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Right Ventricular Myofilament Functional Differences in Humans with Systemic Sclerosis-associated versus Idiopathic Pulmonary Arterial Hypertension

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Abstract

Background—Patients with systemic sclerosis-associated pulmonary arterial hypertension (SSc-PAH) have a far worse prognosis than those with idiopathic PAH (IPAH). In the intact heart, SSc-PAH exhibits depressed rest and reserve right ventricular (RV) contractility as compared to IPAH. We tested whether this disparity involves underlying differences in myofilament function.

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DISCLOSURES

- R.J.T. is a member of an Abbott Pulmonary Hypertension Study steering committee (modest) and is an Actelion core lab consultant (significant).
- All other authors have no relevant disclosures to report.

Methods—Cardiac myocytes were isolated from RV septal endomyocardial biopsies from patients with SSc-PAH, IPAH, or SSc with exertional dyspnea but no resting PAH (SSc-d); control RV septal tissue was obtained from non-diseased donor hearts (6–7/group). Isolated myocyte passive length-tension and developed tension-calcium relationships were determined and correlated with in vivo RV function and reserve. RV septal fibrosis was also examined.

Results—Myocyte passive stiffness from length-tension relations was similarly increased in IPAH and SSc-PAH over control, although SSc-PAH biopsies had more interstitial fibrosis. More striking disparities were found between active force-calcium relations. Compared to controls, maximal calcium-activated force (F_{max}) was 28% higher in IPAH but 37% lower in SSc-PAH. F_{max} in SSc-d was intermediate between control and SSc-PAH. The calcium concentration required for half-maximal force (EC_{50}) was similar between control, IPAH, and SSc-d, but lower in SSc-PAH. This disparity disappeared in myocytes incubated with the active catalytic subunit of protein kinase A. Myocyte F_{max} directly correlated with in vivo RV contractility assessed by end-systolic elastance (Ees, R^2=0.46, P=0.002) and change in Ees with exercise (R^2=0.49, P=0.008), and was inversely related with exercise-induced chamber dilation (R^2=0.63, P<0.002), the latter also a marker of depressed contractile reserve.

Conclusions—A primary defect in human SSc-PAH resides in depressed sarcomere function, whereas this is enhanced in IPAH. These disparities correlate with in vivo RV contractility and contractile reserve, and are consistent with worse clinical outcomes in SSc-PAH. The existence of sarcomere disease prior to developing resting PAH in SSc-d patients suggests that earlier identification and intervention may prove useful.

Keywords

RV; pulmonary arterial hypertension; scleroderma; myofilament; PKA; tension

BACKGROUND

Pulmonary arterial hypertension (PAH) is a progressive disorder that imposes high resistive and pulsatile loads on the right ventricle (RV). The extent of RV compensation is a major determinant of PAH clinical outcome, and its decompensation is a primary cause of death.[1,2] Among World Health Organization Group I PAH subtypes, systemic sclerosis (SSc)-associated PAH carries a particularly poor prognosis.[3] Systemic sclerosis, or scleroderma (SSc), is an autoimmune inflammatory disorder characterized by excess fibrosis and depressed microvascular perfusion and angiogenesis, and affects multiple organs including the ventricular myocardium.[4] As compared to patients with idiopathic PAH (IPAH), SSc-PAH subjects are less responsive to therapy[3,5] and display higher pro-brain natriuretic peptide levels, indicative of increased myocardial stress.[6]

Conventional measures of RV function, including echocardiographic indices, invasive hemodynamic assessments of afterload and compliance, ejection fraction, RV dimensions and mass do not distinguish between SSc-PAH and IPAH.[4,7–9] However, in 2013 we reported that SSc-PAH patients have an intrinsic defect in RV chamber contractility as compared to IPAH, and that this results in adverse RV-pulmonary artery coupling at rest.[7] In a subsequent study, we showed SSc-PAH subjects also have depressed RV force-frequency

responses and RV contractile reserve with exercise versus IPAH.[8] A consequence of this disparate reserve is that the RV dilates during exercise in SSc-PAH but not IPAH patients.[8] In contrast to systolic measures, diastolic chamber abnormalities, including relaxation and end-diastolic pressure were similarly altered in both groups.

These studies turned a spotlight away from the pulmonary vasculature and more on the RV as a critical pathophysiologic feature of SSc-PAH. Many factors could underlie the contractile differences, though the presence of systolic depression without corresponding relaxation defects in SSc-PAH[7] led us to hypothesize that the sarcomere may itself be prominently involved. The dependence of myofilament force on calcium has only been reported in a few PAH studies, with only one conducted with human tissue, and the results have varied.[10–12] Pulmonary artery constriction in the rat lowered maximal Ca^{2+}-activated tension,[11] whereas this was unaltered in hypoxia-induced PAH induced in calves,[10] and was increased in human PAH.[12] The human study mostly examined tissue from patients with PAH from congenital heart disease, and did not include any with SSc-PAH.

The current study tested the hypothesis that RV sarcomere defects differentiate SSc-PAH from IPAH, and predict intact RV contractility and reserve function. As echo-Doppler studies have reported RV defects in SSc patients without PAH,[13] we also examined myocardium from SSc patients who presented with exertional dyspnea but no resting PAH (SSc-d). Control heart RV septal tissue was provided from explanted but unused donor hearts. We find a striking depression of RV maximal Ca^{2+}-dependent tension in SSc-PAH, directionally opposite to increased tension in IPAH. SSc-d had intermediate abnormalities. Maximal sarcomere tension corresponds with intact RV rest and reserve function measured in the same patients. Thus, defects of the RV sarcomere are a core feature differentiating two of the most common forms of PAH, and pose a potential therapy target for patients with SSc-PAH and SSc-d.

METHODS

Study Subjects

We prospectively enrolled 24 patients clinically referred for right heart catheterization (RHC) to evaluate pulmonary arterial hypertension (PAH) or exercise-induced PH at the Johns Hopkins Hospital (JHH) from November 2012 to March 2017. SSc-PAH patients and SSc patients with exertional dyspnea but without resting PAH (SSc-d) were enrolled, as were subjects with idiopathic PAH (IPAH). Diagnostic and exclusionary criteria for these groups have been previously described.[8] All patients consented to the procurement of endomyocardial biopsies from the RV septum, obtained under echocardiographic guidance. A subset of patients separately consented to invasive right ventricular pressure-volume (PV) relation analysis, with data measured both at rest and during supine bicycle exercise. Details of the PV methods and intact patient results have been previously reported.[8] Endomyocardial tissue was obtained in 7 IPAH patients, 11 SSc-PAH patients, 6 SSc-d patients, and all compared to 7 RV septal samples obtained from age-matched control donor hearts. The protocols were approved by the Johns Hopkins University Institutional Review Board (IRB), and informed consent obtained in all patients. Not all procedures and assays could be performed in each subject (details in Supplemental Table 1). Donor RV septum was

obtained using cold-cardioplegia and open chest surgical excision, and tissue rapidly dissected and frozen. The University of Pennsylvania IRB and the Gift-of-Life donor program in Philadelphia, PA approved the donor tissue protocol, with consent for research use of donated heart tissue obtained from the families of the deceased. The data and study materials will not be available to other researchers as they are sensitive human subjects data.

Study Design

Demographic information and clinical characteristics of the study group were obtained prospectively by PH-specialists employing standardized assessments. Six-minute walk tests and other laboratory data were measured either at or within six months prior to RHC. After routine catheterization study, 3–4 endomyocardial biopsies (~2–3 mg/biopsy) were obtained from the RV septum using a 7 Fr endomyocardial bioptome (Argon Medical, Plano, TX). Each piece was rapidly frozen in liquid nitrogen immediately upon removal, with one first placed in OCT (optimal cutting temperature) solution prior to freezing to improve tissue preservation. This piece was used for the myofilament studies. In a subset of patients another piece was placed in formalin for histopathology. Flash frozen donor RV septal tissue was shipped by overnight express and analyzed in our laboratory.

Invasive PV assessments at rest and during supine bicycle ergometry were performed as previously described.[8] PV analyses include data from these as well as additional patients, the latter obtained using the Inca System (Millar, Houston, TX) and CD Leycom PV catheters (RP-CA-41103-PN, Millar, Houston, TX), as the previously employed catheters were discontinued.[8] All PV data were analyzed with custom WinPVAN software.

Myofilament Function

Myocardium was frozen at −140°C until studied. Methods for force-calcium analysis of biopsy-derived skinned myocytes have been previously reported.[14] Briefly, biopsies are incubated with 0.3% Triton X-100 in isolation buffer with protease inhibitors (Sigma-Aldrich, St. Louis, MO) and phosphatase inhibitors (PhosSTOP, Roche Applied Science, Penzberg, Germany) and then homogenized by low-speed pulverization to generate a skinned cellular preparation. After washing in isolation buffer, myocytes are affixed to a force transducer-length controller (Aurora Scientific, Ontario, Canada) using ultraviolet-activated adhesive (Norland Products, Cranbury, NJ) (Supplemental Figure 1). Sarcomere length (SL) measured by Fourier transform of digital images (IPX-VGA210, Imperx, Boca Raton, FL) is adjusted by micro-manipulators (Newport, Corporation, Irvine, CA). Force was normalized to myocyte cross sectional area (CSA) to derive tension expressed as mN/mm^2.

Passive tension-SL relations were measured in relaxing solution (0 μM Ca^{2+}) over a range of SL. SL was then set to 2.1 μm, and active tension-Ca^{2+} relations generated by varying Ca^{2+} concentration from 0–46.8 μM (latter being saturating conditions). Steady-state force versus log[Ca^{2+}] (F-Ca^{2+}) plots were fit to the Hill equation: $F = F_{max} \times Ca^{h}/(EC_{50}{}^{h} + Ca^{h})$ yielding maximal force (F_{max}, μN), calcium sensitivity (EC_{50}, i.e. [Ca^{2+}] required to achieve 50% maximal force, μM), and cooperativity (Hill) coefficient (h). In a subset of myocytes,

we exposed cells to the active catalytic subunit of Protein Kinase A (0.125 units/ml, 20 minutes; Sigma-Aldrich) and repeated measurements of the F-Ca^{2+} dependence.

Histology

Heart biopsies fixed in 10% formalin were embedded in paraffin, and 10 μm sections cut and stained with Masson Trichrome. Histology was analyzed blinded to group using Aperio Imagescope (Leica Biosystems, Nussloch, Germany) and percent fibrosis determined by calculating the total area of blue-hued staining as a percentage of total area. Standardized settings (hue 0.66 and threshold 0.33) were used to define positive fibrosis staining.

Statistical Analysis

Data are presented as mean ± standard deviation in the tables and text, and mean ± standard error of the mean in figures, unless otherwise indicated. Parametric tests were used when possible; non-parametric tests were employed when normality criteria was not met. Fibrosis, as defined by an excess of 2.5% of the biopsy area, was compared between groups using Fisher's exact and Kruskal-Wallis analysis of variance (ANOVA). Passive tension at each sarcomere length was compared between groups using one-way ANOVA with post-hoc testing using the Tukey test. For cell mechanics studies, 2–3 myocytes were tested from each biopsy (majority was 3 per biopsy) yielding a total of 68 myocytes. Hill equation parameters (F_{max}, EC_{50}, h) and myocyte cross-sectional area (CSA) were compared by a mixed-model ANOVA to account for the random effects of multiple myocyte measurements per individual biopsy. Post-hoc comparisons were done using the Tukey test. Group mean comparisons were made using Wilcoxon rank sum testing, while proportions were compared with Fisher exact tests. Within disease groups, the effect on measurements pre- and post-protein-kinase A (PKA) was tested using paired student T-test; group comparisons and differential PKA effects between disease groups were tested using two-way ANOVA and an interaction term between disease and PKA. Myocyte F_{max} was compared to in vivo pressure-volume parameters were made using Pearson correlation coefficients and linear regression. Statistical analysis and graphical depiction were performed using commercial software (Stata-15, StataCorp, College Station, TX; Prism 7, Graphpad, La Jolla, CA).

RESULTS

Study Population

Demographic and clinical data for each patient group are provided in Supplemental Table 2. The groups were matched in nearly all parameters, with somewhat fewer female controls and somewhat greater body surface area in IPAH subjects. Clinical and cardiac magnetic resonance-based features of IPAH and SSc-PAH subjects studied in the current cohort were very similar to that previously reported.[7,8] Resting RV contractility as assessed by ventricular end-systolic elastance (Ees), and right ventricular-pulmonary arterial (RV-PA) coupling assessed by the ratio of Ees to arterial elastance (Ees/Ea), were both lower in SSc-PAH compared to IPAH (Table 1). SSc-d subjects had significantly lower right atrial and pulmonary artery pressures and pulmonary afterload (resistance and Ea) as compared to SSc-PAH and IPAH, and their Ees and Ees/Ea were similar to that in IPAH (Table 1). Measures of in vivo diastolic function (time constant of relaxation and peak flow rate

normalized to EDV) were similar between groups. When available, the hemodynamic response to exercise is also provided in Table 1. Both PAH groups displayed abnormal pulmonary artery pressure rise. SSc-d patients did not have resting PH, but on average displayed exercise-induced PH, as defined by total pulmonary resistance >3 Wood Units and mean pulmonary artery pressure >30 mmHg during exercise.[15,16]

Passive myocyte stiffness is similarly increased in SSc-PAH and IPAH

Interstitial fibrosis is observed in patients with PAH, and is considered a particular feature of SSc. Interstitial fibrosis in formalin fixed endocardial biopsies exceeded a threshold of 2.5% in two-thirds of SSc-PAH subjects but in none of IPAH or SSc-d patients ($P<0.01$ by Fischer Exact Test; $P<0.05$ for Kruskal-Wallis test comparing the 3 groups) (Figure 1A-B). Tissue stiffness could not be assessed from the biopsy, but isolated myocyte stiffness was possible. Myocytes were stiffer compared to both forms of PAH versus controls ($P<0.05$ from sarcomere lengths of 2.2 to 2.6 μm), but this increase was virtually identical between SSc-PAH and IPAH ($P>0.75$ from sarcomere lengths of 2.2 to 2.6 μm) (Figure 1C).

Maximal Tension and Calcium Sensitivity Differs between SSc-PAH, IPAH, and SSc

Unlike passive myocyte stiffness, we found marked differences in active F-Ca^{2+} dependence between the PAH groups, each being different from controls (Fig. 2A). Maximal Ca^{2+}-activated tension (e.g. normalized maximal force, or F_{max}) was 28% higher in IPAH versus controls (23.4±2.7 vs. 18.2±2.7 mN/mm^2, $P<0.05$), whereas in SSc-PAH, F_{max} was depressed by 37% (11.4±2.6 mN/mm^2, $P<0.05$ versus both other groups). The calcium required for half-maximal activation (EC_{50}) was similar between IPAH, controls, and SSc-d (1.73±0.21, 1.85±0.25, and 1.88±0.28 μM respectively, $P=0.35$); however SSc-PAH had a lower EC_{50} (1.35±0.26 μM, $P<0.05$), shown by the left-shifted F-Ca^{2+} relation when data are normalized to their respective F_{max} (Figure 2B). Figure 2C compares SSc-d to SSc-PAH and controls. SSc-d displayed an intermediate F_{max} (15.1±3.5 mN/mm^2, $P<0.05$ vs. control and SSc-PAH); other parameters were similar to control. Summary Hill equation parameters for all groups are provided in Figure 2D. Myocyte cross-sectional area is also shown and was similar among the four groups (Figure 2D). Human myocyte resting length in our experimental setup was generally 1.70 to 1.80 μm.

Protein Kinase A Exposure Equalizes Calcium Sensitivity Among Patient Groups

In both LV and RV failure, increased Ca^{2+} sensitivity (reduced EC_{50}) has been reported and linked to depressed protein kinase A (PKA) phosphorylation of myofilament proteins, notably of troponin I.[10,17,18] Only SSc-PAH patients displayed this, and we hypothesized this too might reflect depressed PKA activation. Myocytes from both PAH groups and controls were therefore further exposed to the catalytically active PKA subunit, and data before and after exposure compared. In IPAH and controls, PKA increased EC_{50} slightly ($P<0.05$ versus non-PKA); however this rise was significantly greater in SSc-PAH myocytes, resulting in near-identical EC_{50} values after PKA exposure (P-value for interaction between PAH group and PKA treatment = 0.002; Figure 3A-B). F_{max} was unaltered by PKA (pre- vs. post PKA F_{max} in controls, 17.3±1.0 vs. 17.2±1.0 mN/mm^2, $P=0.8$; IPAH, 22.4±0.7 vs. 22.3±0.7 mN/mm^2, $P=0.8$; SSc-PAH, 10.0±1.1 vs. 11.7±1.7 mN/mm^2; $P=0.4$).

PKA also reduces passive myocyte tension by phosphorylating the sarcomeric protein titin,[19] and reduced titin phosphorylation has been implicated as a mechanism of increased passive myocyte stiffness in the RV of patients with PAH.[12,18] We therefore assessed the impact of PKA in both PAH groups by change in passive tension at a fixed SL of 2.1 μm. This similarly declined after PKA in both PAH groups, but was unchanged in controls ($P<0.05$ for both, Figure 3C).

F_{max} Predicts RV Rest Contractility and Exercise-Reserve Response

To test if the lower F_{max} in SSc-PAH, partial decline in SSc-d, and rise in IPAH correlated with in vivo RV systolic function, we regressed F_{max} and resting chamber contractility (Ees), the latter derived from invasive RV pressure-volume analysis in the same patients. Figure 4A shows these variables directly correlated (R^2 =0.46, P=0.002). We also tested if F_{max} correlated with RV exercise performance. As previously reported,[8] RV contractile reserve (change in Ees) is depressed in SSc-PAH subjects. This was associated with chamber dilation (higher RV end-diastolic volumes, RV EDV) at submaximal exercise whereas IPAH patients showed no dilation at the same exercise level (Stage 2, 25 Watts). F_{max} positively correlated with Ees change during exercise (R^2=0.49, P=0.008), and inversely correlated with chamber dilation (%Δ RV EDV, R^2=0.63, P<0.002).

DISCUSSION

The current study reveals that patients with SSc-PAH have depressed sarcomeric function manifest prominently by a substantial decline in maximal force-calcium dependence. The exact opposite, an increase in this dependence, is observed in IPAH. This disparity parallels and predicts corresponding differences in global RV chamber systolic function at rest and upon exertional demand. As the latter has been found to correlate with clinical outcome, the new results surprisingly focus the spotlight on the RV sarcomere rather than pulmonary vasculature in explaining SSc-PAH versus IPAH differences. This is further strengthened by the finding that SSc-d patients who do not yet have resting PAH also exhibit abnormal sarcomere function. While prior efforts to treat both SSc-PAH and IPAH have focused on pulmonary load, our results suggest tailored treatment focusing on RV sarcomeric disease maybe particularly useful for SSc-PAH.

To our knowledge, only one prior study by Rain and colleagues has reported on human RV force-calcium relations in PAH.[12] In this report, the authors found myocytes were passively stiffer, much as we observed. From their F-Ca^{2+} data, they found F_{max} was ~40% above that in non-failing controls, and EC_{50} was slightly lower. This study primarily examined tissue from congenital heart disease-PAH patients; with only 3/11 having IPAH and none with SSc-PAH. Congenital heart disease involves complex mechanical loads and chronic disease history, so comparisons with the current IPAH and SSc-PAH groups are difficult. The prior study was also based on tissue obtained at time of heart-lung transplantation, so patients all had end-stage disease.[12] The current results are the first to include both SSc-PAH and SSc-d, and perform analysis from heart biopsies reflecting an earlier disease stage. Our findings in IPAH are similar to those of Rain et al.,[12] suggesting this form of PAH retains RV sarcomere compensation even at late stages.

In any form of heart disease involving marked increases in chamber load, the net result depends on the interaction between load severity and the myocardium. As pulmonary arterial load was similar in both PAH groups, unique RV features linked to SSc would appear more important for the specific maladaptive response and disease severity. Whether SSc patients without any abnormal pulmonary vascular load have similar sarcomere defects remains unknown, as these patients are not referred for RHC or biopsy. However, non-invasive echo Doppler data such patients have found sub-clinical systolic and diastolic defects based on strain-based measurements.[13] The intermediate defect in SSc-d may indicate that even intermittent PAH as induced during exercise is sufficient to manifest a sarcomere defect. While the underlying cause remains unknown, SSc-related immune activation with vasculopathy and inflammation could be factors leading to damaged myofibrillar proteins.

The EC_{50} is actively regulated by post-translational changes in regulatory thin filament proteins including troponins I, C, and T (TnI, TnC, TnT), myosin binding protein C, myosin light chain, and tropomyosin.[20] It is controlled by multiple kinases, including phosphorylation by PKA,[17] PKG,[21] PKC,[22] PKD,[23] and AMPK[24] (particularly targeting TnI and TnT), and by oxidation,[25] nitrosylation, and acetylation.[26] Many of these factors lead to Ca^{2+}-desensitization (increase EC_{50}), although some (e.g. TnI phosphorylation by AMPK and PKCβ2) result in sensitization. EC_{50} often declines with LV failure, and this has been attributed to reduced TnI phosphorylation by PKA due to down-regulation of β-adrenergic signaling, since PKA activation restores it to control levels.[17] Such sensitization may in part compensate for depressed calcium transients in heart failure, but it may also worsen diastolic dysfunction.[27] Evidence that RV PKA deficiency occurs with PAH has been reported.[10,12,18] We found evidence for PKA deficiency in active force-Ca^{2+} dependency in SSc-PAH, as observed in forms of LV failure. This suggests another component of abnormal systolic reserve in these patients versus IPAH, perhaps related to greater inhibition of sympathetic stimulation. This remains to be tested in vivo. PKA-effects on passive myocyte stiffness were similar between groups, though our capacity to detect differences may have been limited by having restricted the analysis to a SL of 2.1 μm, where stiffness differences are slight.

Unlike EC_{50} or passive stiffness, F_{max} is not modified by PKA, and indeed most kinases that alter sarcomere function leave F_{max} intact. Prior studies have found that F_{max} is depressed by proteolysis of regulatory thin filament proteins and perturbations in structural sarcomere integrity.[28] For example, F_{max} declines after ischemic injury in conjunction with troponin I proteolysis by calpain-1 at the C-terminus.[29,30] Mice expressing a mutant TnI with this truncation exhibit a depressed F_{max} and develop heart failure[31] Another protein that can undergo destructive proteolysis is myosin binding protein C, and in this case, the cleaved by-product serves as a poison peptide to depress F_{max}.[32] F_{max} also declines with oxidative stress,[33] and in a canine tachypacing heart failure model, reduced F_{max} was correlated with structural disruption of the sarcomere with reduced force generation by ~40% of myofibrils.[34] At present, we do not know what modifies F_{max} in SSc-PAH and to a smaller extent in SSc-d, but ongoing studies in additional patients hope to reveal factors to further guide precision therapy. However, even without a specific molecular explanation for the F_{max}

defect in SSc-PAH, its presence and correlation to intact RV physiology suggests that small molecule inotropes designed to enhance sarcomere function[20] may be particularly useful.

We previously described that the RV contractile response to faster heart rates (force-frequency response, FFR) and calcium cycling also predicted to be diminished in SSc-PAH versus IPAH.[8] However, as calcium transients could not be measured in vivo, we were unable to test if these defects were due to calcium transient disparities or to abnormal sarcomere responses to calcium. The F_{max} and EC_{50} disparities between IPAH and SSc-PAH shown here would be adequate to explain FFR differences as well, since the latter depends on levels of stimulating Ca^{2+}. This was shown in a study employing a calcium sensitizer that modifies F_{max} but not Ca^{2+} cycling to improve FFR.[35] Thus, improving sarcomere dysfunction in SSc-PAH should also help frequency dependent systolic reserve.

Diastolic stiffening of PAH RV myocytes has been described,[12,18] and our results are concordant with such data. Chamber stiffness combines properties of myocytes, the interstitium, and extrinsic factors (e.g. RV-LV interaction, pericardium). In this regard, our in vivo analysis has not revealed significant disparities between IPAH and SSc-PAH. While SSc-PAH had more endocardial fibrosis, this could be consistent with an autopsy study which found SSc fibrosis to be disproportionately reflected in the endocardium and interventricular septum.[36] By contrast, we found T2-weighted cardiac magnetic resonance imaging analysis of fibrosis was similar between IPAH and SSc-PAH.[8] Myocyte stiffness is also similarly increased in both diseases, supporting the net results in intact patients. Thus, while subtle differences may still exist, it is unlikely that diastolic properties are a primary contributor to worsened RV function or clinical course in SSc-PAH.

This study has several limitations. Given its invasive nature, endomyocardial biopsies from healthy controls could not be obtained. The RV septal tissue from non-failing donor hearts can be impacted by the patient history, anesthesia, neurohormonal activation, and other conditions at time of organ procurement. We did not assess the RV free wall given the higher risk of myocardial perforation, but prior studies suggest homogeneity of myofilament function from multiple anatomic sites within a given RV.[10] While the individual sample sizes (number of hearts) were relatively small for each group, we analyzed many cells, which confer their own variability given the extraction procedure. Our analysis was adjusted for the random effects of multiple measurements per biopsy piece. Our ability to correlate the individual results to the same subject’s intact heart data strengthens the conclusions.

Conclusion

We demonstrate that in contrast to the hyper-contractile response of IPAH myofilaments, RV myofilaments from SSc-PAH exhibit a marked decrease in maximal calcium-activated force and abnormal increase in calcium sensitivity. These findings are strengthened by their correlation to measures of in vivo RV contractility and reserve. Abnormalities in calcium sensitivity, but not maximal calcium-activated tension, were restored by PKA. The presence of intermediate defects in SSc-d further supports a primary influence of SSc on the RV. Together, these data highlight the critical role of RV sarcomere dysfunction in SSc-PAH and suggest that the SSc-PAH RV is characterized by a phenotype of RV failure, not compensation. Targeted therapies to enhance RV myofilament function in this PAH

subgroup, with more vigilant surveillance and perhaps early intervention in borderline SSc patients, should be explored.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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CLINICAL PERSPECTIVE

What is new?

- Right ventricular (RV) myofilaments isolated from human subjects with systemic sclerosis-associated pulmonary arterial hypertension (SSc-PAH) exhibit diminished contractile force and abnormal calcium sensitivity versus control myofilaments, in contrast to hyper-contractile compensation in idiopathic pulmonary arterial hypertension.
- SSc patients with dyspnea and only exercise-induced pulmonary hypertension (PH) exhibited an intermediate RV myofilament phenotype.
- These myofilament contractile abnormalities correlate strongly with in vivo right ventricular (RV) function at rest and RV contractile reserve during exercise, suggesting a central role of RV myofilament dysfunction in SSc-PAH.

What are the clinical implications?

- Among patients with PAH, those with SSc-PAH suffer disproportionately poor outcomes, even with treatment using standard PAH-directed therapies.
- These findings uncover key deficiencies in the SSc-PAH RV and suggest that therapies targeted at RV myofilament contractile dysfunction may prove particularly useful for this vulnerable PAH sub-population.
- Furthermore, SSc patients with only exercise-induced PH would appear to comprise an at-risk population that may warrant improved screening and treatment paradigms.

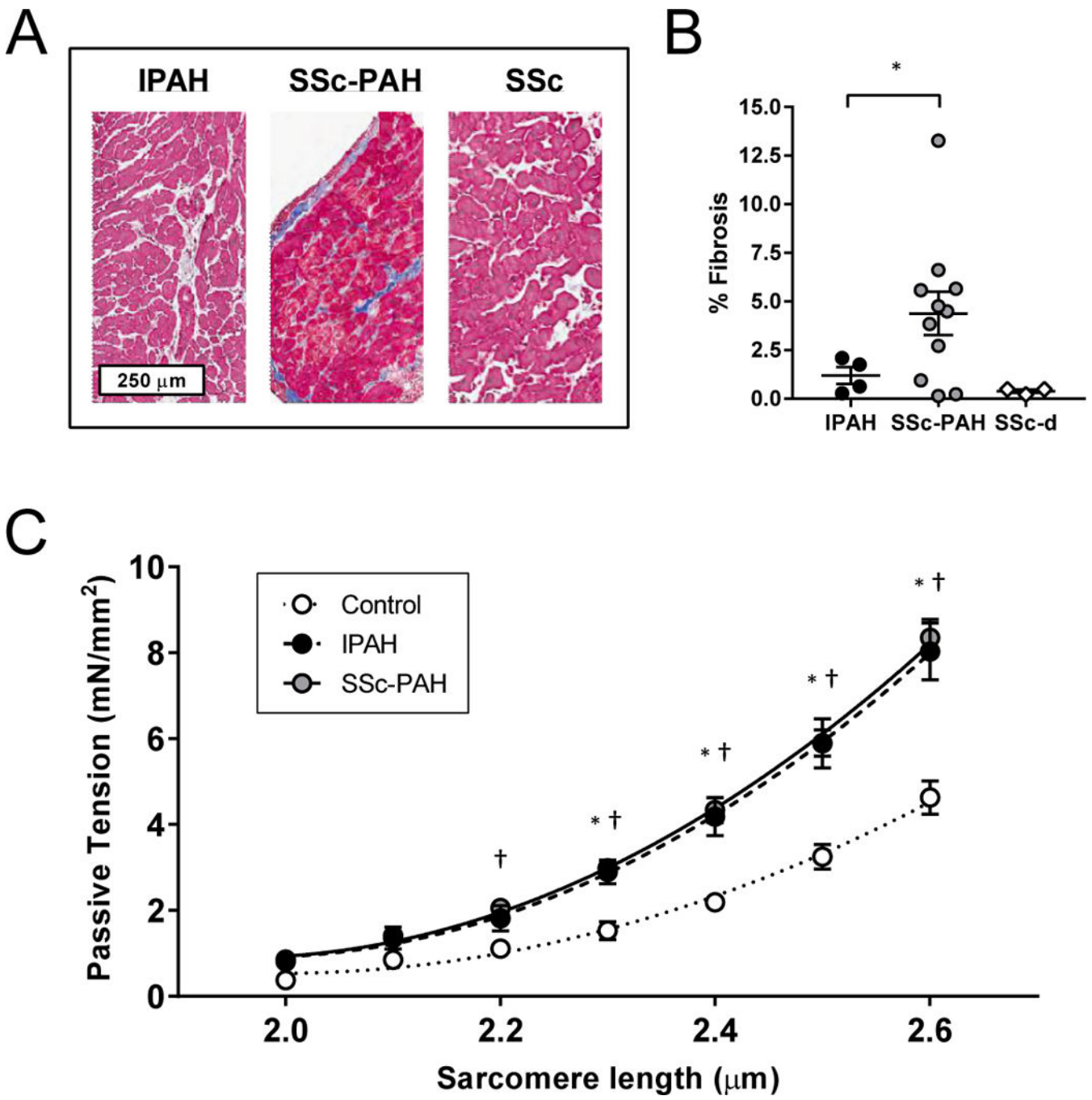

Figure 1.

Effects of SSc-PAH versus IPAH on passive myocyte tension and fibrosis

(A) Representative Masson Trichrome histologic images from IPAH, SSc-PAH, and SSc. (B) Histologic fibrosis measured by Masson Trichrome as a percentage of total biopsy area was increased in SSc-PAH versus IPAH or SSc-d, based on a threshold of 2.5% fibrosis. (C) Passive tension as a function of escalating sarcomere length was measured in IPAH, SSc-PAH, and non-failing control myocytes (n=5 subjects/group). Passive tension between all three groups were compared using one-way analysis of variance (ANOVA) at each SL. *

post-hoc P<0.05 between Control and IPAH; † post-hoc P<0.05 between Control and SSc-PAH.

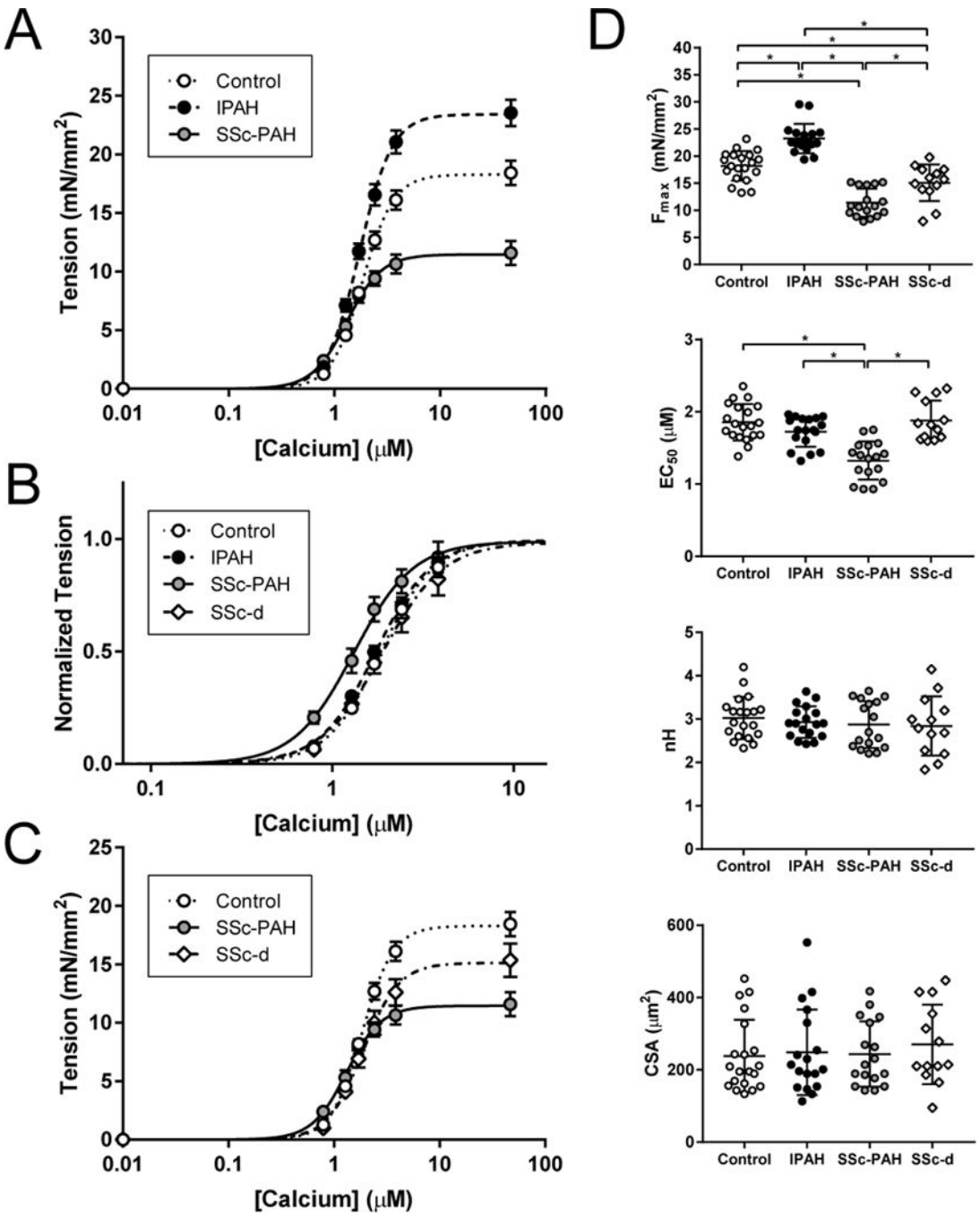

Figure 2.

Force-Calcium curves in Control, IPAH, and SSc myofilaments

(A) Force-calcium data were obtained in non-failing control, IPAH, and SSc-PAH myocytes to obtain maximal calcium-activated tension (F_{max}) and calcium sensitivity (EC_{50}) (n=7 subjects/group). (B) Data were normalized to maximal tension to illustrate shifts in calcium sensitivity (measured as EC_{50}, or the calcium concentration necessary for 50% maximal activation). (C) Myofilament force-calcium data of control, SSc-PAH, and SSc subjects free of PAH (SSc-d). (D) F_{max}, EC_{50}, Hill coefficient (*h*), and myocyte cross-sectional area

(CSA) data; data presented as mean ± SD. F_{max} was increased in IPAH versus controls, whereas it was decreased in SSc-PAH versus both control and IPAH; in SSc-d, F_{max} was intermediate between control and SSc-PAH. EC_{50} was decreased in SSc-PAH versus control, IPAH, and SSc-d. There was no significant difference between groups in Hill coefficient or myocyte cross-sectional area (CSA). * significant difference in post-hoc comparison.

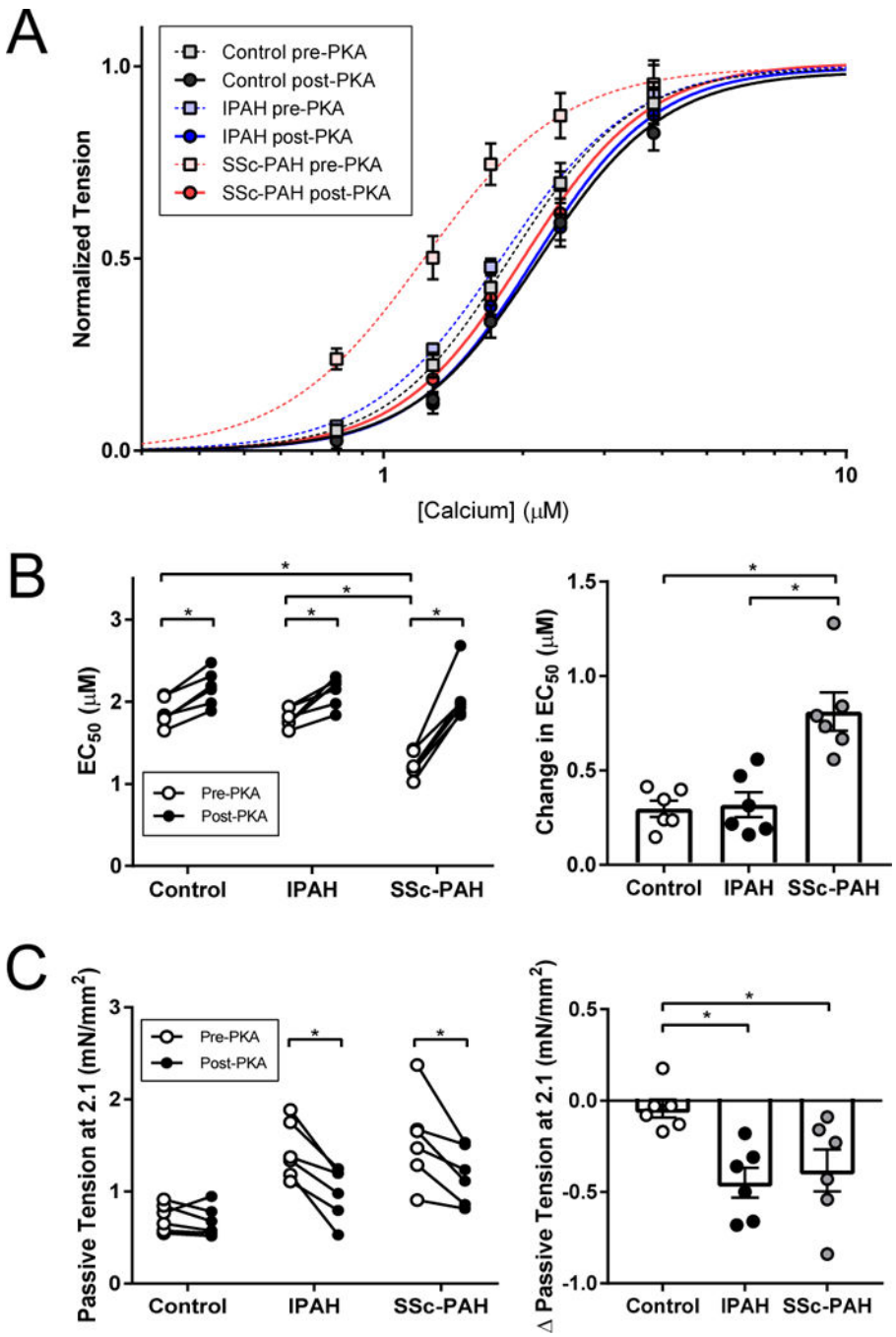

Figure 3.

Effect of PKA treatment on myofilament function and passive myocyte stiffness

(A) Myofilaments from a subset of control, IPAH, and SSc-PAH subjects (3 subjects/group) were directly incubated with protein kinase A (PKA). (B) Baseline calcium sensitivity (EC_{50}) in all three groups mirrored trends of the larger cohort. With PKA, there was a slight increase in EC_{50} in control and IPAH. On the other hand, PKA completely markedly increased EC_{50} in the SSc-PAH group to control levels. Change in EC_{50} was significantly greater in SSc-PAH than in both control and IPAH (Disease × group interaction P=0.002).

(C) PKA treatment led to a decrease in passive tension, at a sarcomere length (SL) of 2.1 μm, in both IPAH and SSc-PAH, but no change in controls. There was no significant difference in PKA-mediated passive tension change between IPAH and SSc-PAH. * $P<0.05$; post-hoc P-value applied where applicable.

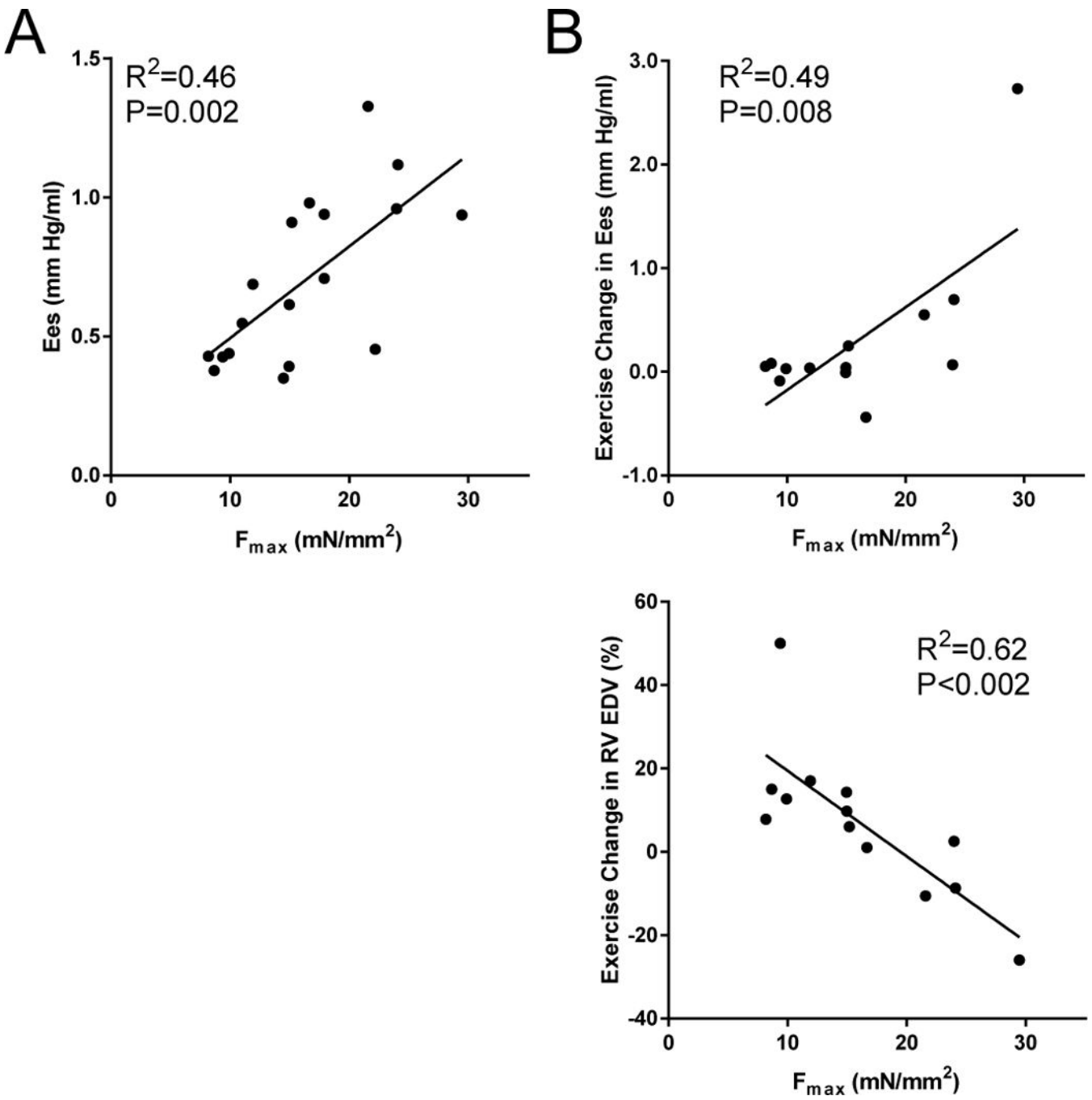

Figure 4.

Myofilament Fmax correlates with in vivo RV function and functional reserve during exercise

(A) Myofilament F_{max} correlated strongly with resting RV chamber-level contractility (Ees) (R^2=0.46, P=0.002). (B) F_{max} also correlated strongly with contractile reserve, or change in Ees with exercise (R2=0.49, P=0.008), as well as exercise RV chamber dilation, as measured

by percent change in RV end-diastolic volume (RV EDV) after reaching a 25-Watt workload (R^2=0.63, P<0.002).

Table 1

Resting and Exercise Hemodynamics in IPAH, SSc-PAH, and SSc-d

Hemodynamics	IPAH	SSc-PAH	P-value*	SSc-d	P-value†
Resting	**N=7**	**N=11**		**N=6**	
RAP (mmHg)	9 ± 7	8 ± 5	0.93	3 ± 1	0.02
PASP (mmHg)	70 ± 18	58 ± 20	0.17	33 ± 6	0.005
PADP (mmHg)	30 ± 8	23 ± 7	0.10	12 ± 4	0.003
mPAP (mmHg)	44 ± 10	36 ± 12	0.22	18 ± 5	0.002
PAWP (mmHg)	9 ± 4	10 ± 3	0.78	8 ± 2	0.08
CO (L/min)	4.2 ± 1.0	4.4 ± 1.0	0.59	4.2 ± 1.0	0.65
PVR (Wood units)	8.9 ± 4.3	7.3 ± 5.6	0.30	3.0 ± 1.9	0.04
Ees (mmHg/ml)	0.96 ± 0.32	0.44 ± 0.14	0.01	0.71 ± 0.29	0.08
Ea (mmHg/ml)	0.97 ± 0.60	1.06 ± 0.73	0.78	0.48 ± 0.17	0.02
Ees/Ea	1.39 ± 0.85	0.49 ± 0.16	0.08	1.53 ± 0.66	0.001
τ(Suga)	39.0 ± 7.9	32.2 ± 7.7	0.11	41.8 ± 5.3	0.11
PFR/EDV (s^{-1})_	2.9 ± 1.2	3.3 ± 1.6	0.69	4.2 ± 1.9	0.27
Exercise	N=4	N=10		N=4	
Peak VO_2 (ml/kg/min)	11.5 ± 2.4	9.9 ± 3.2	0.40	11.0 ± 4.2	0.74
Peak CO (L/min)	11.2 ± 3.3	10.1 ± 5.6	0.48	9.7 ± 4.3	0.89
Peak mPAP (mmHg)	64 ± 24	52 ±19	0.36	38 ± 8	0.20
TPR (Wood Units)	6.5 ± 4.1	7.7 ± 6.4	0.94	5.4 ± 4.6	0.78

Resting and exercise hemodynamics of IPAH, SSc-PAH, and SSc without resting PAH (SSc-d) patients. RAP, right atrial pressure; PASP, PA systolic pressure; PADP, PA diastolic pressure; mPAP, mean PA pressure; PAWP, PA wedge pressure, CO, cardiac output; PVR, pulmonary vascular resistance; Ees, end-systolic elastance; Ea, effective arterial elastance; Ees/Ea, RV-PA coupling ratio; τ(Suga), relaxation time constant; PFR/EDV, peak flow rate normalized to end-diastolic volume; TPR, total pulmonary resistance; peak VO_2, peak oxygen consumption. Comparisons between groups made using Wilcoxon rank-sum test.

*P-value comparing IPAH vs. SSc-PAH,

†P-value comparing SSc-PAH vs SSc-d.