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Etiology-dependent impairment of relaxation kinetics in right ventricular end-stage failing human myocardium

Jae-Hoon Chung[a,1], Brit L. Martin[a,1], Benjamin D. Canan[a,1], Mohammad T. Elnakish[a], Nima Milani-Nejad[a], Nancy S. Saad[a], Steven J. Repas[a], J. Eric J. Schultz[a], Jason D. Murray[a], Jessica L. Slabaugh[a], Rachel L. Gearinger[a], Jennifer Conkle[a], Tallib Karaze[a], Neha Rastogi[a], Mei-Pian Chen[a], Will Crecelius[a], Kyra K. Peczkowski[a], Mark T. Ziolo[a,b], Vadim V. Fedorov[a,b], Ahmet Kilic[c], Bryan A. Whitson[c], Robert S.D. Higgins[c], Sakima A. Smith[b,d], Peter J. Mohler[a,b,d], Philip F. Binkley[b,d], Paul M.L. Janssen[a,b,d,*]

[a] Department of Physiology and Cell Biology, The Ohio State University, Columbus, OH, United States
[b] Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH, United States
[c] Department of Surgery, The Ohio State University, Columbus, OH, United States
[d] Department of Internal Medicine, The Ohio State University, Columbus, OH, United States

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ABSTRACT

Background: In patients with end-stage heart failure, the primary etiology often originates in the left ventricle, and eventually the contractile function of the right ventricle (RV) also becomes compromised. RV tissue-level deficits in contractile force and/or kinetics need quantification to understand involvement in ischemic and non-ischemic failing human myocardium.

Methods and results: The human population suffering from heart failure is diverse, requiring many subjects to be studied in order to perform an adequately powered statistical analysis. From 2009-present we assessed live tissue-level contractile force and kinetics in isolated myocardial RV trabeculae from 44 non-failing and 41 failing human hearts. At 1 Hz stimulation rate (in vivo resting state) the developed active force was not different in non-failing compared to failing ischemic nor non-ischemic failing trabeculae. In sharp contrast, the kinetics of relaxation were significantly impacted by disease, with 50% relaxation time being significantly shorter in non-failing vs. non-ischemic failing, while the latter was still significantly shorter than ischemic failing. Gender did not significantly impact kinetics. Length-dependent activation was not impacted. Although baseline force was not impacted, contractile reserve was critically blunted. The force-frequency relation was positive in non-failing myocardium, but negative in both ischemic and non-ischemic myocardium, while the β-adrenergic response to isoproterenol was depressed in both pathologies.

Conclusions: Force development at resting heart rate is not impacted by cardiac pathology, but kinetics are impaired and the magnitude of the impairment depends on the underlying etiology. Focusing on restoration of myocardial kinetics will likely have greater therapeutic potential than targeting force of contraction.

1. Introduction

End-stage heart failure impact over 6 million people in the US alone, and is a growing and very costly problem. End-stage heart failure is typically characterized by contractile dysfunction of the ventricles. In vivo, ventricular contractility is impaired resulting in overall reduced pump function. Ventricular contraction is mainly determined by two factors: the number of myocytes contributing to contraction and the average contractile force they produce. In addition to ventricular contraction, the rate (i.e. shortening and re-lengthening speed of the tissue) at which this contractile process takes place is critically important for function; when the contractile and relaxation speed is insufficient, a component of diastolic dysfunction arises and further deteriorates overall pump function.

Investigation of myocardial kinetics of viable human myocardium has been limited [1]. The majority of previous studies have focused on

* Corresponding author at: Department of Physiology and Cell Biology, The Ohio State University, 304 Hamilton Hall, 1645 Neil Avenue, Columbus, OH 43210-1218, USA.
E-mail address: janssen.10@osu.edu (P.M.L. Janssen).
[1] Contributed equally

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contractile strength, thus, contractile kinetics were not always assessed or reported. A second factor is that the diversity in the human population, especially when compared to inbred laboratory mice, increases the variability in assessed parameters. It is thus necessary to investigate a larger number of subjects to achieve the same statistical power as studies using animal-based models. Live contracting human myocardial tissue is logistically much harder to obtain than tissue from animal models of disease, specifically in large numbers required. Thus, most past studies were performed on a relatively small patient/sample population, allowing only for large differences to be detected as statistically significant. Another complicating factor is that to quantify parameters obtained in the failing heart, a comparison with healthy tissue parameters obtained in an identical manner to the failing tissue is paramount. Non-failing viable myocardium is often even more difficult to obtain than failing tissue, and most past studies, including our own, have typically included a very low sample size of non-failing controls, or none at all. Again, the vast differences even among healthy humans (due to size, race, age, gender, etc.) result in a broad spectrum of quantifiable myocardial contraction and kinetic parameters which severely limits statistical power. Although all these past studies [2–16] have made significant contributions to further understanding myocardial dysfunction at the tissue level, the low numbers of subjects, mixed etiologies, lack of non-failing controls, and gender-indifferent studies, have produced conflicting results regarding contractile function and kinetic behavior of end-stage failing myocardium. Moreover, although experimental conditions of most studies were chosen to be close to in vivo conditions, several studies were performed at non-physiological temperatures, or non-physiological pacing rates, possibly further contributing to conflicting results.

Consequently, we lack sufficiently-powered quantitative data on force development and myocardial kinetics of viable RV tissue, as it relates to end-stage failing hearts of ischemic versus non-ischemic etiologies. Moreover, little is known about the impact of gender on human myocardial contractility. Therefore, we set out to quantify the kinetics of relaxation in failing right ventricular myocardium, wherever possible stratified by non-ischemic and ischemic etiology and gender. We performed the study over 7.5 years on a large patient/sample group (44 non-failing hearts, 41 end-stage failing hearts) to allow for the detection of significant, functional differences that would be clinically relevant. Moreover, we investigated the impact of disease on contractile force and kinetics encompassing the three main mechanisms of contractile regulation [17]: the length-dependent activation [18,19], frequency-dependent activation [20], and β-adrenergic stimulation [21].

2. Methods

2.1. Human tissue collection

All explanted hearts were obtained directly in the operating room and immediately flushed with cardioplegic solution after removal from donors/patients as described previously [2,22]. The hearts were transferred to the laboratory (within 10–30 min) in cold cardioplegic solution containing (in mM): 110 NaCl, 16 KCL, 16 $MgCl_2$, 10 $NaHCO_3$, and 0.5 $CaCl_2$. All hearts were procured and treated with identical protocols, solutions and timing regardless of their source. All human tissues were experimented on with approval from the Institutional Review Board (IRB) of The Ohio State University and conform to the declaration of Helsinki. Informed consents were acquired from cardiac transplant patients. All end-stage failing hearts ($n = 41$) were acquired from patients in the operating room undergoing cardiac transplantation at The Ohio State University Wexner Medical Center. Non-transplantable donor hearts ($n = 44$) were acquired in the operating room in collaboration with Lifeline of Ohio Organ Procurement. The biometric characteristics of these hearts are provided in Tables 1 and 3. Characteristics of the dimensions of the hearts, as well as wall-thickness is given in Tables 2 and 4. The age of the donors was 48 ± 15 (SD) years, the patients with failing ischemic hearts were 59 ± 7 years old, and the failing non-ischemic patients were 53 ± 10 years of age. Body mass index was also similar: 29.6 ± 8.0, 26.8 ± 4.3, and 29.4 ± 4.3 resp. Heart weight, on average, was 19.7% higher in failing hearts compared to the donor hearts, with no significant difference by etiology.

Table 1
Characteristics of non-failing hearts.

ID #	Sex	Etiology	Age	Race	BMI	Heart weight (g)
118,258	M	Non-Failing	38	Caucasian	29.9	575
147,381	M	Non-Failing	58	Caucasian	32.1	512
156,910	F	Non-Failing	62	Caucasian	35.5	478
168,021	F	Non-Failing	62	Caucasian	26.0	896
219,852	F	Non-Failing	30	Caucasian	24.2	299
240,603	F	Non-Failing	51	Caucasian	23.9	320
294,050	M	Non-Failing	23	Caucasian	33.9	462
313,956	F	Non-Failing	38	Caucasian	31.0	406
331,253	F	Non-Failing	42	Hispanic	29.6	390
364,587	M	Non-Failing	19	Caucasian	24.5	300
380,071	F	Non-Failing	43	Caucasian	60.9	603
394,176	M	Non-Failing	57	Caucasian	32.0	748
402,879	M	Non-Failing	54	Caucasian	30.9	474
415,217	M	Non-Failing	42	Caucasian	28.6	508
435,578	M	Non-Failing	20	Caucasian	25.9	324
442,404	M	Non-Failing	69	Caucasian	40.3	659
452,192	F	Non-Failing	55	African American	24.5	350
460,025	F	Non-Failing	69	Caucasian	29.6	435
474,083	F	Non-Failing	41	African American	37.7	
476,074	F	Non-Failing	29	Caucasian	20.2	271
481,041	M	Non-Failing	41	African American	20.3	455
481,043	F	Non-Failing	65	Caucasian	26.0	451
488,240	F	Non-Failing	51	Caucasian	39.7	
507,207	F	Non-Failing	72	Caucasian	30.5	456
514,489	F	Non-Failing	42	Caucasian	28.3	327
600,245	F	Non-Failing	51	Caucasian	20.6	507
618,200	F	Non-Failing	58	Caucasian		499
632,941	F	Non-Failing	68	Caucasian	31.1	402
685,884	M	Non-Failing	36	Caucasian	25.2	415
694,855	F	Non-Failing	46	Caucasian	22.3	356
712,301	M	Non-Failing	67	Caucasian	29.2	527
749,693	M	Non-Failing	65	African American	20.8	643
753,820	F	Non-Failing	43	African American	20.9	605
785,258	F	Non-Failing	51	Caucasian	23.0	335
809,108	M	Non-Failing	60	Caucasian	33.3	842
845,013	M	Non-Failing	26	Caucasian	24.1	497
872,295	M	Non-Failing	56	Caucasian	31.5	539
921,821	M	Non-Failing	22	African American	26.3	383
925,852	F	Non-Failing	34	African American	50.4	672
947,200	F	Non-Failing	63	Caucasian	34.5	608
958,987	M	Non-Failing	40	Caucasian	33.7	615
984,478	F	Non-Failing	54	African American	21.1	348
987,692	F	Non-Failing	34	Caucasian	29.0	313

2.2. Trabeculae isolation

The RV of each heart was transferred from the cardioplegic solution to a cold modified Krebs-Henseleit solution (K-H) bubbled with 95% O_2–5% CO_2 containing (in mM): 137 NaCl, 5 KCl, 0.25 $CaCl_2$, 20 $NaHCO_3$, 1.2 NaH_2PO_4, 1.2 $MgSO_4$, 10 dextrose, and 20 BDM (2,3-butanedione monoxime) and pH of 7.4. Linear, small, and free-running trabeculae were isolated with the aid of a stereo dissection microscope, and kept in this solution at 0–4 °C until the time of the experiment. Thin, free running trabeculae were chosen since they contain all cell-types found in the heart, and their mechanical properties very closely match those of the ventricle, including inotropic properties of ejection [23], and they have a long experimental life [24], whereas papillary muscles (often used in studies using mice and rats), are way too big in humans to ensure a properly oxygenated preparation. Muscles were transferred into custom-made setups as previously described for animal models [25] and the perfusion solution was changed to another modified K-H without BDM. This solution was maintained at 37 °C and