Passive Stiffness of Myocardium From Congenital Heart Disease and Implications for Diastole

Rajiv R. Chaturvedi, MRCP, MD, PhD; Todd Herron, PhD; Robert Simmons, PhD; Darryl Shore, FRCS; Pankaj Kumar, FRCS; Babulal Sethia, FRCS; Felix Chua, MRCP, PhD; Efstathios Vassiliadis, MSc; Jonathan C. Kentish, PhD

Background—In ventricular dilatation or hypertrophy, an elevated end-diastolic pressure is often assumed to be secondary to increased myocardial stiffness, but stiffness is rarely measured in vivo because of difficulty. We measured in vitro passive stiffness of volume- or pressure-overloaded myocardium mainly from congenital heart disease.

Methods and Results—Endocardial ventricular biopsies were obtained at open heart surgery (n=61; pressure overload, 36; volume-overload, 19; dilated cardiomyopathy, 4; normal donors, 2). In vitro passive force-extension curves and the stiffness modulus were measured in skinned tissue: muscle strips, strips with myofilaments extracted (mainly extracellular matrix), and myocytes. Collagen content (n=38) and titin isoforms (n=16) were determined. End-diastolic pressure was measured at cardiac catheterization (n=14). Pressure-overloaded tissue (strips, extracellular matrix, myocytes) had a 2.6- to 7.0-fold greater force and stiffness modulus than volume-overloaded tissue. Myocyte force and stiffness modulus at short stretches (0.05 resting length, L₀) was pressure-overloaded >normal=volume-overloaded>dilated cardiomyopathy. Titin N2B:N2BA isoform ratio varied little between conditions. The extracellular matrix contributed more to force at 0.05 L₀ in pressure-overloaded (35.1%) and volume-overloaded (17.4%) strips than normal myocardium. Stiffness modulus increased with collagen content in pressure-overloaded but not volume-overloaded strips. In vitro stiffness modulus at 0.05 L₀ was a good predictor of in vivo end-diastolic pressure for pressure-overloaded but not volume-overloaded ventricles and estimated normal end-diastolic pressure as 5 to 7 mm Hg.

Conclusions—An elevated end-diastolic pressure in pressure-overloaded, but not volume-overloaded, ventricles was quantifying diastolic chamber stiffness in vivo is extremely difficult. An increased end-diastolic pressure in pressure-overloaded, but not volume-overloaded, ventricles was related to increased myocardial stiffness. The greater stiffness of pressure-overloaded compared with volume-overloaded myocardium was due to the higher stiffness of both the extracellular matrix and myocytes. The transition from normal to very-low stiffness myocytes may mark irreversible dilatation. (Circulation. 2010;121:979-988.)

Key Words: diastole • heart defects • congenital • pressure overload • titin • volume overload

An abnormal cardiac filling caused by altered diastolic chamber stiffness is often proposed as a contributor to the pathophysiology of a range of cardiac diseases, especially ventricular hypertrophy and dilatation. However, quantifying diastolic chamber stiffness in vivo is extremely difficult and requires invasive measurement of the end-diastolic pressure-volume relationship (EDPVR). This is particularly problematic in ventricles with complex geometry (eg, the right ventricle, congenital heart disease).

Clinical Perspective on p 988

Myocardial stiffness is usually a major determinant of ventricular chamber stiffness, although forces exerted by tissues and cavities surrounding the heart (eg, pericardium, pleura, intrathoracic pressure) can affect and even, in some clinical syndromes, dominate chamber stiffness. An alternative to in vivo quantification of myocardial stiffness is direct in vitro measurement by stretching myocardial samples to different lengths and recording the force response.

This technique has established that, in normal rat cardiac muscle, titin is responsible for ~90% of passive force at physiological sarcomere lengths. Titin is a huge elastic protein that extends across each half-sarcomere and is stretched in diastole when the sarcomere relaxes. In the heart, 2 isoforms of titin, N2B and N2BA, may be expressed. They differ in length in the I-band portion and influence tissue stiffness; myocardium expressing mainly the stiffer N2B isoform has a higher stiffness than myocardium in which...
N2BA is coexpressed. At long sarcomere lengths, additional elastic components increasingly contribute to stiffness (eg, collagen in the extracellular matrix [ECM]). However, extrapolation from the behavior of myocardium from normal rodents to that of human diseases is problematic. Although muscles with less stiff titin isoforms are in general less stiff, a survey of 37 different rabbit striated muscles found that the contribution of titin to total passive force and stiffness varied between muscles. Thus, the relative contribution of titin and the ECM to myocardial stiffness requires assessment for each family of diseases. Heart muscle from only a few conditions has been examined: ischemic and dilated cardiomyopathy (DCM), diabetic heart failure, and diabetest mellitus.

Congenital heart disease offers many examples of ventricular hypertrophy or dilatation. We used the right ventricle of tetralogy of Fallot (TOF), double-chambered right ventricle (DCRV), stenotic right-ventricle-to-pulmonary-artery conduits, and the left ventricle of aortic stenosis (AS) as our models of hypertrophy caused by pressure overload. Our models of ventricular dilatation were the right ventricle of atrial septal defects (ASD), pulmonary regurgitation (PR), and the left ventricle of aortic regurgitation. Included for comparison was left ventricular tissue from normal donor hearts and severely dilated ventricles with DCM and end-stage heart failure that have been reported previously.

This range of tissue enabled the characterization of the effect of pressure or volume overload on myocardial passive force and stiffness.

**Methods**

Detailed methods and patient information are provided in the online-only Data Supplement. In brief, endocardial ventricular biopsies were obtained with full consent and ethics approval (Royal Brompton, Harefield, and NHLI ethics protocols 01-006 and 01-194) and without any complications. Consent for donor hearts was obtained from the recipient (protocol 01-194). Biopsies were obtained within 10 minutes of cardioplegic arrest of the heart, except for donor hearts in which the ischemic time under cardioplegic arrest was 0.7 to 4 hours. Tissue was rapidly dissected into thin strips and either snap-frozen in liquid nitrogen or placed in glycerol/relaxing solution at 0°C. All of the solutions contained protease inhibitors. Collagen was quantified by hydroxyproline content. Titin isoforms were separated in a vertical 15% agarose gel electrophoresis system. Titin bands were confirmed in some samples by in-gel tryptic digestion and liquid chromatography–tandem mass spectrometry.

The force response to passive stretch was evaluated only in skinned (permeabilized) preparations to determine the contribution of different structural components: skinned muscle strips, the most physiological preparation with both ECM and myocytes; skinned muscle strips with thick and thin filaments and titin extracted, a predominantly ECM preparation; and skinned myocytes. The force response to passive stretch of striated muscle has a viscous component (increases with stretch speed) and an elastic component (increases with the size of the stretch). Although stress relaxation is due to the viscous component, the peak force was predominantly an elastic response.

Collagenous regions were removed from muscle strips; they were skinned in relaxing solution containing 1% Triton X-100 and dissected to 0.1 to 0.25 mm wide and 1 to 2 mm long. Each muscle strip was mounted to a force transducer and servomotor with a servomotor. This immersion removed virtually all of the myofilaments but left collagen intact and allowed evaluation of the ECM. Some extracted and nonextracted muscles were fixed in 2% glutaraldehyde while still attached to the transducers and processed for electron microscopy. Myocytes were prepared by homogenizing the frozen tissue in relaxing solution and skimming in 1% Triton X-100. Myocyte fragments 120 μm in length and 20 to 30 μm in diameter were used. Skinned myocytes were attached to a sensitive force transducer and a servomotor. The mechanical protocol was similar to that for muscle strips except that mean sarcomere length was recorded with a 240-Hz charge-coupled device camera and commercial software. Cardiac catheterization was performed at the discretion of the responsible cardiologist.

**Data Analysis**

A nonlinear stiffness can be estimated by the slope of the force-extension curve (Δforce/Δextension in N/m) of the material. In this study, force was normalized to cross-sectional area (force/area=stress in N/m²), and stress was expressed as a dimensionless fractional extension: (L–L₀)/L₀=Lagrangian strain, where L₀ is the resting length and L is the stretched length. The slope of our force-extension (stress-strain) curves has units of Newtons per square meter and is the elastic modulus, elasticity or Young’s modulus of elasticity (in N/m²) of the material. To maintain consistency with the use of stiffness, this slope is called the stiffness modulus.

Hence, passive stiffness, in the sense of the resistance of a material to stretch, is evaluated here by the peak passive force in response to a stretch and the slope (stiffness modulus in N/m²) of the force-extension curves. Curves were fitted to force-extension data using splines, and the slope was calculated from the fitted curves using a finite-difference method. Note that compliance (used in the text) is the reciprocal of stiffness.

The mechanical data for strips in the Table were obtained from the median, minimum, and maximum of all of the subjects with a particular type of load (pressure overload, volume overload). Mechanical data for the strips for each subject were calculated from the force-extension curve that was fitted to the pooled data points from all of the strips from that subject.

Yield force of muscle strips was measured only from force-extension curves that had a clear inflection point and a plateau in the force-extension curve with the stiffness modulus dropping to ~0 (Figure IA in the online-only Data Supplement). The force at this inflection point/plateau was taken as the yield force. In the filament-extraction experiments, the postextraction force-extension data reflect mainly the ECM mechanics and are labeled force and elastic modulus at extension 0.05 (ECM) and extension 0.2 (ECM) in the Table and VO₂,ECM and PO₂,ECM in Figure 5.

**Results**

Ventricular endocardial biopsies were obtained from 61 patients (35 male, 26 female patients), and the pressure-overloaded tissue (age range, 0.4 to 72.6 years) came from younger patients than volume-overloaded tissue (age range, 1.4 to 65.6 years) in all experiments The summary data from all of the experiments are given in the Table, and subject details are provided in the online-only Data Supplement.
Muscle Strips

Ninety-one strips were studied from 10 pressure-overloaded (9 right ventricles, 1 left ventricle) and 13 volume-overloaded (9 right ventricles, 4 left ventricles) hearts. There was insufficient tissue from DCM or donor hearts for muscle strip experiments. For most patients, several strips were studied, and the pooled force-extension data for that patient were fitted with a single curve that covered the full extension range.

Small rapid stretch of a strip produced a force response with a largely elastic upstroke followed by stress relaxation (Figure 1A and 1B). The force-extension relationship, derived from a series of stretches, showed a steep nonlinear rise in force ($F = F_0 + k(L - L_0)^n$) and after a maximum or an inflection

---

Table. Summary of Results

<table>
<thead>
<tr>
<th></th>
<th>Pressure Overload</th>
<th>Normal</th>
<th>Volume Overload</th>
<th>DCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strips</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects, n</td>
<td>10</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>1.6 (0.4, 72.6)</td>
<td>28.8 (7.1, 65.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extension 0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Force, kN/m²</td>
<td>5.7 (4.5, 14.8)</td>
<td>2.3 (1.3, 3.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stiffness modulus, kN/m²</td>
<td>137.2 (86.5, 272.5)</td>
<td>51.4 (29.4, 90.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extension 0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Force, kN/m²</td>
<td>20.2 (11.1, 29.6)</td>
<td>5.0 (4.1, 7.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stiffness modulus, kN/m²</td>
<td>85.5 (13.8, 268.5)</td>
<td>18.8 (0.2, 27.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yield force, kN/m²</td>
<td>17.4 (9.7, 25.1)</td>
<td>4.3 (2.9, 6.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Myocytes

|                      |                   |        |                 |      |
|----------------------|-------------------|--------|                 |      |
| Subjects, n          | 3                 | 2      | 3               | 3    |
| Age, y               | 2.3 (1.5, 18.8)   | 35.0 (19, 53) | 46.4 (13.3, 60.4) | 53 (51, 56) |
| Extension 0.05       |                   |        |                 |      |
| Force, kN/m²         | 5.6 (3.3, 10.7)   | 2.5 (1.2, 2.8)  | 1.4 (1.2, 2.4)  | 0.6 (0.3, 1.8) |
| Stiffness modulus, kN/m² | 145.6 (106.8, 253.0) | 59.1 (22.6, 65.3) | 20.7 (17.0, 34.8) | 7.1 (5.7, 46.2) |
| Extension 0.2        |                   |        |                 |      |
| Force, kN/m²         | 47.8 (24.1, 97.3) | 6.6    | 6.3 (5.8, 6.8)  | 1.7  |
| Stiffness modulus, kN/m² | 90.3 (142.6, 1353.6) | 47.8 | 46.8 | 9.9 |
| Force 0.75, kN/m²    | 1.97 (1.77, 2.17) | 2.09 (1.88, 2.21) | 2.16 (2.04, 2.28) | 2.31 (2.18, 2.45) |
| Sarcomere length, μm |                   |        |                 |      |
| Titin isoforms       |                   |        |                 |      |
| Subjects, n          | 10                | 2      | 1               | 3    |
| Age, y               | 4.0 (0.3, 56.0)   | 19.0, 53.0 | 14.0 | 53.0 (51.0, 56.0) |
| N2B, %               | 59.0 (54.4, 74.0) | 64.5 (62.3, 66.6) | 67.3 | 59.5 (55.3, 68.9) |
| Filament-extracted strips |               |        |                 |      |
| Subjects, n          | 3                 | 7      |                 |      |
| Age, y               | 0.8 (0.3, 21.0)   | 45.4 (18.4, 58.8) |     |
| Extension 0.05 (ECM) |                   |        |                 |      |
| Force, kN/m²         | 2.0 (1.4, 3.1)    | 0.4 (0.3, 1.5)  |     |
| Stiffness modulus, kN/m² | 28.1 (18.6, 29.2) | 10.7 (6.0, 33.7) |     |
| Extension 0.2 (ECM)  |                   |        |                 |      |
| Force, kN/m²         | 11.4 (9.0, 13.7)  | 3.8 (2.8, 4.7)  |     |
| Stiffness modulus, kN/m² | 97.8 (90.4, 146.6) | 43.6 (32.6, 54.6) |     |
| Collagen             |                   |        |                 |      |
| Subjects, n          | 25                | 13     |                 |      |
| Age, y               | 1.4 (0.4, 72.6)   | 22.4 (1.4, 65.6) |     |
| Content, mg/g        | 8.6 (2.4, 30.4)   | 15.2 (1.2, 23.1) |     |

Values are median (minimum, maximum). Pressure-overloaded strips were stiffer than volume-overloaded ones at extension 0.05 (force and stiffness modulus, $P<0.001$) and extension 0.2 (force, $P=0.003$; stiffness modulus, $P=0.03$). Pressure-overloaded myocytes at extension 0.05 were stiffer than donor myocytes (force and stiffness modulus, $P<0.02$), volume-overloaded myocytes (force and stiffness modulus, $P<0.01$), and DCMs (force and stiffness modulus, $P<0.05$). After filament extraction, pressure-overloaded strips were stiffer than volume-overloaded at extension 0.05 (force, $P=0.03$; stiffness modulus, $P=0.05$).
transitioned (0.05 to 0.1 $L_0$) to a shallower curve, which rose more steeply at $H_0$. The data were more sparse for large than for short stretches. A striking feature is the clustering by load rather than clinical diagnosis or ventricle of origin. Note the biphasic responses and the higher forces of pressure-overloaded vs volume-overloaded strips. Volume-overloaded symbols: □, aortic regurgitation; ○, ASD; and △, PR. Pressure-overloaded symbols: ◦, TOF; ▽, AS; and ×, DCRV. D, Stiffness modulus-force curves of volume-overloaded (red) and pressure-overloaded (blue) strips. Note the similar slopes of volume-overloaded and pressure-overloaded strips at low forces (solid lines, short stretches). There is greater scatter at higher tensions (dashed lines). For clarity, the intermediate range of tension is not shown (see the online-only Data Supplement).

Figure 1. Muscle strips. Comparable stretches (0.01, 0.03, 0.05 $L_0$) in a muscle strip from a volume-overloaded right ventricle (ASD; A) result in forces approximately half those of a pressure-overloaded right ventricle (DCRV; B). C, Force-extension curves of volume-overloaded (red) and pressure-overloaded strips (blue). Data points group by load rather than clinical diagnosis or ventricle of origin. Note the inflection and plateau in the force-extension relationship, if present, were taken as the yield force. This yield force was lower in volume-overloaded (n=7) than pressure-overloaded (n=3) strips (Table), and the estimated force on titin molecules was 3.1 to 4.1 pN and 9.3 to 16.2 pN per titin, respectively (see the online-only Data Supplement).

Sarcomere length in the strips was not measureable by laser diffraction or confocal fluorescence microscopy. Thus, the relative disposition of the curves with respect to sarcomere length was unknown. To remove any sarcomere length–dependent lateral displacement of force-extension plots, the data were compared in stiffness modulus–force plots (Figure 1D, and in the online-only Data Supplement). Pressure-overloaded and volume-overloaded curve slopes were similar at low forces, although the pressure-overloaded curves reached higher stiffness moduli, confirming the differences between the passive mechanics of pressure-overloaded and volume-overloaded strips.
Figure 2. Stiffness modulus at 0.05 L₀ increases with collagen content in pressure-overloaded but not volume-overloaded strips (dashed lines, SE of the fit). Pressure-overloaded symbols: blue ○, TOF; blue ×, DCRV; blue △, pulmonary infundibular stenosis; and ▽, AS. Volume-overloaded symbols: red ○, ASD; red △, aortic regurgitation; and red ▽, PR.

Contribution of the ECM

The contribution of collagen to passive mechanics of muscle strips was evaluated by measuring collagen content and assessing the effect of myofilament and titin extraction on passive mechanics. Stiffness modulus at 0.05 L₀ increased with collagen content in pressure-overloaded strips but not in volume-overloaded strips (Figure 2). However, there was no difference in overall collagen content between the pressure-overloaded and volume-overloaded groups (Table). Immersion in KCl and KI resulted in massive depletion of thick and thin filaments and titin with failure of activation with calcium (pCa²⁺, 4.5; not shown). Compared with preextraction values, resting force fell (Figure 3b), force (P=0.03) and stiffness modulus (P=0.05) decreased at short stretches (0.05 L₀; Table and Figure 3), but larger stretches of 0.1 to 0.2 L₀ were little affected (P=0.16; Figure 3C). Similarly, the stiffness modulus-force relationship was affected only at low forces (Table I in the online-only Data Supplement). After filament extraction, the stiffness modulus of the pressure-overloaded strips was still 2 to 4 times that of the volume-overloaded strips over the range of stretches used, demonstrating that the ECM was also stiffer in pressure-overloaded than volume-overloaded tissue.

The contribution of the ECM to the passive force of muscle strips was calculated as the ratio of the median of all of the postextraction strips/median of all of the nonextracted force at 0.05 L₀ stretch. The ECM contribution to force at 0.05 L₀ stretch was 35.1% for pressure-overloaded and 17.4% for volume-overloaded strips.

Myocytes

The myocyte force-extension curves were similar to those for muscle strips (compare Figure 4C and 4D with 1C); curves for pressure-overloaded myocytes lay at shorter sarcomere lengths than those for the donor, volume-overloaded, and DCM myocytes (Figure 4C and 4D), and the maximum force reached was at least 2 times higher. The volume-overloaded and donor myocyte curves overlapped (Figure 4D). In contrast, DCM myocytes were shifted to the right relative to the donor and volume-overloaded cells. There were important differences between the force (P=0.006) and stiffness modulus (P=0.004) of the groups at 0.05 L₀, and the pressure-overloaded cells had a higher force and stiffness modulus than the donor (P=0.016), volume-overloaded (P=0.008), and DCM (P=0.036) cells. A comparison of the resting sarcomere length of the myocytes was made by calculating for each cell the sarcomere length required to reach a given low force (0.75 kN/m²; Table): pressure overloaded< donor=volume overloaded<DCM.

Myocytes were largely responsible for the passive force and stiffness modulus of muscle strips at short stretches (<0.05 L₀). Strips and myocytes had similar forces and stiffness moduli at 0.05 L₀ stretch (Table and Figure 5), and filament extraction led to a fall in resting force, and a loss of the early component of the force-extension curve resulting in a monophasic rather than biphasic curve (Figure 3).

To examine whether titin isoform variation accounted for differences in myocyte stiffness modulus, the titin N2B:N2BA ratio was determined for 16 patients (online-only Data Supplement). The relative amounts of the N2B and N2BA isoforms did not vary consistently between the pressure-overloaded, volume-overloaded, donor, and DCM tissue and are unlikely to account for differences in myocyte passive mechanics (Table).

Comparison With In Vivo Hemodynamics

In vivo EDP, measured by cardiac catheterization, was available in 14 patients. In these pressure-overloaded ventricles, in vivo EDP increased with myocardial stiffness modulus at 0.05 L₀ stretch (spline fit to the pressure-overloaded points; Figure 6). In contrast, in the volume-overloaded ventricles, myocardial stiffness modulus was within the normal range and did not explain an elevated EDP. Note that our fit gives an excellent estimate for normal in vivo EDP of 5 to 7 mm Hg for ventricles with normal myocardial stiffness modulus.

Discussion

Myocardium is a composite material, simplified in this study to myocytes and the ECM. The dominant stiffness of myocardium shifts from myocytes to the ECM as stretch size increases, and hemodynamic load affects both components. Within the range of tissue examined, the load on the ventricle (pressure-overload, volume-overload) was a dominant factor in the passive mechanical behavior, beyond that exerted by ventricle of origin and anatomic lesion.

Pressure-overloaded tissue (muscle strips, ECM, myocytes) had a 2.6- to 7.0-fold higher force and stiffness modulus than the comparable volume-overloaded tissue (Table). The ECM made a larger contribution to the force response at short stretches (pressure overload, 35.1%; volume overload, 17.4% at 0.05 L₀ stretch) of our muscle strips than in normal rat myocardium (≈10%). Our DCM cells had low stiffness, similar to DCM myofibrils and strips.
contrast, myocytes from moderately dilated ventricles (ASDs) had forces and stiffness moduli comparable to those of normal donors. This finding of normal passive mechanics with moderate ventricular dilatation in contrast to the extremely low stiffness tissue of severely dilated cardiomyopathic hearts is striking.

Mechanisms of Variation of Stiffness

The similar slopes of the stiffness modulus-force plots of volume-overloaded and pressure-overloaded strips at low forces suggest that similar elastic units are responsible in both tissues at short stretches/low forces (see the online-only Data Supplement). An increased number of elastic units in parallel may underlie the higher stiffness modulus of pressure-overloaded strips. We found that an increase in at least 1 component, collagen, was associated with an increase in stiffness modulus of pressure-overloaded muscle strips at short stretches (Figure 2). Although collagen levels were similar in the pressure-overloaded and volume-overloaded muscle strip groups as a whole, comparable to other diseases, the higher stiffness modulus of the ECM in pressure-overloaded muscle strips (Figure 3 and Table) suggests that changes in the collagen microarchitecture within strips (eg, increased cross-linking), may have occurred. Collagen cross-linking

---

**Figure 3.** Filament-extracted strips. A, Electron micrographs of pressure-overloaded muscle (TOF) before (A) and after (B and C) filament extraction. Collagen fibrils are intact (B, labeled C) despite depletion of myofilaments. The Z and M lines are largely intact (B and C). Sarcomere length is 2.26 to 2.36 μm. Magnification 10 000. B, Force transients in a volume-overloaded muscle strip before (left; 0.01, 0.03, 0.04 L₀) and after (right; additional 0.1 L₀ stretch) filament extraction. The postextraction 0.1 L₀ stretch fails to reach the force of a preextraction 0.04 L₀ stretch. C, Filament extraction in a strip from a volume-overloaded right ventricle (ASD) results in a monophasic force-extension curve (broken line). D, After filament extraction, the pressure-overloaded (blue) strips still reach higher forces (d) than those from volume-overloaded strips (red), with loss of the initial component of the control curve (solid line, C).
by advanced glycation end products can occur independently of hyperglycemia and may contribute to the increased stiffness modulus of the ECM.\textsuperscript{16,25}

The yielding of our myocardial strips occurred at forces similar to rabbit skeletal split fibers (0.9 to 25 pN per titin\textsuperscript{7}). This transition may occur in vivo because the estimated end-diastolic wall stress in dilated ventricles is 5 to 30 kN/m\textsuperscript{2}\textsuperscript{23,26} or 4 to 25 pN per titin molecule. Two potential mechanisms for muscle strip yielding are titin immunoglobulin unfolding (150 to 250 pN\textsuperscript{27,28}) and bond rupture between myocyte surface receptors and their ECM ligands (\(\alpha_5\beta_1\), integrin-collagen, 65 pN; \(\alpha_5\beta_1\), integrin-fibronectin, 35 to 60 pN)\textsuperscript{29}. Hence, myocytes are likely to uncouple from the ECM first.

The large differences in myocyte stiffness, especially the high stiffness of pressure-overloaded cells, remain unexplained. There was no systematic variation in titin isoforms across disease states (Table). Further studies are required to explore posttranslational modification of titin. Phosphorylation of titin in the N2B region by protein kinase A and G or the PEVK region by protein kinase C\textsuperscript{30–33} can decrease or increase the stiffness of titin, respectively. Additional studies should examine the stiffness of single pressure-overloaded myofibrils to determine whether a high stiffness
modulus is a property of pressure-overloaded sarcomeres and should investigate the increased stiffness resulting from nonmyofibrillar structures such as an increase in the network density of the extrasarcomeric cytoskeleton, changes in microtubules, or an increase in desmin.

Several mechanisms are pertinent to the low stiffness of DCM tissue. First, because compliances in series are additive, longer myocytes (more sarcomeres in series) or longer protein isoforms (more amino acids in series [eg, titin N2BA]) will increase compliance (and decrease stiffness). Second, force-induced bond breakage can disrupt networks of structural proteins or uncouple myocytes from the ECM. Third, the force required to break molecular bonds increases with the speed of stretch. The slow stretch over years in chronic volume-overload or DCM may drop bond rupture forces to that attainable in vivo. Fourth, unfolding of a titin immunoglobulin domain increases its contour length, introducing an additional compliance in series, and refolding occurs only if the applied force drops to nearly 0. Because end-diastolic wall stress is never 0, unfolded domains will be trapped in the unfolded state and progressively accumulate. Unfolded immunoglobulin/fibronectin domains may also accumulate in other mechanically important proteins (eg, fibronectin, tenascin).

**Implications for In Vivo Cardiac Function**

These direct measurements of in vitro passive mechanics of myocardial strips and myocytes may extrapolate to in vivo ventricular performance. Figure 6 suggests that ventricular EDP increases with myocardial stiffness modulus in pressure-overloaded ventricles (ie, they have a steeper EDPVR). However, an elevated EDP in moderately dilated volume-overloaded ventricles can occur independently of an increased myocardial stiffness modulus by a raised end-diastolic volume shifting the ventricle to a steeper part of a normal EDPVR. In contrast, the myocytes of end-stage heart failure have an extremely low stiffness modulus, resulting in a shallow EDPVR. These severely dilated ventricles operate at the extreme right of their EDPVR at high end-diastolic volumes and, we hypothesize, at long sarcomere lengths (see the online-only Data Supplement). The transition from moderate volume-overload with normal stiffness myo-

---

**Figure 5.** Comparison of force (A) and stiffness modulus (B) at 0.05 L₀ stretch in different preparations. Pressure-overloaded (PO) tissues (strips, cells, ECM) have higher force and stiffness modulus than other tissues. Muscle strips and cells from the same loading condition (volume-overloaded or pressure-overloaded) had similar forces and stiffness moduli at 0.05 L₀. Muscle strip filament extraction experiments were used to obtain the ECM (postextraction) values.

**Figure 6.** Relationship between in vitro stiffness modulus at 0.05 L₀ and in vivo EDP (dashed lines, SE; vertical dotted lines, 95% confidence intervals for normal stiffness modulus). Increased myocardial stiffness modulus is associated with an elevated EDP in pressure-overloaded but not volume-overloaded ventricles. Normal EDP is estimated as 5 to 7 mm Hg. Red ○ indicates ASD (strips); red ●, ASD (cells); red △, PR; blue ○, TOF; blue ▲, AS; blue □, pulmonary infundibular stenosis; and blue ●, conduit stenosis.
cytes to the very-low stiffness myocytes of end-stage heart failure may be a marker of irreversible dilatation.

Volume-overloaded hearts have elevated end-diastolic wall stress uncompensated for by increased wall thickness. We hypothesize that a persistently elevated end-diastolic wall stress may result in a less stiff myocardium and drive cardiac dilatation. As the heart dilates and myocytes operate at longer sarcomere lengths, a higher end-systolic wall stress will be required to generate the same systolic ventricular pressure (law of Laplace). If the required end-systolic wall stress is not achieved, then end-diastolic volume and wall stress may increase further and progressively drive dilatation.

**Study Limitations**

This was an observational study from the perspective of clinical diagnosis, ventricular load, sex, and age at surgery. Stiffness of myocytes or ventricles is usually found to increase with age if all else is kept constant. However, our pressure-overload group had stiffer tissue and was younger than the volume-overload group. Although age is likely to have an impact within each clinical diagnostic category, across the categories in this study, load appeared to have the dominant effect.

A consequence of the large number of conditions evaluated is that the sample sizes are small, resulting in low statistical power. The Table shows how this applies to the myocytes (n = 17 from 11 subjects), titin isoforms (16 subjects), and filament extraction experiments (10 subjects). However, although our data are more sparse in the myocyte and titin isoform studies, they are supported by previous reports.

Sarcomere length was not measurable in muscle strips, and this was tackled with 3 methods. First, we set each strip to the same low resting force rather than the same resting sarcomere length. When muscles with different passive properties are compared, only one of these can be set constant at a time. Higher force in the pressure-overloaded than the volume-overloaded strips did not arise from a longer initial sarcomere length because the stiffer pressure-overloaded strips reached the same force at shorter sarcomere lengths than volume-overloaded strips, as found in the myocyte experiments. Second, there were large differences between the volume-overloaded and pressure-overloaded strips throughout the extension range, which should overcome any small variation in initial sarcomere length. Third, the stiffness modulus-force plots (Figure 1D, and in the online-only Data Supplement), which exclude sarcomere length dependence, also demonstrated systematic differences between the pressure-overloaded and volume-overloaded strips.

Human myocytes or myofibrils are usually prepared by homogenization of biopsies. Hence, it is difficult to exclude a small amount of ECM on the cells or between the bundles of 2 to 3 myofibrils that are typically used. However, any ECM component in our myocytes was small. The numeric values of force-extension curves of the donor and the DCM myocytes in this study are similar to previously published values for myofibrils and myocytes. In addition, the mechanical behavior of the myocytes was extremely different from that of the strips. Stretching the strips by 0.06 to 0.1 L0 led to irreversible changes, whereas the myocytes showed complete recovery.

Ideally, the relationship between wall stress and stiffness modulus would have been explored. However, the majority of our catheterization data are from right ventricles with congenital heart disease for which quantitative models of fiber architecture do not exist.

**Conclusions**

Either an increase (pressure-overload) or a decrease (severe-volume overload/DCM) in passive myocardial stiffness can be detrimental. Systolic pressure-overload results in increased stiffness of myocytes and ECM, with the diastolic consequence a stiffer ventricle and potential for impaired filling. The elevated end-diastolic wall stress of severe volume-overload results in a less stiff and more easily stretched myocardium and ultimately ventricular dilatation with systolic consequences. In the most common form of heart failure with a dilated ventricle, rather than the heart dilating as it fails, it may be more precise to say that it fails as it dilates.

**Source of Funding**

This study was funded by the British Heart Foundation.

**Disclosures**

None.

**References**


12. Chaturvedi et al Passive Stiffness of Myocardium From CHD
Changes in myocardial stiffness may contribute to the pathophysiology of cardiac disease. However, myocardial stiffness is difficult to measure in vivo, especially in the right ventricle or in congenital heart disease. Myocardial stiffness was measured in vitro from biopsies obtained from pressure-overloaded, volume-overloaded, donor, and dilated cardiomyopathy hearts. Pressure-overloaded tissue was stiff, suggesting that a raised end-diastolic pressure in these ventricles is due to a steeper-than-normal end-diastolic pressure-volume relationship. In contrast, the tissue from moderately dilated ventricles had relatively normal stiffness, implying that a raised end-diastolic pressure would be secondary to the ventricle operating at higher end-diastolic volumes in the steeper part of a normal end-diastolic pressure-volume curve. The dilated cardiomyopathy tissue had low stiffness, suggesting that the transition from normal to low stiffness may be a marker of irreversible dilatation.
Passive Stiffness of Myocardium From Congenital Heart Disease and Implications for Diastole
Rajiv R. Chaturvedi, Todd Herron, Robert Simmons, Darryl Shore, Pankaj Kumar, Babulal Sethia, Felix Chua, Efstatios Vassiliadis and Jonathan C. Kentish

Circulation. 2010;121:979-988; originally published online February 16, 2010;
doi: 10.1161/CIRCULATIONAHA.109.850677
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/121/8/979

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2010/02/12/CIRCULATIONAHA.109.850677.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/
SUPPLEMENTAL MATERIAL

Supplement to Chaturvedi et al “Passive stiffness of myocardium from congenital heart disease and implications for diastole”
Methods

The study was approved by the Royal Brompton Hospital Clinical Research Ethics Committee (Protocol 01-006 and 01-194).

Solutions

All solutions were made with ultrapure water and contained protease inhibitors (1 tablet/25 ml solution; Complete Protease Inhibitor Tablets, Roche). Relaxing solution had pH 7.1 and ionic strength 200 mM (1). The skinning solution was relaxing solution with 1% Triton X-100 v/v. Activating solution (pCa 4.5) was relaxing solution with replacement of K₂EGTA by 10 mM CaEGTA. The transport solution was a 1:1 v/v mixture of glycerol and relaxing solution with pH adjusted to be 7.1 at 0°C. Storage solution was 1:1 v/v mixture of glycerol and a relaxing solution with phosphate buffer with pH 7.1 at -20°C. Solutions of 0.6M KCL and 1.0M KI were used for the filament extraction experiments (2). All experiments were performed at 20 °C.

Tissue collection and processing

Endocardial biopsies were obtained without any complications. Endocardial biopsies of a few millimetres length were taken from the right or left ventricle within 10 minutes of cardioplegic arrest of the heart. Tissue from the donor hearts was obtained within an ischaemic period under cardioplegic arrest of 0.7-4 hours. Tissue was rapidly dissected in the transport solution on ice into thin strips and either snap-frozen in polypropylene vials (Nalgene) in liquid nitrogen within 30-60 seconds of harvesting, or transferred into transport solution in vials packed in ice in a vacuum flask. Tissue reached the laboratory within 20-30 minutes.

Muscle strips

The tissue in transport solution was kept on ice and dissected into finer strips. Collagenous regions in the biopsies were excised. These strips were skinned for 6 hours at 4°C, washed in relaxing solution to remove detergent, dissected further into finer strips and kept in storage solution at -20°C. Studies were performed within 7 days of obtaining tissue. Experiments were performed in a ten-well anodised aluminium muscle bath (3) secured on an air table. Both ends of a muscle strip were wrapped in aluminium T-clips, and these
were mounted onto the hooks of the force transducer (AE-801 SensoNor, Horten, Norway) and servomotor (Cambridge Technology 300S), and secured by painting on cyanoacrylate glue. A low resting tension of 5 \( \mu \text{N} \) was applied to prevent the strip from going slack. A subset of muscle strips underwent salt extraction of filaments using a protocol that removed virtually all myosin, actin, titin and \( \alpha \)-actinin but left collagen intact (2). The strips were sequentially immersed in 0.6 M KCl (40 minutes) and then 1.0 M KI (40 minutes) whilst mounted between the force transducer and servomotor. Subsequent mechanical measurements were performed in relaxing solution of normal ionic strength.

For the mechanical measurements, the servomotor triggered by software (pClamp 6.0, Axon Instruments) produced length changes. Force and muscle length signals were low-pass filtered (2 kHz) and acquired on a computer with a 12-bit A/D board (Digidata 1200). A constant velocity ramp stretch (10 \( \mu \text{m/ms} \)) was followed by a length-clamp of 1 second and constant velocity (10 \( \mu \text{m/ms} \)) ramp down to baseline. Stretches were separated by 10 minutes, to allow muscle recovery. Stretches were up to 0.05 \( L_0 \) of muscle length; beyond this, irreversible changes occurred in the tension-extension relationship. Since there was a finite amount of tissue and each strip underwent only one stretch \( > 0.05 \ L_0 \), the data for large extensions was more sparse than that for short stretches. It proved impossible to measure sarcomere length from the muscle strips.

**Single myocytes**

The snap-frozen tissue was transferred to a -80\(^\circ\)C freezer, and was used for myocyte experiments as previously described (1). Myocytes were homogenized in ice-cold relaxing solution, then skinned for 30 minutes and washed twice in relaxing solution. Myocyte preparations \( \geq 120 \ \mu\text{m} \) in length and 20-30 \( \mu\text{m} \) diameter were used. The single cell apparatus has been previously described (4, 1). Myocytes were tied into 25G stainless steel troughs with monofilament nylon sutures (4), and suspended between a capacitance-gauge force transducer (Model 400A, Cambridge Technology) and servomotor (Cambridge Technology). The mechanical protocol was identical to that used for muscle strips, except that sarcomere length could be recorded from the myocytes. For this a 240 Hz CCD camera and software were used to measure mean sarcomere length from the power spectrum of the light intensity of the video scan lines (IonOptix). Sarcomere length trace lags behind the force transient during ramps due to the low video sampling frequency. Myocytes could be stretched beyond 0.05 \( L_0 \) without causing irreversible changes to the tension-extension relationship.
This video frequency is acceptable as laser diffraction in several skeletal (5, 6, 7, 8) and cardiac muscle (9, 10) preparations has shown there is no fast component to the sarcomere length response to passive stretches up to sixty times faster than this study.

**Collagen measurements**

The method of Campa et al. (11) was used. Frozen tissue samples were crushed into a powder with a mortar and pestle, with occasional addition of liquid nitrogen to keep them frozen. Samples were hydrolysed with 6M HCL, decolorized and then dried in an oven. The dried hydrolysates were derivatized for high performance liquid chromatography and absorbance was monitored at 495 nm. The absorbance-time integral was used to quantify hydroxyproline content by comparison with samples from standard solutions.

**Titin gels**

Snap-frozen tissue stored at -80°C was powdered and the method of Warren et al. (12) was followed with a vertical 1% agarose gel electrophoresis system and Coomassie Blue staining. N2B and N2A bands were demonstrated by using lanes with rat cardiac muscle or rat skeletal muscle. The identity of some N2B and N2BA bands was confirmed by tryptic in-gel digestion, liquid chromatography-tandem mass spectrometry with identification of proteins by correlation of tandem mass spectra to the SwissProt/TREMBL database (13).

**Electron microscopy**

Whilst still attached to the transducers, muscle strips underwent 2% glutaraldehyde fixation. T-clips were removed from the prior to washing the strips in phosphate buffer solution and fixing with a 4% osmium tetroxide solution (30 minutes). Strips were dehydrated in ethanol and embedded in Araldite. Longitudinal sections were made with a Leica ultramicrotome. Sections were transferred to Formvar/carbon-coated copper grids for microscopy.

**Data analysis**

Data analysis was performed in R (www.r-project.org). The mgcv package was used to perform nonlinear regression using penalized regression splines with smoothing parameters determined by generalized cross validation (14).
Calculation of force on titin molecules

Titin density is estimated at $2.4 \times 10^9$ molecules/mm$^2$ in end filaments (15, 16). If the fraction of cross-sectional area of myocardium that is myocytes is $\text{myocyte}_{\text{csa}}$, the fraction of cross-sectional area of myocytes that is myofibrils is $\text{myofibril}_{\text{csa}}$, and tension is $T$ (kN/m$^2$), then the force on titin is $T/(\text{myocyte}_{\text{csa}} \times \text{myofibril}_{\text{csa}} \times 2.4)$ pN/molecule. In patients with aortic stenosis $\text{myocyte}_{\text{csa}}$ is 0.75-0.8 and $\text{myofibril}_{\text{csa}}$ is 0.6 (17).

Sarcomere length in heart failure

Sarcomere length in end-stage heart failure remains contentious. To date, direct measurements of sarcomere lengths in dilated beating hearts have not been made. However in dogs with LV dilatation for 3 - 12 weeks sarcomere lengths up to 2.27 $\mu$m were found in rapidly fixed tissue (18). Resting sarcomere length measured in tissue sections (19) or in single myocytes from biopsies (20) did not differ between human disease states. However, the sarcomere lengths in the autopsy tissue sections (1.36 - 1.45 $\mu$m) were too short for large mammal muscle and probably reflect the effects of rigor and shortening during fixation, and the slack sarcomere lengths of isolated myocytes in suspension cannot be assumed to be representative of their in vivo length.

Normal human myocardium

Completely normal human myocardium is essentially impossible to obtain. Tissue from donor hearts is an approximation to normal, but hearts harvested from patients with brain death are known to have both systolic and diastolic ventricular abnormalities and may have been exposed to inotropes for many days (21, 22). Post-implantation ventricular failure, although multifactorial in origin, is a well recognised clinical phenomenon. Tissue from donor hearts is scarce and we were unable to obtain sufficient tissue to perform filament extraction or muscle strip experiments. Hence the estimates of ECM contribution to passive tension at 0.05 $L_0$ could not be performed in donor heart tissue and the reference used was normal rat myocardium (2).
Table 1: Patient details

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Load</th>
<th>Biopsy</th>
<th>Strips</th>
<th>Myocytes</th>
<th>Extraction</th>
<th>Collagen</th>
<th>Titin</th>
<th>Clinical details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.7</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TOF, BT shunt, RV EDP 7 mmHg</td>
</tr>
<tr>
<td>2</td>
<td>0.8</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td>TOF</td>
</tr>
<tr>
<td>3</td>
<td>72.6</td>
<td>PO</td>
<td>LV</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>AS, LV EDP 23 mmHg, LV mass 373 g/m², severe diastolic dysfunction</td>
</tr>
<tr>
<td>4</td>
<td>0.8</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>TOF</td>
</tr>
<tr>
<td>5</td>
<td>14.9</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>DCRV</td>
</tr>
<tr>
<td>6</td>
<td>1.1</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>TOF, hypercyanotic episodes, RV EDP 10 mmHg</td>
</tr>
<tr>
<td>7</td>
<td>0.4</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>TOF, RV EDP 7 mmHg</td>
</tr>
<tr>
<td>8</td>
<td>1.4</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>TOF, hypercyanotic episodes</td>
</tr>
<tr>
<td>9</td>
<td>56.1</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>PIS gradient 124 mmHg, RV EDP 10 mmHg, severe RVH, VO₂ 25.5 ml/kg/min</td>
</tr>
<tr>
<td>10</td>
<td>4.2</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>TOF, hypercyanotic episodes</td>
</tr>
<tr>
<td>11</td>
<td>9.4</td>
<td>VO</td>
<td>LV</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>AR, prolapse non-coronary cusp, LVEDD 55 mm</td>
</tr>
<tr>
<td>12</td>
<td>15.7</td>
<td>VO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ASD 33 mm, RV EDP 16 mmHg, Qp:Qs 3:1</td>
</tr>
<tr>
<td>13</td>
<td>47.9</td>
<td>VO</td>
<td>LV</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>AR</td>
</tr>
<tr>
<td>14</td>
<td>28.8</td>
<td>VO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>PR, PA + VSD, microdeletion 22q11</td>
</tr>
<tr>
<td>15</td>
<td>7.1</td>
<td>VO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>ASD 20 mm</td>
</tr>
<tr>
<td>16</td>
<td>29.5</td>
<td>VO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>PR, TOF + PA, RV dilatation, RV EDP 9 mmHg</td>
</tr>
<tr>
<td>17</td>
<td>58.8</td>
<td>VO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td>Secundum ASD x 2, RV EDP 4 mmHg, Qp:Qs 2:1</td>
</tr>
<tr>
<td>18</td>
<td>65.6</td>
<td>VO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Large primum ASD, RVEDP 6 mmHg, Qp:Qs 2.1:1, VO₂ 25.1 ml/kg/min</td>
</tr>
<tr>
<td>19</td>
<td>22.4</td>
<td>VO</td>
<td>RV</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>PR, PA + VSD, PR fraction 45%</td>
</tr>
<tr>
<td>20</td>
<td>18.3</td>
<td>VO</td>
<td>LV</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>AR, LVEDD 65 mm</td>
</tr>
<tr>
<td>21</td>
<td>20.2</td>
<td>VO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>PR, Trisomy 21, TOF, RV EDP 12 mmHg, PR fraction 30 %</td>
</tr>
<tr>
<td>22</td>
<td>57.7</td>
<td>VO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>sinus venosus ASD + PAPVD (both right veins), RV EDP 12 mmHg, Qp:Qs 3.9:1</td>
</tr>
<tr>
<td>23</td>
<td>45.4</td>
<td>VO</td>
<td>LV</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td>AR, LVEDD 69 mm</td>
</tr>
<tr>
<td>24</td>
<td>0.3</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td>TOF</td>
</tr>
<tr>
<td>Patient</td>
<td>Age</td>
<td>Load</td>
<td>Biopsy site</td>
<td>Strips</td>
<td>Myocytes</td>
<td>Extraction</td>
<td>Collagen</td>
<td>Titin</td>
<td>Clinical details</td>
</tr>
<tr>
<td>---------</td>
<td>-----</td>
<td>------</td>
<td>-------------</td>
<td>--------</td>
<td>----------</td>
<td>------------</td>
<td>----------</td>
<td>-------</td>
<td>------------------</td>
</tr>
<tr>
<td>25</td>
<td>21.0</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>Conduit stenosis, RV EDP 6 mmHg</td>
</tr>
<tr>
<td>26</td>
<td>46.4</td>
<td>VO</td>
<td>RV</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>sinus venosus ASD + PAPVD, RV EDP 13 mmHg, Qp:Qs 2.2:1</td>
</tr>
<tr>
<td>27</td>
<td>13.3</td>
<td>VO</td>
<td>RV</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>sinus venosus ASD</td>
</tr>
<tr>
<td>28</td>
<td>60.4</td>
<td>VO</td>
<td>RV</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>Primum ASD + PAPVD, RV EDP 7 mmHg, Qp:Qs 2.1:1</td>
</tr>
<tr>
<td>29</td>
<td>18.8</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>Conduit stenosis, Rastelli, RV EDP 15 mmHg</td>
</tr>
<tr>
<td>30</td>
<td>53</td>
<td>Donor</td>
<td>LV</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Donor heart</td>
</tr>
<tr>
<td>31</td>
<td>51</td>
<td>DCM</td>
<td>LV</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>DCM</td>
</tr>
<tr>
<td>32</td>
<td>56</td>
<td>DCM</td>
<td>LV</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>DCM</td>
</tr>
<tr>
<td>33</td>
<td>53</td>
<td>DCM</td>
<td>LV</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>DCM</td>
</tr>
<tr>
<td>34</td>
<td>19</td>
<td>Donor</td>
<td>LV</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>Donor LV, cystic fibrosis (heart-lung transplantation)</td>
</tr>
<tr>
<td>35</td>
<td>1.5</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TOF</td>
</tr>
<tr>
<td>36</td>
<td>2.2</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>TOF</td>
</tr>
<tr>
<td>37</td>
<td>1.8</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>TOF</td>
</tr>
<tr>
<td>38</td>
<td>1.0</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>TOF</td>
</tr>
<tr>
<td>39</td>
<td>6.8</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>PS</td>
</tr>
<tr>
<td>40</td>
<td>14.7</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Conduit stenosis, TOF</td>
</tr>
<tr>
<td>41</td>
<td>2.7</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TGA + VSD + PS</td>
</tr>
<tr>
<td>42</td>
<td>56.1</td>
<td>PO</td>
<td>LV</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AS</td>
</tr>
<tr>
<td>43</td>
<td>54</td>
<td>DCM</td>
<td>LV</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DCM</td>
</tr>
<tr>
<td>44</td>
<td>0.5</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TOF</td>
</tr>
<tr>
<td>45</td>
<td>0.4</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TOF</td>
</tr>
<tr>
<td>46</td>
<td>1.5</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PA + VSD</td>
</tr>
<tr>
<td>47</td>
<td>0.9</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TOF</td>
</tr>
<tr>
<td>48</td>
<td>1.2</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TOF</td>
</tr>
<tr>
<td>49</td>
<td>7.4</td>
<td>PO</td>
<td>RV</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TOF</td>
</tr>
<tr>
<td>50</td>
<td>0.5</td>
<td>PO</td>
<td>LV</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AS</td>
</tr>
<tr>
<td>Patient</td>
<td>Age</td>
<td>Load</td>
<td>Biopsy site</td>
<td>Strips</td>
<td>Myocytes</td>
<td>Extraction</td>
<td>Collagen</td>
<td>Titin</td>
<td>Clinical details</td>
</tr>
<tr>
<td>---------</td>
<td>-----</td>
<td>------</td>
<td>-------------</td>
<td>--------</td>
<td>-----------</td>
<td>------------</td>
<td>----------</td>
<td>-------</td>
<td>------------------</td>
</tr>
<tr>
<td>51</td>
<td>1.2</td>
<td>PO</td>
<td>RV</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>TOF</td>
</tr>
<tr>
<td>52</td>
<td>1.3</td>
<td>PO</td>
<td>RV</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>TOF</td>
</tr>
<tr>
<td>53</td>
<td>16.4</td>
<td>VO</td>
<td>LV</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>AR</td>
</tr>
<tr>
<td>54</td>
<td>2.0</td>
<td>PO</td>
<td>RV</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>TOF</td>
</tr>
<tr>
<td>55</td>
<td>57.2</td>
<td>PO</td>
<td>LV</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>AS</td>
</tr>
<tr>
<td>56</td>
<td>7.7</td>
<td>PO</td>
<td>RV</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>TGA + VSD + PS, BT shunt</td>
</tr>
<tr>
<td>57</td>
<td>0.6</td>
<td>PO</td>
<td>RV</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>TOF</td>
</tr>
<tr>
<td>58</td>
<td>27.2</td>
<td>VO</td>
<td>RV</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>PR</td>
</tr>
<tr>
<td>59</td>
<td>1.4</td>
<td>PO</td>
<td>RV</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>TOF</td>
</tr>
<tr>
<td>60</td>
<td>1.4</td>
<td>VO</td>
<td>RV</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>PR, Pulmonary valvotomy, RVOTO resection</td>
</tr>
<tr>
<td>61</td>
<td>1.6</td>
<td>PO</td>
<td>RV</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>TOF</td>
</tr>
</tbody>
</table>

Abbreviations: AS, aortic stenosis; AR, aortic regurgitation; ASD, atrial septal defect; BT, Blalock-Taussig; DCRV, double-chambered right ventricle; EDP, end diastolic pressure; LVEDD, left ventricular end diastolic dimension; PAPVD, partial anomalous pulmonary venous drainage; PIS, pulmonary infundibular stenosis; PO, pressure overload; PR, pulmonary regurgitation; PS, pulmonary stenosis; TOF, tetralogy of Fallot; VSD, ventricular septal defect.
Figure legends

Figure 1. Strips underwent short (≤ 0.05L₀; green and red lines), long (> 0.05 L₀; blue lines) or the full range (green and red lines) of stretches as shown in Supplement Figure 1 (a). The curve with lowest force at 0.2 L₀ (green) shows an inflection point and a plateau in its force response to stretch. The yield force for this curve can be estimated from the inflection point/plateau force.

The corresponding Stiffness-Force plot is in (b). The transition from short to long stretches has the sparsest amount of data, and for clarity and to emphasise the differences in short and long stretches this transition is not shown in (c) and in the main manuscript (Figure 1d).

Figure 2. The figure is similar to Figure 1d in the manuscript, but without the fitted regression lines. It shows the impact of load (volume-overload or pressure-overload) on the force-extension relationship. Note that data clusters by load rather than ventricle of origin or clinical diagnosis. Force-extension data of volume-overload (red) and pressure-overload patients (blue). Note the higher forces of the strips from pressure-overload as compared to the volume-overload patients. Volume-overload symbols: AR, square; ASD, circle; PR, triangle point up. Pressure-overload symbols: TOF, diamond; AS, triangle point down; DCRV, cross.

Figure 3. Typical gel showing human titin isoforms. Identity of titin isoforms was confirmed by a combination of running lanes with rat skeletal muscle and rat cardiac muscle and also liquid chromatography-tandem mass spectrometry. Note multiple breakdown bands were often seen (T2, T3).
References


