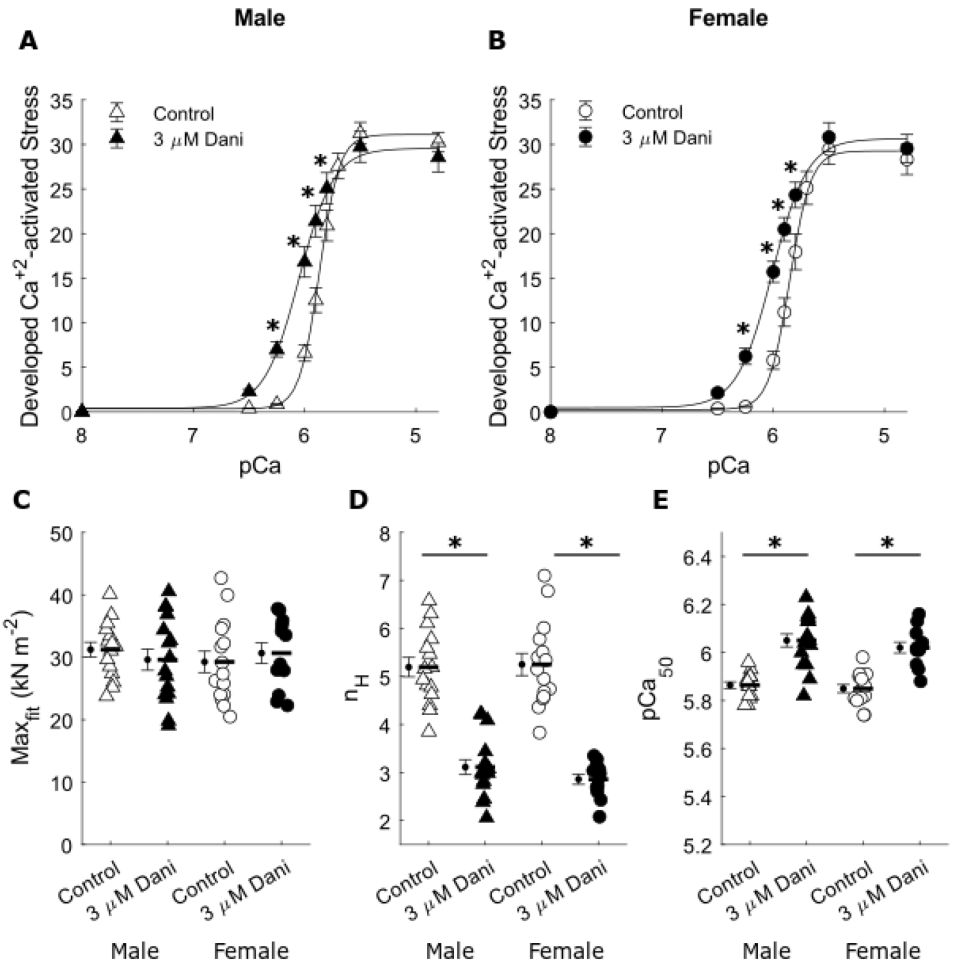


**Fig. 1.** $Ca^{2+}$-activated stress is plotted against pCa for myocardial strips from (A) male and (B) female rats. Lines represent fits to a 3-parameter Hill equation (see legend of Table 1). Steady-state isometric tension increased as [$Ca^{2+}$] increased for all myocardial strips. There were not statistical differences in maximal force between groups (individual $Max_{fit}$ values shown in panel C). Force-pCa relationships were shifted to the left with danicamtiv, showing increased $Ca^{2+}$-sensitivity ($pCa_{50}$ values shown in panel E) for both sexes. Danicamtiv also produced a lower slope, or Hill coefficient ($n_H$ values shown in panel D). Number of biological and technical replicates for each condition are listed at the bottom of Table 1. * post-hoc effect of danicamtiv at $p < 0.001$.

**Table 1:** Characteristics of tension-pCa relationships.

| | Male | | Female | | Male and female combined | |
|---|---|---|---|---|---|---|
| Treatment | Control | 3 μM Dani | Control | 3 μM Dani | Control | 3 μM Dani |
| $T_{min}$ (kN m$^{-2}$) | 2.38±0.21 | 2.15±0.25 | 2.40±0.32 | 2.67±0.28 | 2.39±0.19 | 2.38±0.19 |
| $T_{max}$ (kN m$^{-2}$) | 32.63±1.16 | 30.71±1.75 | 30.72±1.87 | 32.21±1.74 | 31.68±1.09 | 31.35±1.24 |
| $T_{dev}$ (kN m$^{-2}$) | 30.26±1.10 | 28.55±1.63 | 28.32±1.70 | 29.54±1.58 | 29.29±1.01 | 28.97±1.13 |
| $pCa_{50}$ | 5.86±0.01* | 6.05±0.03 | 5.85±0.02* | 6.02±0.02 | 5.86±0.01* | 6.04±0.02 |
| $n_H$ | 5.19±0.20* | 3.11±0.16 | 5.25±0.23* | 2.86±0.11 | 5.22±0.15* | 3.00±0.10 |
| $Max_{fit}$ (kN m$^{-2}$) | 31.23±1.17 | 29.61±1.70 | 29.26±1.71 | 30.66±1.65 | 30.25±1.04 | 30.06±1.18 |
| n (strips) | 15 | 16 | 15 | 12 | 30 | 28 |
| N (# of hearts) | 4 | | 4 | | 8 | |

Values are means ± SEM, $T_{min}$, absolute tension value at pCa 8.0., $T_{max}$, absolute tension value at pCa 4.8., $T_{dev}$, $Ca^{2+}$-activated, developed tension ($T_{max}$ - $T_{min}$). $Max_{fit}$, $pCa_{50}$, and $n_H$ represent fit parameters to a 3-parameter Hill equation: $T(pCa) = \frac{Max_{fit}}{[1+10^{n_H(pCa-pCa_{50})}]}$ for the developed tension vs. pCa relationships shown in Fig. 1. * post-hoc effect of danicamtiv at $p < 0.001$.

*Viscoelastic myocardial stiffness*

Elastic and viscous modulus values were measured at relaxed (pCa 8.0, Fig. 2) and maximally activated (pCa 4.8, Fig. 3) conditions. Under relaxed conditions elastic and viscous moduli values increased as oscillatory frequency increased (main effect of frequency at $p<0.001$ for males, females, and the combined data sets), which arises from the viscoelastic stiffness of the tissue increasing as it is oscillated faster at higher frequencies. There were statistically significant effects of danicamtiv on elastic and viscous moduli under relaxed conditions, although the minor differences are unlikely to influence physiological contractility. Elastic moduli values decreased slightly for the males with danicamtiv treatment (main effect of treatment at $p=0.024$, Fig. 2A). Viscous moduli values increased for both sexes with danicamtiv treatment (main effect of treatment at $p<0.001$ for males, females and the combined data set, Fig. 2D-F). There was not a significant main effect of sex nor any significant interaction for elastic or viscous moduli at pCa 8.0. The small, albeit consistent, increases in viscous modulus suggest subtle, yet detectable, increases in cross-bridge binding and altered cross-bridge cycling for the danicamtiv treated strips at low [$Ca^{2+}$].

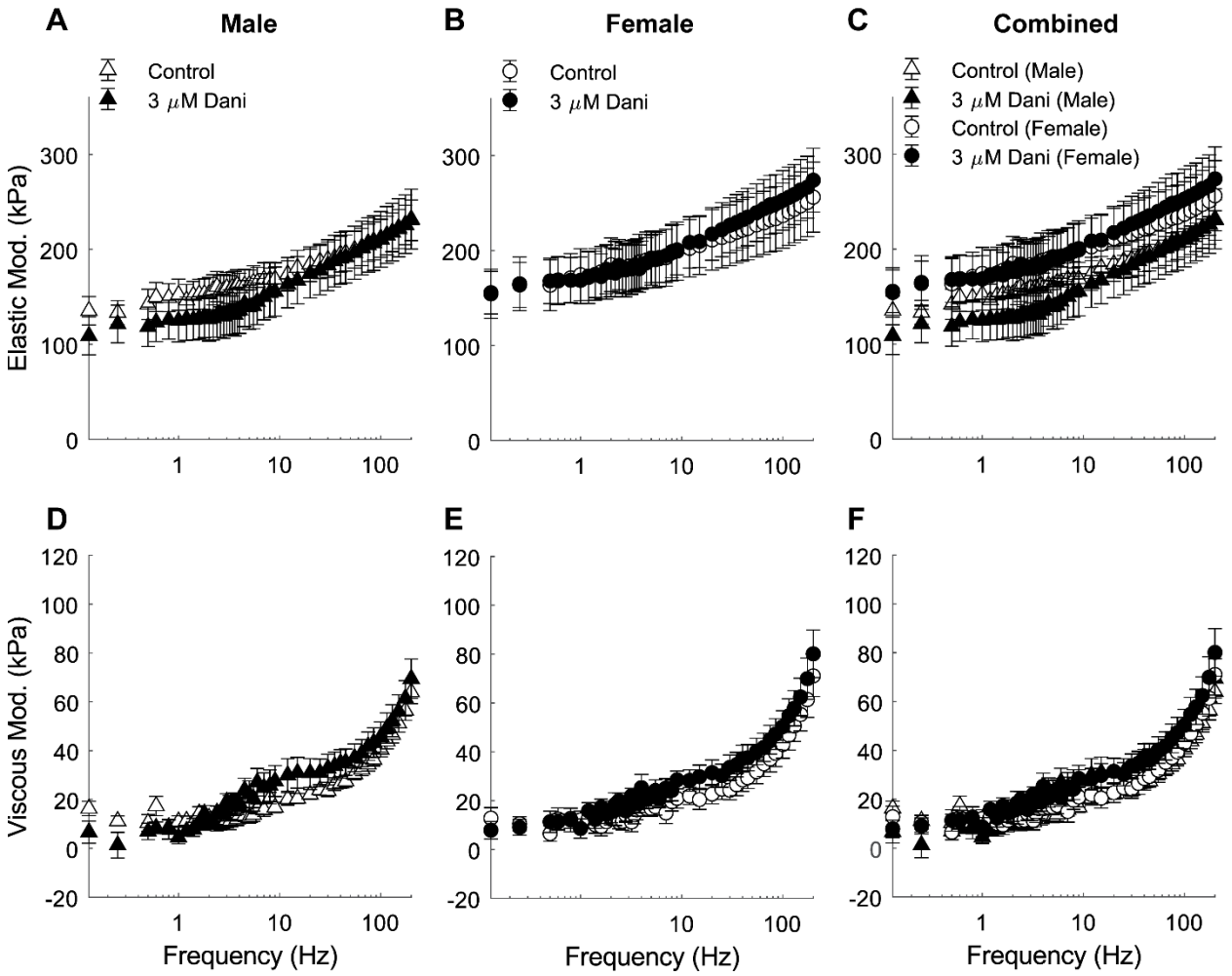


**Fig. 2.** (A-C) Elastic and (D-F) viscous moduli are plotted against frequency under relaxed conditions (pCa 8.0) for males, females, and combined for both sexes. Number of biological and technical replicates for each condition are listed at the bottom of Table 1.

At maximal $Ca^{2+}$-activation (pCa 4.8), elastic moduli values were greater for danicamtiv treated strips above 8 Hz for both sexes, compared to controls (main effects of frequency, treatment, and a frequency*treatment interaction at $p<0.001$ for both sexes and the combined data, Fig. 3A-C). Viscous moduli values were also greater for danicamtiv treated strips between 2-10 Hz, but less than control strips above 35 Hz (main effects of frequency, treatment, and a frequency*treatment interaction at $p<0.001$ for both sexes and the combined data, Fig. 3D-F). Both the elastic and viscous modulus vs. frequency relationships were shifted leftward, towards lower frequencies for both sexes. This leftward shift in the moduli-frequency relationships indicates slower overall cross-bridge cycling in danicamtiv treated strips vs. control strips. The "dip frequency", or smallest elastic modulus value, is shifted from ~8-9 Hz for control strips to ~4-5 Hz for danicamtiv-treated strips, which suggests that danicamtiv slows cross-bridge attachment. Similarly, the maximal viscous modulus value is shifted from ~30 Hz for control strips to ~10 Hz

for danicamtiv treated strips, which indicates that danicamtiv also slows cross-bridge detachment. The remarkable similarities between male and female myocardial strips in the presence and absence of danicamtiv indicates that danicamtiv produces the same chemomechanical or biophysical effects on cross-bridge binding, cycling, and force production in myocardium from male and female rats (Fig. 3C and 3F).

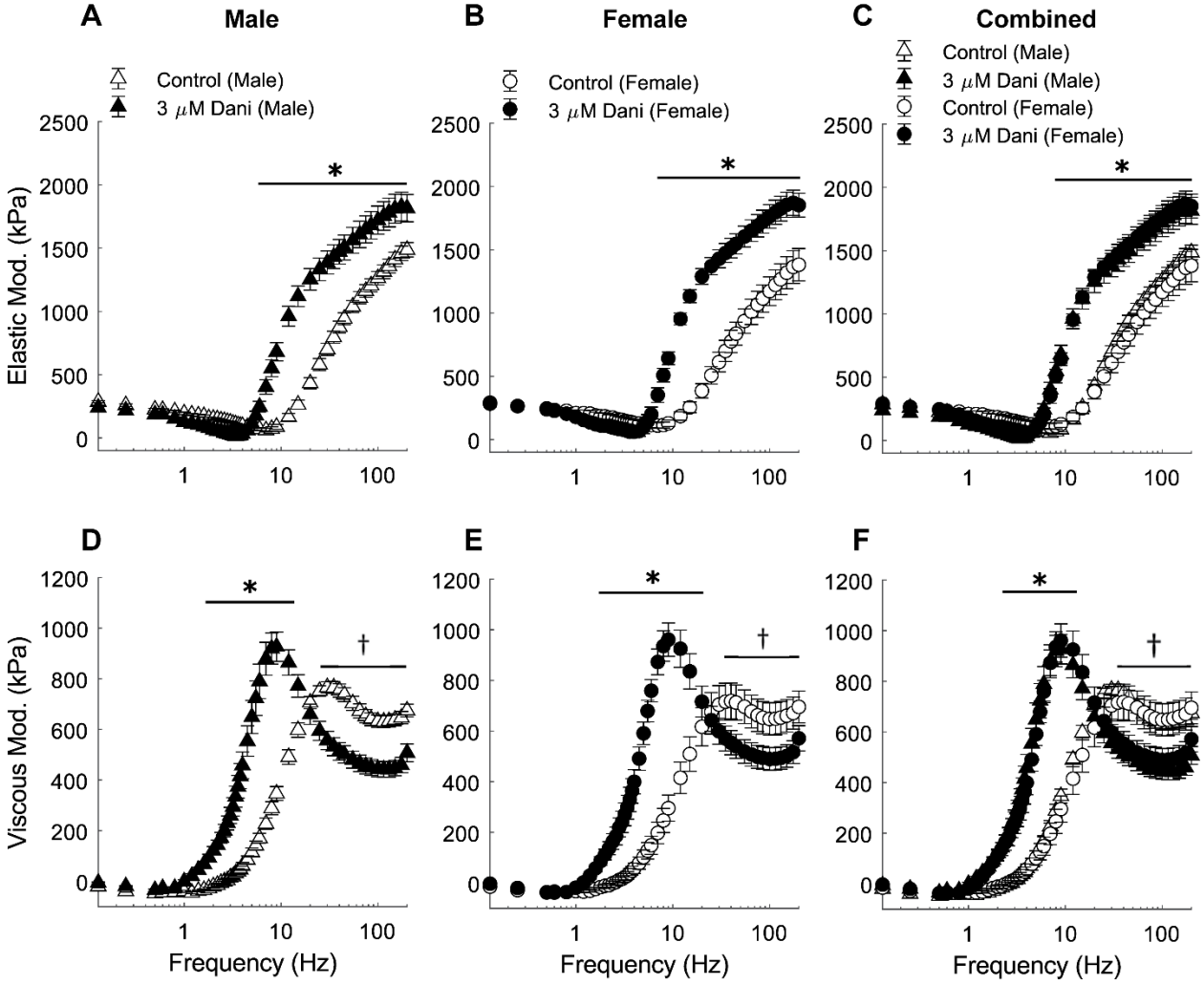


**Fig. 3.** (A-C) Elastic and (D-F) viscous moduli are plotted against frequency under maximally activated conditions (pCa 4.8) for males, females, and combined for both sexes. Number of biological and technical replicates for each condition are listed at the bottom of Table 1. * post-hoc effect of danicamtiv at $p < 0.001$. † post-hoc effect of danicamtiv at $p < 0.05$.

## Discussion

Danicamtiv targets cardiac myosin to augment contractility, and it represents a sub-set of small molecules that are under development to target myofilament protein function as potential therapies for heart disease (Alsulami and Marston 2020; Lehman et al. 2022). Compared to some more studied myotropes, such as omecamtiv mecarbil (a myosin activator), (Malik et al. 2011; Woody et al. 2018; Kieu et al. 2019) or mavacamten (a myosin inhibitor), (Stern et al. 2016; Awinda et al. 2020), danicamtiv is still in clinical trials (Voors et al. 2020). Only a few mechanistic studies have investigated effects of danicamtiv on contractility in cardiac muscle (Shen et al. 2021; Ráduly et al. 2023; Kooiker et al. 2023; Choi et al. 2023); these have not investigated sex as a biological variable, with limited data related to any effects of danicamtiv on contractility between males and females. Herein we systematically assessed the effects of danicamtiv on myocardial function using male and female rat hearts. While this is a focused study on a few metrics of contraction (isometric tension, viscoelastic myocardial stiffness, and cross-bridge kinetics), the remarkable similarities in myocardial function between sexes suggest that danicamtiv influences molecular and cellular mechanisms of contraction the same for both sexes.

We show that danicamtiv increases $Ca^{2+}$-sensitivity of contraction, but does not increase maximal force production (Fig. 1). The increase in sub-maximal force agrees with prior observations in permeabilized myocardial strips or myofibrils from pigs and humans (Voors et al. 2020; Kooiker et al. 2023; Choi et al. 2023). However, data showing increases in maximal force with danicamtiv treatment are mixed. Like Choi et al. 2023, we did not see any increases in maximal force, but others have seen increases in maximal force (Kooiker et al. 2023; Voors et al. 2020). Although we did not observe increased maximal force, danicamtiv-treated strips had greater myocardial viscoelastic stiffness; this could imply that danicamtiv increases the number of strongly-bound cross-bridges that contribute to stiffness, but not to tension. Given that in vivo cardiac contraction and intact myocardial twitches typically occur at sub-maximal calcium levels, these data also agree with prior observations that danicamtiv augments systolic function in mouse and dog models of heart failure (Voors et al. 2020; Kooiker et al. 2023), rats (Ráduly et al. 2023), and human engineered heart tissue (Shen et al. 2021). There is no evidence that danicamtiv affects $Ca^{2+}$-binding to troponin C (Kooiker et al. 2023) and it did not alter intracellular $Ca^{2+}$-transients in canine cardiomyocytes (Ráduly et al. 2023). However, it is possible that danicamtiv could alter thin-filament regulation via binding to tropomyosin or actin, which we have not ruled out with our measurements herein and could not confirm through previously published studies. Nonetheless, much of our data suggests that danicamtiv affects cross-bridge behavior, consistent with the idea of a ‘myotrope’ being designed to affect myosin function.

The leftward shift in the peak viscous modulus value from 30 Hz in control strips to 10 Hz in danicamtiv treated strips (Fig. 2D) suggests that danicamtiv slows cross-bridge cycling. Although we did not explicitly measure ADP dissociation nor ATP association rates herein, this observation likely follows from slowed cross-bridge detachment (Kawai and Halvorson 1989; Kawai et al. 1993) Moreover, this interpretation is supported by our prior measurement of danicamtiv prolonging cross-bridge attachment duration due to slowed ADP release (Kooiker et al. 2023). Thus, it is likely that the increases in sub-maximal force production partially stem from prolonged cross-bridge binding, which augments cross-bridge contributions to thin-filament activation (Bremel and Weber 1972; Smith et al. 2009). Once full activation (or saturating $Ca^{2+}$-activation)

along the thin filament is reached, however, cross-bridge contributions to thin-filament activation may have already saturated, whereby danicamtiv treatment no longer augments force production at maximal $Ca^{2+}$-activation (Fig. 1).

It is likely that danicamtiv increases sub-maximal force production by two separate, yet complementary, mechanisms. The first one we introduced just above, related to prolonged cross-bridge binding due to slowed ADP release. The second one was introduced by Kookier *et al*., (Kooiker et al. 2023) showing that danicamtiv changes myosin head positioning along the thick-filament. This conformational change reduced the population of myosin heads in the OFF state (super-relaxed state), leading to more myosin heads populating the ON state (or disordered relaxed state); the ON state is capable of binding actin to form a cross-bridge. A portion of this could follow from danicamtiv amplifying the mechanosensitive thick-filament regulation pathway as force production begins to increase at sub-maximal calcium levels (Ait-Mou et al. 2016; Fusi et al. 2016; Piazzesi et al. 2018; Park-Holohan et al. 2021). However, it is possible that increases in sub-maximal force could also follow from danicamtiv simply disorganizing the OFF state, which also agrees with the x-ray diffraction data from in pig myocardial strips (under relaxed conditions) (Kooiker et al. 2023). Any of these structural effects of danicamtiv are likely to be amplified as $Ca^{2+}$ increases throughout (systolic) contraction as more cross-bridges begin to generate force.

Recent efforts have found discrepancies in the pathophysiology of heart disease between males and females (Bui et al. 2011; Sciomer et al. 2020; Lala et al. 2022). Despite this, large gaps have remained in assessing, preventing, and treating cardiovascular disease in both sexes. Females have remained underrepresented in randomized clinical trials assessing myotropic efficacy in recent years, with females only comprising 19-32% of total participants (Sullivan et al. 2021; Wang et al. 2023; Pabon et al. 2023). Data from these limited trials have alluded to differences in the response to therapy between sexes, contributing to modified optimal dose levels of therapeutics. This could arise from altered presentation and manifestation of cardiovascular disease as well as differences in metabolism and drug clearance. Thus, it is important to assess the sex-specific efficacy and safety of clinical treatments. Here, we have assessed the effects of danicamtiv on healthy male vs female rats to understand any sex differences that may arise at a genetic or molecular level. Our study shows no significant changes in the mechanism by which danicamtiv augments myocardial contraction between sexes, although further characterization of sex-differences under pathophysiological conditions may still be warranted to better inform future clinical trials.

## Acknowledgments

This work was supported by grants from the American Heart Association (23TPA1074093, https://doi.org/10.58275/AHA.23TPA1074093.pc.gr.172307), National Science Foundation (IOS-2312925 to BCWT), and National Institutes of Health (R01HL149164, R01HL164795 and R21AG077125).

## Author Contributions

Conceptualization: P.O.A., K.L.T., B.C.W.T.; Performed experiments: P.O.A., B.J.V.T., K.L.T.; Analyzed and curated data: P.O.A., B.J.V.T., K.L.T., B.C.W.T.; Drafted original manuscript:

P.O.A., B.C.W.T.; Reviewed, edited, and revised manuscript: P.O.A., B.J.V.T., K.L.T., B.C.W.T.; All authors approved the final version submitted for review.

**Statements and Declarations**

The authors have nothing to disclose and no conflicting interests.

**References**

Ait-Mou Y, Hsu K, Farman GP, et al (2016) Titin strain contributes to the Frank-Starling law of the heart by structural rearrangements of both thin- and thick-filament proteins. Proc Natl Acad Sci U S A 113:2306–11. https://doi.org/10.1073/pnas.1516732113

Alsulami K, Marston S (2020) Small molecules acting on myofilaments as treatments for heart and skeletal muscle diseases. Int J Mol Sci 21:1–31. https://doi.org/10.3390/ijms21249599

Awinda PO, Bishaw Y, Watanabe M, et al (2020) Effects of mavacamten on Ca2+ sensitivity of contraction as sarcomere length varied in human myocardium. Br J Pharmacol 177:5609–5621. https://doi.org/10.1111/bph.15271

Bremel R, Weber A (1972) Cooperation within actin filament in vertebrate skeletal muscle. Nat New Biol 238:97–101. https://doi.org/10.1038/newbio238097a0

Bui AL, Horwich TB, Fonarow GC (2011) Epidemiology and risk profile of heart failure. Nat Rev Cardiol 8:30–41. https://doi.org/10.1038/nrcardio.2010.165

Choi J, Holmes JB, Campbell KS, Stelzer JE (2023) Effect of the Novel Myotrope Danicamtiv on Cross-Bridge Behavior in Human Myocardium. J Am Heart Assoc 12:e030682. https://doi.org/10.1161/JAHA.123.030682

Fusi L, Brunello E, Yan Z, Irving M (2016) Thick filament mechano-sensing is a calcium-independent regulatory mechanism in skeletal muscle. Nat Commun 7:13281. https://doi.org/10.1038/ncomms13281

Godt RE, Lindley BD (1982) Influence of temperature upon contractile activation and isometric force production in mechanically skinned muscle fibers of the frog. J Gen Physiol 80:279–297. https://doi.org/10.1085/jgp.80.2.279

Grillo MP, Markova S, Evanchik M, et al (2021) Preclinical in vitro and in vivo pharmacokinetic properties of danicamtiv, a new targeted myosin activator for the treatment of dilated cardiomyopathy. Xenobiotica 51:222–238. https://doi.org/10.1080/00498254.2020.1839982

Kawai M, Halvorson HR (1989) Role of MgATP and MgADP in the cross-bridge kinetics in chemically skinned rabbit psoas fibers. Study of a fast exponential process (C). Biophys J 55:595–603. https://doi.org/10.1016/S0006-3495(89)82857-7

Kawai M, Saeki Y, Zhao Y (1993) Crossbridge scheme and the kinetic constants of elementary steps deduced from chemically skinned papillary and trabecular muscles of the ferret. Circ Res 73:35–50. https://doi.org/10.1161/01.res.73.1.35

Kieu TT, Awinda PO, Tanner BCW (2019) Omecamtiv Mecarbil Slows Myosin Kinetics in

Skinned Rat Myocardium at Physiological Temperature. Biophys J 116:2149–2160. https://doi.org/10.1016/j.bpj.2019.04.020

Kooiker KB, Mohran S, Turner KL, et al (2023) Danicamtiv Increases Myosin Recruitment and Alters Cross-Bridge Cycling in Cardiac Muscle. Circ Res. https://doi.org/10.1161/CIRCRESAHA.123.322629

Lala A, Tayal U, Hamo CE, et al (2022) Sex Differences in Heart Failure. J Card Fail 28:477–498. https://doi.org/10.1016/j.cardfail.2021.10.006

Lehman SJ, Crocini C, Leinwand LA (2022) Targeting the sarcomere in inherited cardiomyopathies. Nat Rev Cardiol 19:353–363. https://doi.org/10.1038/s41569-022-00682-0

Malik FI, Hartman JJ, Elias KA, et al (2011) Cardiac myosin activation: a potential therapeutic approach for systolic heart failure. Science 331:1439–43. https://doi.org/10.1126/science.1200113

Pabon M, Cunningham J, Claggett B, et al (2023) Sex Differences in Heart Failure With Reduced Ejection Fraction in the GALACTIC-HF Trial. JACC Heart Fail 11:1729–1738. https://doi.org/10.1016/j.jchf.2023.07.029

Park-Holohan S-J, Brunello E, Kampourakis T, et al (2021) Stress-dependent activation of myosin in the heart requires thin filament activation and thick filament mechanosensing. Proc Natl Acad Sci U S A 118:. https://doi.org/10.1073/pnas.2023706118

Piazzesi G, Caremani M, Linari M, et al (2018) Thick Filament Mechano-Sensing in Skeletal and Cardiac Muscles: A Common Mechanism Able to Adapt the Energetic Cost of the Contraction to the Task. Front Physiol 9:736. https://doi.org/10.3389/fphys.2018.00736

Pulcastro HC, Awinda PO, Breithaupt JJ, Tanner BCWW (2016a) Effects of myosin light chain phosphorylation on length-dependent myosin kinetics in skinned rat myocardium. Arch Biochem Biophys 601:56–68. https://doi.org/10.1016/j.abb.2015.12.014

Pulcastro HC, Awinda PO, Methawasin M, et al (2016b) Increased Titin Compliance Reduced Length-Dependent Contraction and Slowed Cross-Bridge Kinetics in Skinned Myocardial Strips from $Rbm^{20\Delta RRM}$ Mice. Front Physiol 7:. https://doi.org/10.3389/fphys.2016.00322

Ráduly AP, Sárkány F, Kovács MB, et al (2023) The Novel Cardiac Myosin Activator Danicamtiv Improves Cardiac Systolic Function at the Expense of Diastolic Dysfunction In Vitro and In Vivo: Implications for Clinical Applications. Int J Mol Sci 24:1–17. https://doi.org/10.3390/ijms24010446

Sciomer S, Moscucci F, Salvioni E, et al (2020) Role of gender, age and BMI in prognosis of heart failure. Eur J Prev Cardiol 27:46–51. https://doi.org/10.1177/2047487320961980

Shen S, Sewanan LR, Jacoby DL, Campbell SG (2021) Danicamtiv enhances systolic function and frank-starling behavior at minimal diastolic cost in engineered human myocardium. J Am Heart Assoc 10:1–3. https://doi.org/10.1161/JAHA.121.020860

Smith L, Tainter C, Regnier M, Martyn DA (2009) Cooperative cross-bridge activation of thin filaments contributes to the Frank-Starling mechanism in cardiac muscle. Biophys J

96:3692–702. https://doi.org/10.1016/j.bpj.2009.02.018

Stern JA, Markova S, Ueda Y, et al (2016) A Small Molecule Inhibitor of Sarcomere Contractility Acutely Relieves Left Ventricular Outflow Tract Obstruction in Feline Hypertrophic Cardiomyopathy. PLoS One 11:e0168407. https://doi.org/10.1371/journal.pone.0168407

Sullivan K, Doumouras BS, Santema BT, et al (2021) Sex-Specific Differences in Heart Failure: Pathophysiology, Risk Factors, Management, and Outcomes. Can J Cardiol 37:560–571. https://doi.org/10.1016/j.cjca.2020.12.025

Tanner BCW, Breithaupt JJ, Awinda PO (2015) Myosin MgADP release rate decreases at longer sarcomere length to prolong myosin attachment time in skinned rat myocardium. Am J Physiol Circ Physiol 309:H2087–H2097. https://doi.org/10.1152/ajpheart.00555.2015

Tanner BCW, Wang Y, Maughan DW, et al (2011) Measuring myosin cross-bridge attachment time in activated muscle fibers using stochastic vs. sinusoidal length perturbation analysis. J Appl Physiol 110:1101–1108. https://doi.org/10.1152/japplphysiol.00800.2010.-The

Voors AA, Tamby JF, Cleland JG, et al (2020) Effects of danicamtiv, a novel cardiac myosin activator, in heart failure with reduced ejection fraction: experimental data and clinical results from a phase 2a trial. Eur J Heart Fail 22:1649–1658. https://doi.org/10.1002/ejhf.1933

Wang X, Vaduganathan M, Claggett BL, et al (2023) Sex Differences in Characteristics, Outcomes, and Treatment Response With Dapagliflozin Across the Range of Ejection Fraction in Patients With Heart Failure: Insights From DAPA-HF and DELIVER. Circulation 147:624–634. https://doi.org/10.1161/CIRCULATIONAHA.122.062832

Woody MS, Greenberg MJ, Barua B, et al (2018) Positive cardiac inotrope omecamtiv mecarbil activates muscle despite suppressing the myosin working stroke. Nat Commun 9:3838. https://doi.org/10.1038/s41467-018-06193-2