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# Assessment of sarcomere shortening and calcium transient in primary human and dog ventricular myocytes

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ABSTRACT

Understanding translation from preclinical observations to clinical findings is important for evaluating the efficacy and safety of novel compounds. Of relevance to cardiac safety is profiling drug effects on cardiomyocyte (CM) sarcomere shortening and intracellular $Ca^{2+}$ dynamics. Although CM from different animal species have been used to assess such effects, primary human CM isolated from human organ donor heart represent an ideal non-animal alternative approach. We performed a study to evaluate primary human CM and have them compared to freshly isolated dog cardiomyocytes for their basic function and responses to positive inotropes with well-known mechanisms. Our data showed that simultaneous assessment of sarcomere shortening and $Ca^{2+}$-transient can be performed with both myocytes using the IonOptix system. Amplitude of sarcomere shortening and $Ca^{2+}$-transient (CaT) were significantly higher in dog compared to human CM in the basic condition (absence of treatment), while longer duration of sarcomere shortening and CaT were observed in human cells. We observed that human and dog CMs have similar pharmacological responses to five inotropes with different mechanisms, including dobutamine and isoproterenol (β-adrenergic stimulation), milrinone (PDE3 inhibition), pimobendan and levosimendan (increase of $Ca^{2+}$sensitization as well as PDE3 inhibition). In conclusion, our study suggests that myocytes obtained from both human donor hearts and dog hearts can be used to simultaneously assess drug-induced effects on sarcomere shortening and CaT using the IonOptix platform.

## 1. Introduction

Myocardial sarcomere shortening is the innate ability of the heart muscle to contract and generate force for pumping and delivering blood, which is essential for the function of living organs. Sarcomere shortening and $Ca^{2+}$-transient (CaT) measurements using primary isolated cardiomyocytes (CM) and image-based techniques have been commonly used for assessing the potential of novel compounds to directly modulate heart function during preclinical drug development, while ensuring compliance with 3Rs framework (Abi-Gerges et al., 2020; Beuckelmann, Nabauer, & Erdmann, 1992; Harmer et al., 2012; Mewes & Ravens, 1994; Ren & Wold, 2001). For example, the cardiomyocyte sarcomere shortening-based model was shown to successfully identify positive and negative inotropic drugs with different mechanisms of action (Abi-Gerges, Indersmitten, et al., 2020; Gao et al., 2018; Harmer et al., 2012; Horváth et al., 2017; Nickola et al., 2000). When measured simultaneously, sarcomere shortening and CaT provide valuable mechanistic insights into the effect of novel compounds on the heart's function. Moreover, drug-induced changes in cardiomyocyte function can be evaluated independently of the influence of heart rate, coronary flow, and neuro-hormonal factors.

Because of their similarity to human hearts in terms of their anatomical structure, gene expression, electrophysiology, and hemodynamics, dogs have been commonly used as a large animal model in academic research and biopharmaceutical drug development (Camacho, Fan, Liu, & He, 2016; Gralinski, 2003). It is also well accepted that dog hearts represent much better human cardiac physiology, pathophysiology, and pharmacology compared to rodent hearts (Milani-Nejad &

*Abbreviations:* CaT, Calcium-transient; ISO, Isoproterenol; Cont-P, Amplitude of sarcomere length shortening; Cont-Dur, Duration of sarcomere shortening measured at 50% of Cont-P; CTAs, Calcium transient amplitude at systole; CTD50, Calcium transient duration measured at 50% CTAs.

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Janssen, 2014). Therefore, we have been using isolated dog ventricular myocytes in our laboratory to understand the mechanistic profiling of inotropic drugs (Gao et al., 2018). Recently, we characterized the mechanism of action of omecamtiv mecarbil, a novel positive myotrope in Phase III clinical trial for the treatment of heart failure with reduced ejection fraction, by evaluating its simultaneous effect on sarcomere shortening and $Ca^{2+}$-transients in isolated dog ventricular myocytes (Gao, Sutherland, Vargas, & Qu, 2020). Our data supports using primary ventricular myocyte preparation in drug development to select the best compound through understanding their mechanisms of action.

While the use of primary myocytes from a wide range of animal species in preclinical drug development has been around for the last two decades, the adoption of human primary cardiomyocytes to drug discovery has been hampered by the limited availability of human cardiac tissue, the knowledge gap in addressing the preservation of myocyte's function for several days following isolation and lack of well-defined shipment conditions that maintain structure and function of cardiomyocytes. Recent studies using human primary myocytes have been performed with sufficient throughput and scalability, but at the site of isolation only (Abi-Gerges, Indersmitten, et al., 2020; Abi-Gerges, Miller, & Ghetti, 2020). Consequently, cell-based assays that utilize human primary cardiomyocytes are yet to be implemented by the wider scientific community. To address potential implementation, we evaluated the feasibility of procuring functional human CMs obtained from a qualified vendor by ground transport (> 150 miles from vendor to testing lab). Dog myocytes (local isolation, i.e., no transport) were used as a reference benchmark to characterize human myocyte responses. Both sarcomere length shortening and CaT were measured simultaneously and five positive inotropes (dobutamine, isoproterenol, milrinone, pimobendan, levosimendan) were characterized under the same experimental conditions.

## 2. Materials and methods

### 2.1. Preparation of human myocytes

All human tissues used for the study were obtained by legal consent from organ donors in the US. AnaBios Corporation's procurement network includes only US based Organ Procurement Organizations and Hospitals. Policies for donor screening and consent are the ones established by the United Network for Organ Sharing (UNOS). Organizations supplying human tissues to AnaBios follow the standards and procedures established by the US Centers for Disease Control (CDC) and are inspected biannually by the Department of Health and Human Services (DHHS). Tissue distribution is governed by internal IRB procedures and compliance with HIPAA regulations regarding patient privacy. All transfers of donor organs to AnaBios are fully traceable and periodically reviewed by US Federal authorities.

Donor organs/tissues were from adults aged 17 to 60 years old with mass index (BMI) between 15.7 and 37.3 and ejection fraction (EF) >45% (50–69.5%). Some donors may be trauma victims but will not include HIV, Donor after cardiac death (DCD), HBV, Ongoing infection, HCV, Downtime >60 min, or MRSA (Table 1).

Donor hearts from males and females were harvested using proprietary surgical techniques and tools and were shipped to AnaBios' laboratory via dedicated couriers. Upon arriving at AnaBios, each heart was assigned a unique identifier number that was reproduced on all relevant medical history files, data entry forms and electronic records.

In current study the average time was 13.9 h from donor heart harvest to Anabios laboratory. Upon arrival at the laboratory, hearts were immediately re-perfused with ice-cold propriety cardioplegic solution. Primary ventricular myocytes were isolated via enzymatic treatment as described previously (Nguyen et al., 2017). The Myocytes were preserved in glass vials in ice-cold (4 °C) cardioplegic solution and transported to Amgen via ground shipment by Biocair. Inc. Myocytes were used for recordings either during the same day of shipment or kept at 4 °C for additional recordings in the following days.

**Table 1**
Donor information.

| Donor Identifier | Age | Sex | Ethnicity | BMI | COD | EF (%) |
|---|---|---|---|---|---|---|
| 190811HHA | 56 | M | Caucasian | 25.2 | Anoxia | 60 |
| 200828HHA | 24 | M | Hispanic | 27.8 | Anoxia | 50 |
| 200902HHA | 24 | M | Caucasian | 19.4 | Anoxia | 50–55 |
| 201005HHA | 26 | M | Caucasian | 15.7 | Head trauma | 60–65 |
| 201015HHA | 38 | F | Caucasian | 29.7 | Anoxia | 65 |
| 201023HHA | 60 | F | Hispanic | 28.3 | Head trauma | 50 |
| 201111HHA | 50 | F | Caucasian | 23.3 | CVA/ICH | 77 |
| 201211HHA | 37 | F | American Indian | 26.0 | CVA/ICH/ Stroke | 50 |
| 210209HHB | 53 | F | Caucasian | 28.7 | Anoxia | 69.5 |
| 210328HHA | 53 | F | Caucasian | 25.2 | Anoxia | 60 |
| 210608HHA | 51 | F | American Indian | 37.3 | CVA/ICH | 60 |
| 210710HHB | 43 | F | Caucasian | 27.4 | Head trauma | 65 |
| 210822HHA | 17 | M | Hispanic | 25.3 | Anoxia | 65 |
| 211017HHA | 45 | M | Caucasian | 21.5 | Anoxia | 50–60 |
| 220321HHA | 49 | F | Caucasian | 27.3 | Anoxia | 56 |

### 2.2. Shipment of human myocytes

Human myocytes were typically shipped within 1–2 days post-isolation to ensure that all recordings were conducted within a 5-day window post-isolation. Biocair, Inc. was responsible for transporting the cells from the isolation facility to Amgen via ground shipment. During transportation, the myocytes were preserved in an ice-cold cardioplegic solution and stored in a container with ice. The transportation duration was approximately 3–5 h. Upon receiving the morphology of myocytes were visually inspected under a microscope. After the assessment the myocytes were utilized for recordings either on the same day of shipment or stored at 4 °C for subsequent recordings within the following days. All recordings on human myocytes were performed within 5 days of isolation. Prior to recording, the cardioplegic solution was replaced with 10 mL of Tyrode solution, and the myocytes were allowed to equilibrate at room temperature for 20 min before being washed twice at room temperature.

### 2.3. Preparation of dog myocytes

Adult male beagle dogs (12–15 months old; Marshall BioResources. New York, USA) were.

used in this study. Detailed animal information and myocytes isolation procedure were described previously (Gao et al., 2018). Briefly, heart was submerged in cardioplegic solution after taking from an anesthetized dog and then mounted on a Langendorff perfusion apparatus. The left anterior descending coronary artery was cannulated and perfused with 37 °C Tyrode solution, first with $Ca^{2+}$-free Tyrode solution for 10 min, then followed by 1.1 mg/mL collagenase type II (Worthington Biochemical) plus 0.5 mg/mL bovine serum albumin (BSA, Fraction V, Sigma) and 5 μM $Ca^{2+}$ for 20 min. The collagenase was then washed out with 0.2 mM $Ca^{2+}$-Tyrode for 10 min. A ~ 1.5 cm × 1.5 cm transmural left ventricular wall was excised. The tissue was further minced into smaller pieces, swirled and filtered through a 100 μm Teflon mesh. The dissociated myocytes were collected and stored in storage solution at 4 °C before use (Abi-Gerges et al., 2013). All dog myocytes recordings were carried out within 5 days of isolation.

### 2.4. Solutions

Recordings for both human and dog myocytes were performed in the standard Tyrode's solution containing (in mM): 145 NaCl, 4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 11.1 glucose, and adjusted to pH 7.4 with NaOH. $CaCl_2$ was substituted with equimolar NaCl in the calcium-free-

Tyrode solution. The cardioplegic solution contained in (mM): 27 KCl, 20 NaHCO3, 255 glucose, 63 mannitol, heparin 1000 μ/L. The dog myocyte storage solution was the normal Tyrode solution containing 30 μM N-benzyl-p-toluene sulphonamide (BTS, TCI America-B3082).

### 2.5. Test articles

Five inotropes were evaluated in this study, including dobutamine, isoproterenol, milrinone, pimobendan and levosimendan. They were all purchased from Sigma-Aldrich and with purities ≥97%. The testing concentrations were selected based on our previous experience with isolated myocytes and relevant clinical free plasma exposure with the highest testing concentration to cover the clinical Cmax.

### 2.6. Myocyte sarcomere shortening and CaT measurement

Before recording, human and dog myocytes were loaded with 2 μM Fura-2 AM (Molecular Probes-F1221) for 30 min at room temperature. Then the myocytes were washed three times with standard Tyrode solution. Recordings were performed after 0.5–1 h, during which the dye was de-esterified.

Sarcomere shortening and intracellular CaT were recorded using IonOptix, a video-based edge-detecting system (Gao et al., 2020). Selection criteria of cells and recording procedure were similar to those described previously (Gao et al., 2018). Briefly, myocytes were loaded into the recording chamber mounted on the stage of an inverted microscope (Motic AE31, New York Microscope Co) and perfused with warm (35 ± 1 °C) Tyrode solution via a micro pump (ISMATEC, 827B) to the recording chamber. Myocytes were electrically paced via a MyoPacer (IonOptix LLC, Westwood, MA, USA) at 1 Hz (60 beats/min) pacing frequency and illuminated with an LED alternating dual wavelength (340 nm/380 nm) light. Pacing induced intracellular $Ca^{2+}$-transient fluorescence change (detected at 510 nm with a Hamamatsu H7360 photomultiplier tube) and sarcomere shortening were simultaneously recorded. Data were sampled at a rate of 240 Hz. Background fluorescence was recorded and manually subtracted from the total fluorescence.

After loading into the recording chamber, myocytes were continuously electrically paced. To minimize exposure to UV light, data collection at baseline and each testing concentration was limited to 10–20s, with a 10–12 min pre-baseline assessment performed to confirm stable sarcomere shortening and CaT using short (3–5 s) periodic illumination of F340/F380 fluorescence light. Following baseline measurements, ascending concentrations of test articles were applied for approximately 2 min at each testing concentration prior to data collection. Sarcomere shortening and CaT signals (F340, F380, and ratio) were collected for 10–20s at each concentration.

### 2.7. Data analysis

Data analysis was performed using IonWizard software. Sarcomere shortening was measured from averaged sarcomere waveforms. The following parameters were analyzed for sarcomere shortening:

- Cont-P: Amplitude of sarcomere shortening
- Cont-Dur: Duration of sarcomere shortening measured at 50% of Cont-P

CaT parameters were analyzed from the $Ca^{2+}$-fluorescence ratios (F340/F380) collected at baseline and at each testing concentration. The following parameters were measured from the averaged ratio waveform:

- CTAs: Calcium transient amplitude at systole
- CTD50: Calcium transient duration measured at 50% of CTAs

Drug-related effects for sarcomere shortening and CaT were assessed via time-matched comparison with vehicle control (0.1% DMSO) using one-way ANOVA followed by Bonferroni multiple comparison correction. Statistical significance ($p < 0.05$) was marked with an asterisk (*). Data are presented as mean ± SEM. Mean EC50 values were derived from concentration-response curves fitted with Hill equation using OriginPro 2020b software (OriginLab Corporation).

## 3. Results

### 3.1. Sarcomere shortening and CaT characteristics in isolated human and dog CMs

Baseline properties of myocyte sarcomere shortening and CaT were compared in human and dog CMs. A total of 143 human cardiomyocytes from 15 donors (Table 1: Donor characteristics) and 87 dog cardiomyocytes from 5 dog hearts were recorded and analyzed in the presence of vehicle control (i.e., no pharmacological agents were introduced). For each myocyte, ~10–20 consecutive waveforms were acquired and averaged. The averaged waveforms from each myocyte were then grouped together with other myocytes to generate the average population waveform (Fig. 1). As shown in Fig. 1 and Table 2, human myocytes had smaller amplitude of sarcomere shortening (0.093 μm in human vs. 0.164 μm in dog) and smaller CaT amplitude (0.735 in human vs. 1.721 in dog). The durations of contraction and CaT in human myocytes were relatively longer than that in dog myocytes. The measured duration of contraction was 0.228 ± 0.005 s in human vs. 0.190 ± 0.006 s in dog, and the duration of CaT was 0.341 ± 0.006 s and 0.220 ± 0.003 s in human and dog, respectively. In conclusion, our data indicate that when compared to dog myocytes human myocytes had relatively smaller amplitudes of contraction and CaT with slower relaxation and decay kinetics under the experimental conditions of this investigation.

### 3.2. Stability of recordings over time in human and dog myocytes in the presence of vehicle (0.1% DMSO)

Stable recordings with adequate time window are essential for assessing drug effects. To determine the stability of recordings from human and dog myocytes, we measured sarcomere shortening and CaT first in the presence of vehicle control, 0.1% DMSO, for up to 15 min. As shown in Fig. 2, the amplitude of sarcomere shortening (Con—P) recorded over this period was stable in both human and dog myocytes. Moreover, Ca-transient amplitude (CTAs) did not significantly change over time (decreases of −18% and − 3.9% were seen with human and dog myocytes, respectively). Finally, the duration of sarcomere shortening (Cont-Dur) and CaT (CTD50) remained stable in both human and dog myocytes (Fig. 2).

### 3.3. Effects of β-adrenergic agonists

The pharmacological effects of two β-adrenergic agonists, dobutamine and isoproterenol (ISO), were evaluated and compared in human and dog myocytes. Fig. 3 shows that responses to dobutamine were comparable between human and dog myocytes. For example, dobutamine, at the top testing concentration of 10 μM, increased Cont-P by 198% and 267% in human and dog myocytes, respectively (Fig. 3, middle panel). These increases in Cont-P were accompanied with increases in CTAs, 91% and 39% in human and dog myocytes, respectively (Fig. 3 right panel). When compared to time-match DMSO vehicle control data, dobutamine caused a significant decrease in Cont-Dur in human myocytes only, while significant decreases of CTD50 were observed in both human and dog myocytes (Fig. 3).

Similar to dobutamine, ISO increased Cont-P and CTAs in a concentration-dependent manner in both human and dog myocytes (Fig. 4). For example, ISO, at the top test concentration of 100 nM, increased Cont-P by 367% and 165% in human and dog myocytes,

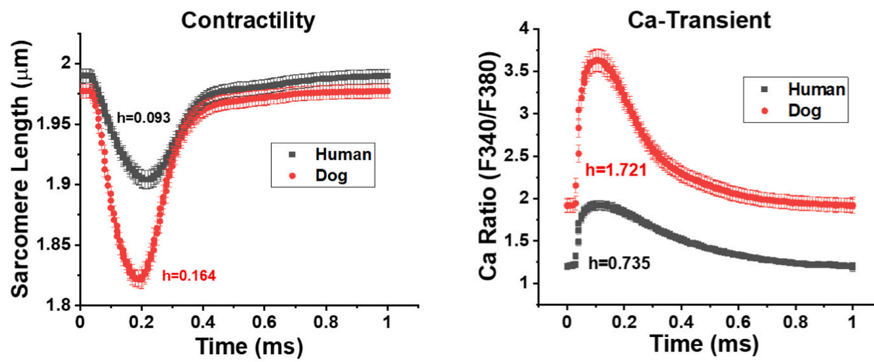


**Fig. 1.** Baseline sarcomere shortening and CaT in isolated human and dog ventricular myocytes. Left: superimposed averaged sarcomere shortening waveforms of human (black) and dog (red) myocytes; Right: superimposed average CaT ratio of human (black) and dog (red) myocytes. Data shown are mean ± SE. $N$ = 143 human myocytes isolated from 15 donor hearts and 87 dog myocytes from 5 dog hearts. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 2**
Baseline parameters of sarcomere shortening and CaT in isolated human and dog ventricular myocytes.

| | Human ($n$ = 143, 15 hearts) | Dog ($n$ = 87, 5 hearts) |
|---|---|---|
| Cont-P (μM) | 0.093 ± 0.005* | 0.164 ± 0.006 |
| Cont-Dur (s) | 0.228 ± 0.005* | 0.190 ± 0.006 |
| CTAs (ratio) | 0.735 ± 0.031* | 1.721 ± 0.091 |
| CTD50 (s) | 0.341 ± 0.006* | 0.220 ± 0.003 |

respectively (Fig. 4 left panel). These increases were accompanied by 66% and 31% increases in CTAs in human and dog myocytes, respectively (Fig. 4 right panel). When compared to time-matched DMSO vehicle control data, ISO caused a significant decrease in Cont-Dur in human myocytes only, while significant decreases of CTD50 were observed in both human and dog myocytes (Fig. 4).

### 3.4. Effects of a phosphodiesterase 3 (PDE3) inhibitor

Fig. 5 shows that milrinone increased Cont-P and CTAs without affecting the duration of contraction in both human and dog myocytes. A slight decrease of CTD50 was observed in human but not dog myocytes. For example, at the top test concentration of 300 μM, milrinone increased Cont-P by 145% and 58%, in human and dog myocytes, respectively (Fig. 5, left panel). This was accompanied with 28% (human) and 19% (dog) increases in CTAs. Small but significant reductions in CTD50 were observed at 100 and 300 uM in human myocytes (e.g., −11% at 300uM) but not in dog myocytes at the same concentrations (e.g., −1% at 300 uM).

### 3.5. Effects of dual Ca-sensitization and PDE3 inhibition

As shown in Fig. 6, pimobendan increased Cont-P and Cont-Dur in both human and dog myocytes in a concentration-dependent manner (Fig. 6). However, pimobendan produced fluorescence signal when

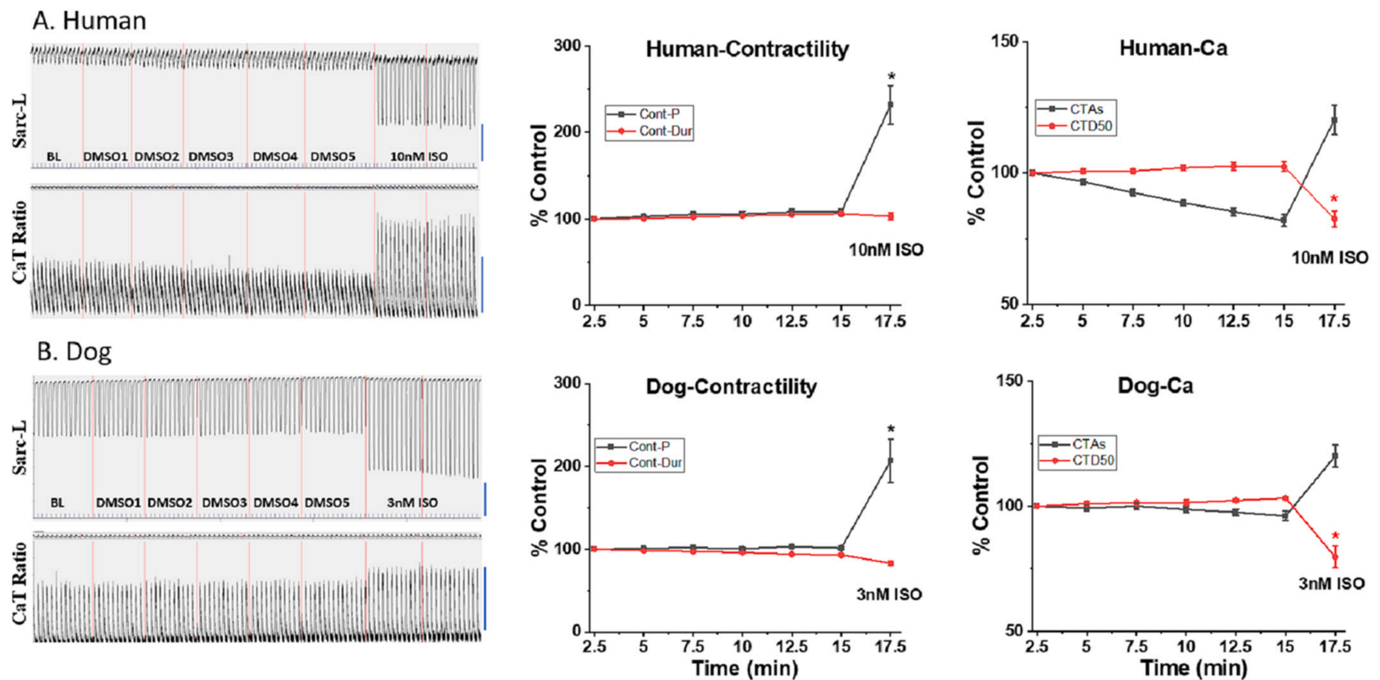


**Fig. 2.** Time-dependent stability of sarcomere shortening and CaT in vehicle control (0.1% DMSO). A: Isolated human ventricular myocytes perfused with 0.1% DMSO. Left panel: raw data trace of sarcomere shortening (upper trace) and CaT (lower trace). Middle panel: sarcomere shortening (Cont-P and Cont-Dur). Right panel: CaT (CTAs and CTD50). B. Isolated dog ventricular myocytes perfused with 0.1% DMSO. Left Panel: raw data trace of sarcomere shortening (upper trace) and CaT (lower trace). Middle panel: sarcomere shortening (Cont-P and Cont-Dur). Right panel: CaT (CTAs and CTD50). A positive inotropic control, Isoproterenol (10 nM in human and 3 nM in dog) was added at the end to verify the condition of the cell. Calibration of sarcomere length: 0.1 μm. Calibration of CaT ratio trace: 1. $N$ = 29 human myocytes from 7 donor hearts and 8 dog myocytes from 1 dog heart. *: statistically significant ($P < 0.05$) compared with baseline.

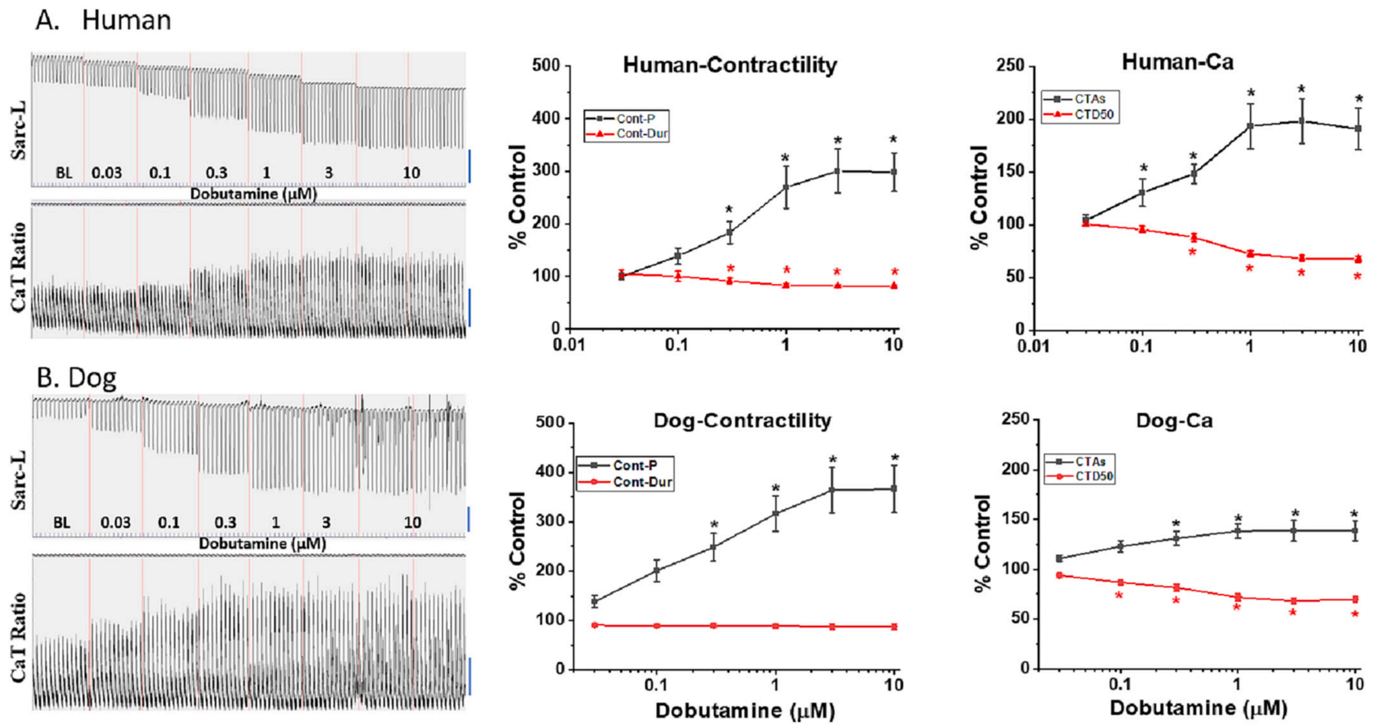


**Fig. 3.** Concentration-dependent effects of dobutamine on sarcomere shortening and CaT in isolated human and dog ventricular myocytes. A: Human myocytes. Left panel: raw data trace of sarcomere shortening (upper trace) and CaT ratio (lower trace) recorded from a single human myocyte; Middle panel: concentration-dependent effects of dobutamine on sarcomere shortening (Cont-P and Cont-Dur); Right panel: concentration-dependent effects of dobutamine on CaT (CTAs and CTD50). B: Dog myocytes. Left panel: raw data trace of sarcomere shortening (upper trace) and CaT ratio (lower race) recorded from a single dog myocyte. Middle panel: concentration-dependent effects of dobutamine on sarcomere shortening (Cont-P and Cont-Dur). Right panel: concentration-dependent effects of dobutamine on CaT (CTAs and CTD50). Calibration for left panels: sarcomere length: 0.1 μm; CaT ratio: 1. $N = 13$ human myocytes from 2 donor hearts and 16 dog myocytes from 2 dog hearts. *: statistically significant ($P < 0.05$) compared with time match vehicle control.

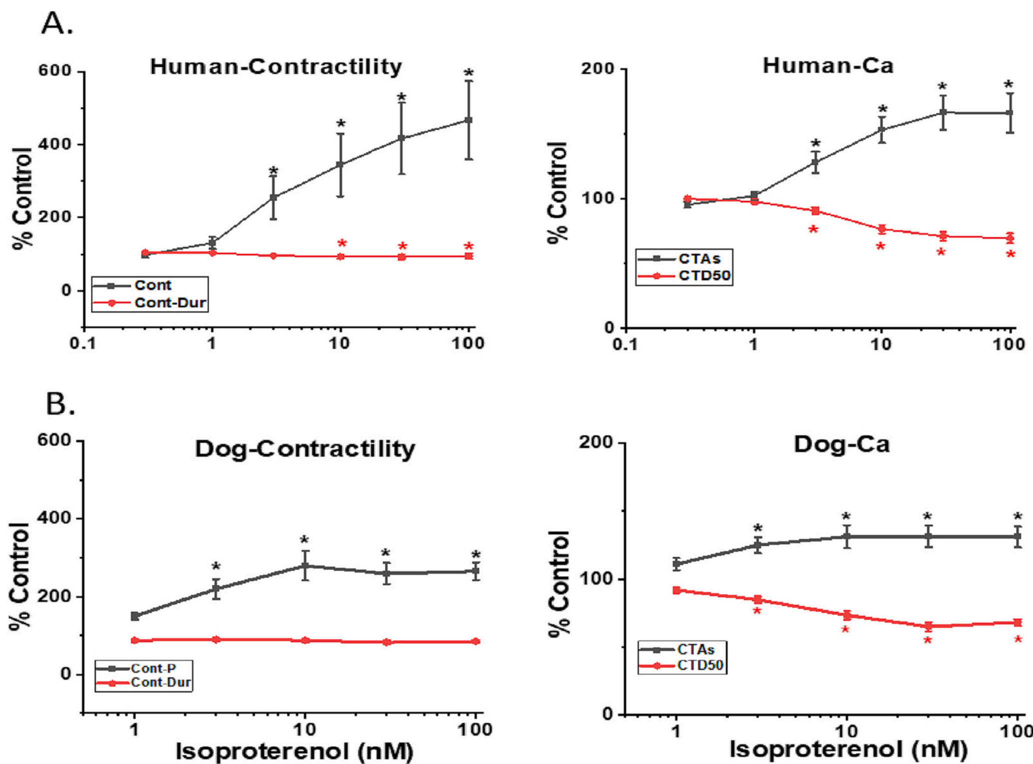


**Fig. 4.** Concentration-dependent effects of isoproterenol (ISO) in isolated human and dog ventricular myocytes. A: Human myocytes. Left panel: effects on sarcomere shortening (Cont-P, Cont-Dur), right panel: effects on CaT (CTAs, CTD50). B: Dog myocytes. Left panel: effects on sarcomere shortening (Cont-P, Cont-Dur), right panel: effects on CaT (CTAs, CTD50). $N = 13$ human myocytes from 2 donor hearts and 11 dog myocytes from 1 dog heat; *: statistically significant ($P < 0.05$) compared with time-match vehicle control.

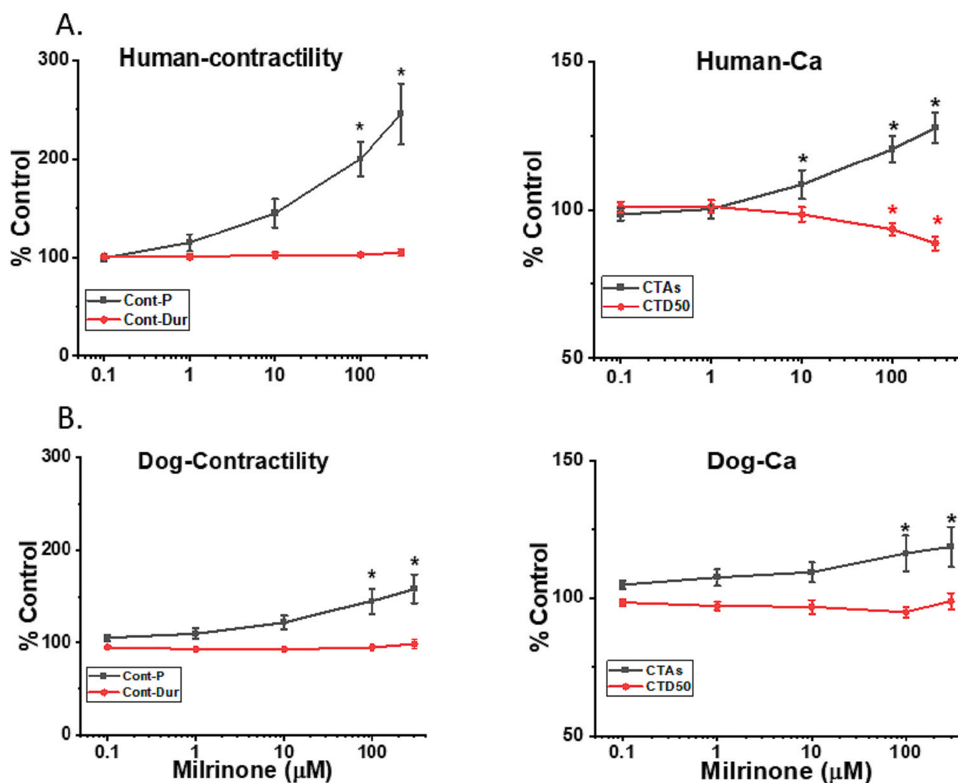


**Fig. 5.** Concentration-dependent effects of milrinone in isolated human and dog ventricular myocytes. A: Human myocytes. Left panel: effects on sarcomere shortening (Cont-P, Cont-Dur), right panel: effects on CaT (CTAs, CTD50). B: Dog myocytes. Left panel: effects on sarcomere shortening (Cont-P, Cont-Dur), right panel: effects on CaT (CTAs, CTD50). $N = 15$ human myocytes from 2 donor hearts and 8 dog myocytes from 1 dog heart; *: statistically significant ($P < 0.05$) compared with time-match vehicle control.

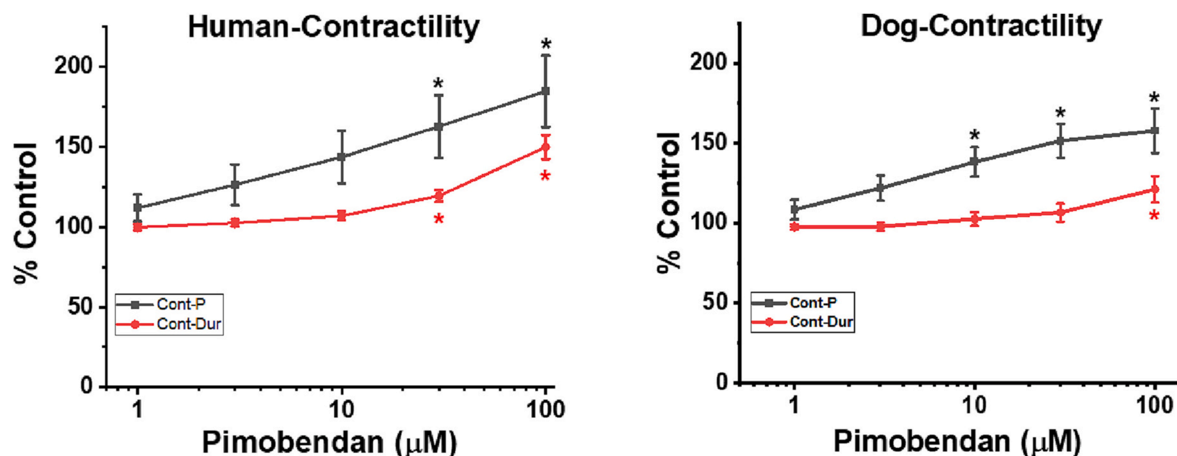


**Fig. 6.** Concentration-dependent effects of pimobendan on sarcomere shortening (Cont-P, Cont-Dur) in isolated human and dog ventricular myocytes. Left panel: Human myocytes; right panel: dog myocytes. $N = 14$ human myocytes from 2 donor hearts and 10 dog myocytes from 1 dog heart. *: statistically significant ($P < 0.05$) compared with time-matched vehicle control.

exposed to alternating F340/F380 wavelength without dye-loading. The predominate change was recorded at F340, with only minimum changes at F380 (data not shown). The other 4 compounds were tested in the same experimental design, no changes in fluorescence signals were detected. This result indicates that pimobendan is a molecule with luminescent property, which prevents us from accurately measuring drug-induced intracellular $Ca^{2+}$ changes using a fura-2 based dye. In the future, a different calcium indicator dye with different excitation spectrum should be investigated for testing pimobendan's effect on intracellular CaT.

Levosimendan had a different profile compared to pimobendan. At testing concentrations up to 10 μM, levosimendan increased Cont-P in both species, however, the effects were small and not statistically significant at any of the testing concentrations (Fig. 7, left panel). Levosimendan did not affect CTAs or CTD50 up to 10 μM in human myocytes. In dog myocytes, CTA was increased significantly at 10 μM without changes in CTD50.

## 4. Discussion

This validation study measured sarcomere shortening and CaT simultaneously from isolated adult human ventricular myocytes and compared to dog ventricular myocytes in the presence and absence of various positive inotropic drugs. The findings from our side-by-side comparison demonstrated that both cell-based models are sensitive to detect drug-induced changes in sarcomere shortening and cytosolic calcium flux, thus are useful models for cardiac safety profiling. The simultaneous measurement of sarcomere shortening with intracellular

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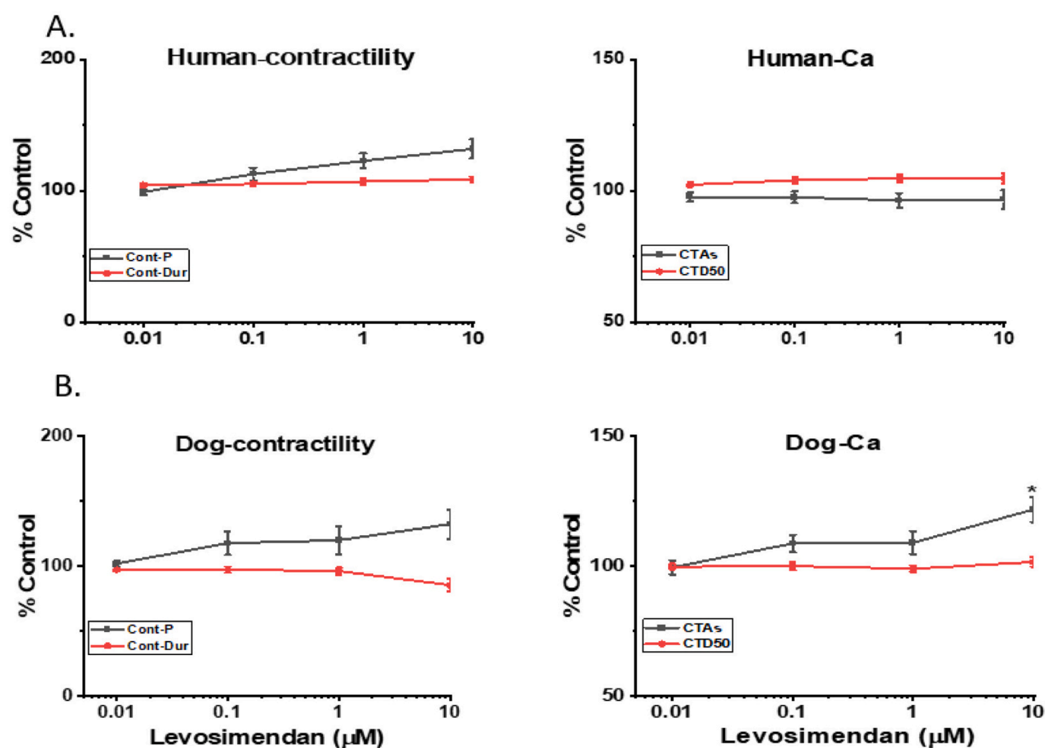


**Fig. 7.** Concentration-dependent effects of levosimendan in isolated human and dog ventricular myocytes. A: Human myocytes. Left panel: effects on sarcomere shortening (Cont-P, Cont-Dur), right panel: effects on CaT (CTAs, CTD50). B: Dog myocytes. Left panel: effects on sarcomere shortening (Cont-P, Cont-Dur), right panel: effects on CaT (CTAs, CTD50). $N = 29$ human myocytes from 4 donor hearts and 9 dog myocytes from 1 dog heart. *: statistically significant ($P < 0.05$) compared with time-match vehicle control.

calcium transients in human CMs is a unique option to provide mechanistic insights on the cardiac effects of novel drugs prior to clinical trials.

### 4.1. *Ventricular myocytes from human donor hearts display good health and functionality after transportation*

Availability of healthy human tissues and cells is a main barrier for inclusion of human data into preclinical drug discovery and safety assessment. The key technical limitation is maintaining the normal function of cardiomyocytes for several days after isolation, which is a challenge. Therefore, the possibility of shipping healthy human cardiomyocytes from the isolation's facility to distant laboratories would greatly alleviate the issue of accessibility. To our knowledge, the current investigation is the first study to demonstrate the feasibility and functional viability of transported human cardiomyocytes in a systematic way. Specifically, our study demonstrated that ground-transported human myocytes (e.g., ≈5 h) displayed stable sarcomere shortening and calcium flux measurement up to 5 days following isolation. Thus, access to human CMs is a realistic option to consider for research and drug evaluation purposes, but additional studies are needed to verify and expand upon these initial findings.

### 4.2. *Baseline properties of sarcomere shortening and Ca transients in human and dog cardiomyocytes*

The shipped human cardiomyocytes and freshly isolated dog myocytes maintained excellent function and rod-shaped morphology with clear striations. Moreover, both cardiomyocytes were quiescent if not electrically stimulated, which indicated that they were calcium tolerant cells. The resting sarcomere length was similar and in agreement with published data for human and dog myocytes (Gerdes et al., 1992; Mukherjee, Crawford, Hewett, & Spinale, 1993; Nguyen et al., 2017). However, the sarcomere shortening amplitude was consistently lower in human cardiomyocytes compared to dog myocytes, which may be due to species difference. These results are consistent with previously published data under similar experimental conditions (Mukherjee et al., 1993; Nguyen et al., 2017). Another plausible explanation for such a difference in sarcomere shortening amplitude could be the smaller calcium-transient we observed in human myocytes, since intracellular calcium is known to be a critical element of the excitation-contraction coupling (Kane, Couch, & Terracciano, 2015). We also found that human myocytes presented longer durations of contraction and calcium-transient when compared to dog myocytes, indicating that human cells had slower relaxation and CaT decay under the experimental conditions of the current study. Our data are consistent with published data reporting longer action potential duration in human myocytes compared to dog myocytes (Nánási et al., 2021; O'Hara & Rudy, 2012). A prolonged depolarization phase in an elongated action potential could maintain intracellular $Ca^{2+}$ longer, which could in turn lengthen contraction.

### 4.3. *Responses of human and dog cardiomyocytes to positive inotropic agents*

To understand the pharmacological similarities and differences of human and dog myocytes, we assessed the potencies of 5 positive inotropic agents for their effects on the amplitude of sarcomere shortening and $Ca^{2+}$-transient. As shown in Table 3, the potency values were found to be similar in both species, indicating that human and dog myocytes have comparable pharmacological responses under our experimental conditions. Moreover, our results are in agreement with previously published reports for human and dog cardiomyocytes (Abi-Gerges, Indersmitten, et al., 2020; Gao et al., 2018; Harmer et al., 2012).

Along with sarcomere shortening assessment we simultaneously measured drug effects on intracellular calcium-transients for 4 out of 5 compounds as shown in Table 2. The potency values of increasing

**Table 3**
Comparison of potencies in human and canine myocytes.

| | Cont-P (EC50, μM) | | CTAs (EC50, μM) | |
|---|---|---|---|---|
| Compound | Human | Dog | Human | Dog |
| Dobutamine | 0.380 | 0.210 | 0.319 | 0.070 |
| Isoproterenol | 0.004 | 0.002 | 0.003 | 0.002 |
| Milrinone | 31.717 | 20.756 | 24.319 | 8.737 |
| Pimobendan | 9.128 | 5.034 | NA | NA |
| Levosimendan | NA | NA | NA | NA |

amplitude of $Ca^{2+}$-transient are similar in human and dog myocytes, which are also consistent with published reports and provided more insights into the mechanism of drug action on the subcellular level (Butler et al., 2015; Nguyen et al., 2017; Shakur et al., 2002).

### 4.4. Limitations

Findings from isolated cardiomyocyte models have a common limitation, that is, they lack the complex multi-cellular environment and structural elements of the intact heart. Therefore, the demonstrated differences between human and dog myocytes are restricted and influenced by the experimental conditions used in the study. First, in contrast with the whole animal condition, isolated myocytes are "unloaded". Despite the limitation of "no loading", the baseline characteristics and pharmacological responses of human and canine myocytes were recorded under the same experimental conditions, the best approach for side-by-side comparison. Second, we cannot exclude experimental bias in choosing suitable cells for use in these functional studies, e.g., only cells that demonstrated regular and forceful contractions were preferred. A high throughput program (non-manual) which can objectively select large population of myocytes for measurement would be needed to reduce cell selection bias. Third, canine myocytes were isolated and recorded without transportation while human myocytes were ground transported after isolation. The best comparison would be to also have the dog myocytes isolated in the same facility and transported in the same way before recording. Finally, this validation was limited by the small number of clinically relevant compounds tested; a larger data set would be needed to confirm the performance of both myocyte models.

## 5. Conclusions

Our studies have provided critical side-by-side comparison in understanding the similarities and differences in physiology and pharmacology of ventricular myocytes isolated from human and dogs. The overall results indicate that simultaneous measurements of sarcomere shortening and CaT are feasible from isolated human and dog myocytes using IonOptix system, which are valuable in-vitro assays for understanding the mechanism of action in preclinical cardiac safety and efficacy.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The data that has been used is confidential.

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