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Role of diastolic properties in the transition to failure in a mouse model of cardiac dilatation

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Abstract

Although the physiological states of hypertrophic remodeling and congestive heart failure have been intensively studied, less is known about the transition from one to the other. The use of genetically engineered murine models of heart failure has proven valuable in characterizing the progression of remodeling and its ultimate decompensation to failure. Mice deficient in the cytoskeletal muscle LIM-only protein (MLP) are known to present with a clinical picture of dilated cardiomyopathy and transition to failure as adults. Longitudinal high-field magnetic resonance (MR) cardiac imaging provided a time course of remodeling where an improvement in ejection fraction and stroke volume (15- vs. 31-wk MLP^{-/-} mice; $P < 0.0001$) was temporally concurrent with an abrupt phase of end-diastolic chamber dilatation. Hemodynamic analysis conducted throughout that dilatation phase showed improved ratio of maximum first derivative of pressure to end-diastolic pressure ($dP/dt_{\max}/EDP$; 15- vs. 31-wk MLP^{-/-} mice; $P < 0.0005$), ratio of minimum first derivative of pressure to EDP ($dP/dt_{\min}/EDP$; 15- vs. 31-wk MLP^{-/-} mice; $P < 0.003$), and developed pressure (15- vs. 31-wk MLP^{-/-} mice; $P < 0.0001$) levels in the MLP^{-/-} mice. Computational modeling techniques were used to estimate the EDP volume relationship, revealing that although MLP hearts possess a stiffer stress-strain relation, chamber compliance increased as a function of dilatation. This detailed physiological characterization during a phase of rapid anatomical remodeling suggests that systolic function in the MLP^{-/-} mice may temporarily improve as a result of alterations in chamber compliance, which are mediated by dilatation. In turn, a balance may exist between exploiting the Frank-Starling mechanism and altering chamber compliance that maintains function in the absence of hypertrophic growth. Though initially compensatory, this process may exhaust itself and consequently transition to a maladaptive course.

Keywords

magnetic resonance imaging; finite element modeling

The discrete, physiological states of hypertrophic remodeling (19, 30) and congestive heart failure (13) have been well studied. The dynamic nature of the heart allows it the plasticity to progressively change structurally to accommodate an inciting stimulus in an attempt to maintain parameters, such as myocardial mass and cardiac output, within physiological ranges (3). This compensatory remodeling has a limit beyond which congestive heart failure may ensue (20).

Although the individual stages of hypertrophy and heart failure have been extensively studied, the transition from one to the other has not been fully characterized. The factors that

influence the inability to further compensate and give way to overt failure remain unclear. The objectives of this study were to quantify the time course of anatomical and functional changes that occur during the transition toward overt failure and to investigate the role of dilatation in altering passive mechanics during that period. A mechanism is then proposed that describes how these dilatation-mediated changes in chamber compliance may maintain function in the absence of hypertrophic growth. A combination of imaging, surgical hemodynamic analysis, and computational modeling methods was employed to allow the assessment of longitudinal changes in global cardiac structure and function in the mouse.

Genetically engineered murine models have become valuable tools in the study of heart failure by providing populations of a similar and reproducible phenotype (31). In particular, the targeted deletion of cytoskeletal proteins, such as the muscle LIM-only protein (MLP), has been shown to consistently lead to dilated cardiomyopathy and heart failure in mice (2, 17). Echocardiography and in vivo pressure-volume (P-V) loop measurements performed on failing adult MLP-deficient (MLP^{-/-}) mice have revealed many of the clinical characteristics associated with congestive heart failure (7). Recent work (20, 26) has investigated whether diastolic function, including passive mechanics, is altered in the MLP^{-/-} model (20, 26). Omens et al. (27) have shown alterations in the passive P-V and pressure-strain relations, concluding a less compliant tissue in the adult dilated hearts versus age-matched controls. Lorenzen-Schmidt et al. (21) have found notable alterations in passive myocardial properties and relaxation times in young MLP^{-/-} mice, which present with a more compliant tissue at 2 wk but normal characteristics for most systolic parameters (21). Although these results suggest that the progression to heart failure in this model may be a consequence of diastolic dysfunction, a driving mechanism that mediates these changes has yet to be revealed. Thus the hypothesis of the present study was that the relative effects of diastolic left ventricular (LV) properties, in particular those due to dilatation and material stiffness, mediate chamber compliance and thus may influence systolic function.

Clearly, MLP^{-/-} mice progress through a distinct evolution in both morphology and function from an early age of virtually normal systolic performance to overt failure. However, little is known about that transition or the stimuli that lead to these changes. In this study, the novel application of longitudinal MRI in conjunction with invasive catheter techniques was used to investigate the time course of remodeling in this clinically relevant heart failure model. Finite element (FE) analysis, integrating data from both techniques, was used to estimate the end-diastolic P-V relationship (EDPVR) and describe the relative balance between factors determining its shape. Results have revealed a distinct transitional period where dilatation is temporally concurrent with an increase in systolic function. The balance between diastolic properties that shifts in favor of dilatation may increase chamber compliance, initially maintaining or improving function but eventually decompensating as overt heart failure ensues.

MATERIALS AND METHODS

Mouse Model

MLP-deficient mice (2) used in this study were obtained from a University of California, San Diego (UCSD), colony. Homozygous MLP^{-/-} mice were inbred so that wild-type littermates were not available. The background strain to the MLP^{-/-} mice was a hybrid cross of 129/Sv and C57BL/6 strains. After 20 generations of intercrossing, genetic marker analysis showed an indistinguishable background between the MLP^{-/-} and 129/Sv genotypes, making age-matched 129/Sv mice (Charles River) an appropriate wild-type control (unpublished data). All protocols were performed according to the National Institutes of Health's (NIH) *Guide for the Care and Use of Laboratory Animals* and approved by the UCSD Animal Subjects Committee.

MRI Study

Animal preparation and monitoring—Eight mice [4 MLP^{-/-} (2 male and 2 female) and 4 129/Sv (2 male and 2 female) mice] were imaged longitudinally at a weekly interval from 2–3 to 32 wk of age with no mortality. Anesthesia was chamber-induced with 5% (vol) inhaled isoflurane in 100% O₂ and then sustained under free-breathing conditions in a custom-built restraint with 1.5% (vol) isoflurane at 1.5 l/min for the remainder of the imaging session. External copper foil or subcutaneous needle ECG leads were placed on the front forelimbs for ECG triggering (HSB-T, Rapid Biomedical), while a rectal thermocouple probe monitored core body temperature that was held constant by heated airflow through the magnet bore. Mice were maintained and observed at 450–550 beats/min and at 36–38°C using a custom-designed physiological monitoring system integrated with a data acquisition network (LabVIEW 6.1, National Instruments).

MRI—In vivo NMR cardiac imaging was performed on a 7-T horizontal-bore magnetic resonance (MR) scanner (Varian, Palo Alto, CA), equipped with a shielded 12-cm bore-gradient system capable of 22-G/cm gradient strength and a 300-μs maximum rise time (MagneX Scientific, Oxford, UK). A 1.9-cm custom-built dual quadrature-driven transverse electromagnetic mode volume coil was used for transmission and reception of the radiofrequency signal.

High-resolution bright blood MRI experiments were conducted using an ECG-triggered fast low angle shot (FLASH) gradient-echo pulse sequence tailored for murine imaging as previously described (8). Scanning parameters optimized for signal to noise were found to be as follows: flip angle = 90°, echo time = 1.8 ms, repetition time ~ R-R interval (~120 ms), receiver bandwidth = 32 kHz, and number of averages = 5. Fractional echo (62.5%) to reduce the echo time and time series averaging were employed, both of which serve to increase the signal-to-noise ratio. A field of view of 25 mm² and data matrix of 128² were prescribed to yield an in-plane resolution of 195 μm².

Each imaging protocol resulted in seven to nine 1-mm-thick short-axis images spanning the whole heart from apex to base. A prospective gating scheme was used where images were acquired at a range of time delays relative to the ECG trigger in temporal increments of 10 ms. Equatorial frames containing the largest and smallest chamber diameters were selected as the diastolic and systolic phases of the cardiac cycle, respectively.

Image analysis—Four two-dimensional contours were manually planimetered for each heart at end diastole and end systole [LV (and right ventricular) epi and endo] using image analysis software (ImageJ, NIH). From calibrated units, contour areas were calculated, multiplied by the slice thickness, and summed together to approximate volumes. LV mass was obtained by subtracting endocardial from epicardial volumes and scaling by the specific gravity of myocardial tissue (1.055 g/ml). Stroke volume was defined as LV end-diastolic volume (LVEDV) – LV end-systolic volume, whereas ejection fraction was calculated as 100 × stroke volume/LVEDV.

Control studies—A validation study was conducted at 36 wk using two 129/Sv controls and two MLP^{-/-} mice to assess the accuracy of MR mass (MR_{mass,mg}) measurement versus wet weight (WW_{mass,mg}), which served as the standard. Immediately after imaging, mice were euthanized, and the hearts were harvested and weighed for comparison. Linear regression analysis showed good correlation between MR and gravimetry [MR_{mass,mg} = WW_{mass,mg} (1.02) – 4.99] where the offset is a measure of the volume artifact associated with blood flow within the ventricle during the time between phase encoding and readout. Oblique flow artifacts can cause artifactual displacement of flow intensities that can overlap

the endocardial wall, resulting in a slight overestimation of the cavity volume (9, 10). These effects were minimized by the use of short echo times.

Hemodynamic and Tissue Analysis

Eighty-seven mice [41 MLP^{-/-} (29 males and 12 females) and 46 129/Sv (24 males and 22 females)] were used in terminal studies at four time points: 8, 15, 22, and 31 wk of age. All 87 mice yielded body, heart, lung, and liver weight data at the time of euthanasia. Forty-eight mice (8 of each strain at each of 3 time points: 15, 22, and 31 wk) underwent hemodynamic analysis.

Millar conductance catheter experiment—Continuous and simultaneous pressure and volume measurements were invasively obtained directly from the LV cavity by a procedure described elsewhere (25). Briefly, mice were induced with 5% (vol) inhaled isoflurane and maintained via nose cone at 2% (vol) while warmed by a circulating waterblanket (K Module, American Pharmaseal). A midline cervical incision exposed the trachea for intubation with a cannula connected to a volume-cycled rodent ventilator (model 687, Harvard Apparatus). The right carotid artery was exposed and isolated via the same incision. A 1.4-Fr Mikro-Tip conductance catheter (SPR-839, Millar Instruments) was introduced into the carotid artery, advanced down the ascending aorta, through the aortic valve, and into the LV. Pressure and volume measurements were recorded under steady-state conditions.

Hemodynamic data were analyzed by using software designed for conductance catheter measurements (PVAN3.3, Millar Instruments). Volume data were calibrated and corrected for parallel conductance by hypertonic saline injection as previously described (33). Relative volume units were calibrated to microliters using heparinized blood in cuvettes of a known volume (no. 910–1049, Millar Instruments). All hemodynamic data were averaged over 10 beats for each animal.

FE Modeling

The chamber compliance of a heart may be influenced by two factors that are related by a highly nonlinear relationship: the stiffness of the myocardium, as reflected in the stress-strain relationship, and the geometry of the ventricle. For modeling purposes, geometry was described with wall thickness and radius measurements. Computational methods make it possible to assess separately the relative contribution of these two components on passive mechanics. To that end, computational FE techniques were used to estimate the EDPVR in each of fourteen mice (7 MLP^{-/-} and 7 129/Sv) before { *week 15* } and after (*week 31*) the dilatation phase using software developed in our laboratory (Continuity 6.3, www.continuity.ucsd.edu). MRI provided geometric data for modeling at end diastole, whereas the hemodynamic analysis supplied pressure and volume data throughout the cardiac cycle. Wall thickness and radius measurements from the MR images yielded wall and cavity volumes at the end-diastolic state, from which volumes of a smaller chamber radius may be extrapolated by unloading the model with pressure while maintaining conservation of mass. A least-squares optimization routine was used to fit two variables, the unloaded volume (V_o) geometry and a single material constant within the constitutive law, to two points known to lie along the EDPVR, the end-diastolic pressure (EDP) volume point and the volume at minimum pressure point.

FE model—The complex cardiac geometry required approximation by an analytical geometrical model. A truncated prolate spheroidal geometry was used to model the LV of the heart, where the inner and outer radii of the mesh at the equatorial plane were set to the measured values from MR image analysis. A high-order [linear (λ , transmural), cubic (μ , longitudinal), and cubic (θ , circumferential)] three-element mesh was used. Similar meshes

have previously been shown to give converged solutions compared with a low-order 70-element mesh (4). Both element and numerical convergence were confirmed by mesh refinement to a 288 element mesh and evaluation of inflation volume to within 3% error. Apical nodes were constrained in λ with respect to the longitudinal element coordinate, apical and basal nodes were constrained in μ with respect to the transmural element coordinate, while the mesh was constrained from rigid body motion and rotation as previously described (4).

Passive mechanical properties—Stress and strain in the LV were modeled with a transversely isotropic exponential strain energy function (Eq. 1), with respect to the fiber coordinate system (fiber, cross-fiber, sheet), that has previously been used to describe resting myocardium (15).

$$W = \frac{1}{2}C(e^Q - 1) + C_{\text{compr}}[I_3(\ln I_3) - I_3 + 1] \quad (1)$$

$$Q = b_{\text{ff}}E_{\text{ff}}^2 + b_{\text{xx}}(E_{\text{cc}}^2 + E_{\text{ss}}^2 + E_{\text{cs}}^2 + E_{\text{sc}}^2) + b_{\text{fx}}(E_{\text{fc}}^2 + E_{\text{cf}}^2 + E_{\text{fs}}^2 + E_{\text{sf}}^2)$$

The constitutive law contains five material constants (C , C_{compr} , b_{ff} , b_{xx} , b_{fx}) that must be fit to strain measurements (E_{ij}^2). The material constant to be estimated was the stress-scaling coefficient (C), which linearly scales the strain energy function (W), and hence the inflation pressure. The remaining four parameters were fixed at values derived from a fit to normal rat myocardium: bulk modulus ($C_{\text{compr}} = 100.0$ kPa, fiber strain exponent ($b_{\text{ff}} = 9.2$, transverse strain exponent ($b_{\text{xx}} = 2.0$, and fiber-transverse shear exponent ($b_{\text{fx}} = 3.7$) (26). The tangential slope of the EDPVR is then directly proportional to the magnitude of C . A larger value for C , therefore, indicates a stiffer curve. By varying one constant in the constitutive law, it is possible to fit the EDPVR to two hemodynamic points and estimate the desired two unknowns (V_0 and C). A passive inflation was modeled for each heart using its unique V_0 geometry and constitutive relation at 2.5 mmHg increments to compute the EDPVR.

Statistical Analysis

All data are expressed as means $\pm$ SE. Statistical comparisons were made by two-way ANOVA followed by Fisher paired least significant difference post hoc analysis over time and genotype. Statistical differences of $P < 0.05$ were considered significant.

RESULTS

Weights

Body and tissue weights measured during the terminal hemodynamic analysis are summarized in Table 1. Although body weights of MLP^{-/-} mice did not differ from wild-type controls, heart, liver, and lung weights per tibial length were increased significantly at each time point ($P < 0.0007$). The wet-to-dry heart weight ratio between strains was also not significant, indicating that the increase in MLP^{-/-} heart mass was due to hypertrophy and not edema.

MRI

A weekly imaging protocol resulted in 142 MRI experiments. For clarity, data are presented in Figs. 1 and 2 at 18 time points, where the mean $\pm$ SE of all mice imaged at the nearest three time points, i.e., 2–4 wks, 5–7 wks, etc., is shown for each genotype.

The time course for a dilatation index, defined as the ratio of LV chamber volume to LV epicardial volume (chamber plus myocardial volume) at end diastole, which serves as a

three-dimensional analog of a radius-to-wall thickness ratio, is illustrated in Fig. 1A. As the chamber radius increases relative to the epicardial surface volume, the index will approach unity, indicating dilatation. In MLP^{-/-} hearts, the epicardial volumes smoothly plateau (data not shown), whereas the endocardial volumes experienced an increase (15- vs. 24-wk MLP^{-/-} mice; $P < 0.0001$), as shown in Fig. 1B, leading to an elevated dilatation index among strains (MLP^{-/-} vs. 129/Sv mice; $P < 0.01$). Of particular note was the abrupt increase in this index. Before ~18 wk of age, MLP^{-/-} hearts had geometrical ratios comparable with wild-type hearts but progressed through a rapid phase of dilatation in the following 8–9 wk to plateau again near *week 27* (15- vs. 24-wk MLP^{-/-} mice; $P < 0.0001$). This accelerated rate of remodeling was reproducible throughout the sample group and marks a clear transition in ventricular geometry.

Although the dilatation index in the MLP^{-/-} mice remained comparable with controls through *week 15*, hypertrophic growth in the LV was observed far earlier, as is reflected by myocardial mass shown in Fig. 2. LV mass peaks and plateaus near *week 15* in both strains (MLP^{-/-} vs. 129/Sv mice; $P < 0.05$), indicating the cessation of hypertrophic growth. Although the mean decreased in the MLP^{-/-} mice from 15 to 31 wk, no statistical difference was found in heart weight (data not shown) or heart weight-to-tibial length ratio (Table 1).

When diastolic MR images with those acquired at end systole are compared, functional information may be derived. The time courses of ejection fraction and stroke volume are described in Fig. 1, C and D. The MLP^{-/-} hearts presented with a normal ejection fraction at 2 wk of age compared with wild-type hearts but quickly declined into failure (MLP^{-/-} vs. 129/Sv mice; $P < 0.05$), as reflected in what is most likely edematous lung and liver tissue (Table 1). Although this relative depression persisted throughout the study, ejection fraction improved in the MLP^{-/-} hearts with a pattern temporally concurrent with dilatation (15- vs. 24-wk MLP^{-/-} mice; $P < 0.005$), as can be seen when the curves are compared on a common time axis. Although the mean in 129/Sv stroke volume and ejection fraction varied with time post maturity (~8 wk), significance between any two time points was only found between the highest and lowest values measured.

Hemodynamic Analysis

The longitudinal MR study revealed a distinct phase of rapid end-diastolic dilatation beginning around *week 18* and continuing through about *week 27* that was accompanied by an apparent intragroup improvement in systolic performance. These trends were also used to define periods of significant change when more invasive and quantitative hemodynamic analysis was conducted. Three such times were chosen to precede (*week 15*), coincide with (*week 22*), and follow (*week 31*) the dilatation phase. The hemodynamic study supplemented the end-diastolic and end-systolic volume measurements from MR with pressure and volume data throughout the cardiac cycle. No statistical difference was found in heart rate between time points or among strains. Representative P-V loop data from 22-wk MLP^{-/-} and 129/Sv hearts are shown in Fig. 3. Note the dilated ventricle and depressed systolic pressure in the MLP^{-/-} hearts. A number of indexes extracted from the P-V data are summarized in Fig. 4. It was again seen that ejection fraction (15- vs. 31-wk MLP^{-/-} mice; $P < 0.0001$) and stroke volume (15- vs. 31-wk MLP^{-/-} mice; $P < 0.0001$) in the MLP^{-/-} hearts increase through the dilatation phase (Fig. 4, A and B), corroborating results of the MR longitudinal study. Additionally, parameters based on pressure measurements were calculated. At *week 15*, the EDP in the MLP^{-/-} hearts was elevated (MLP^{-/-} vs. 129/Sv mice; $P < 0.0001$) (Fig. 4C) but approached control levels at *weeks 22* (MLP^{-/-} vs. 129/Sv mice; $P = 0.19$) and *31* (MLP^{-/-} vs. 129/Sv mice; $P = 0.47$). Within the knockout strain, EDP continued to fall from *weeks 15* to *31* (15- vs. 31-wk MLP^{-/-} mice; $P = 0.0002$). End-systolic pressure in the MLP^{-/-} hearts was significantly depressed versus control hearts (MLP^{-/-} vs. 129/Sv mice; $P < 0.0001$), with little significant change from 15 to 31 wk in

either strain ($P = 0.03$) (data not shown). The ability of $MLP^{-/-}$ hearts to generate pressure from an end-diastolic state (maximum – minimum pressure, Fig. 4D) was depressed compared with wild-type hearts ($MLP^{-/-}$ vs. 129/Sv mice; $P < 0.0001$) but increased from 15 to 31 wk in the $MLP^{-/-}$ hearts (15- vs. 31-wk $MLP^{-/-}$ mice; $P < 0.0001$). Load-independent velocities of both contraction (15- vs. 31-wk $MLP^{-/-}$ mice; $P < 0.0005$) and relaxation (15- vs. 31-wk $MLP^{-/-}$ mice; $P < 0.003$) were both improved in the $MLP^{-/-}$ hearts during the same period, recovering from levels depressed versus wild-type hearts at week 15 ($MLP^{-/-}$ vs. 129/Sv mice; $P < 0.0001$) to those comparable at week 31 ($MLP^{-/-}$ vs. 129/Sv mice; $P > 0.13$) (Fig. 4, E and F). Furthermore, these improving trends are temporally concurrent with the phase of rapid dilatation. The time courses of volume-related parameters from the catheter experiments were consistent with the results of the MRI study. Also, there was good agreement in the statistically significant differences found between strains at corresponding time points where both techniques were used.

EDPVR Modeling

The key results from the FE models are summarized in Table 2, including the calculated values for V_o and C . Of note is the accuracy with which the FE model estimated the end-diastolic volume when inflated to the EDP compared with direct ventricular measurement. The unloaded volumes in 15-wk $MLP^{-/-}$ hearts were comparable with wild-type hearts but increased significantly at 31 wk (15- vs. 31-wk $MLP^{-/-}$ mice, $P = 0.0002$; and 31-wk $MLP^{-/-}$ vs. 129/Sv mice, $P < 0.0001$), most likely as a function of chamber dilatation. These results are in accord with the end-diastolic volumes measured from longitudinal MRI. Myocardial stiffness was seen to increase significantly, both from 15 to 31 wk in the $MLP^{-/-}$ hearts (15- vs. 31-wk $MLP^{-/-}$ mice; $P < 0.05$) and between strains at both time points (15-wk $MLP^{-/-}$ vs. 129/Sv mice, $P < 0.05$; and 31-wk $MLP^{-/-}$ vs. 129/Sv mice, $P = 0.0002$). This agrees with previous results that show older $MLPW^{-/-}$ hearts to have a stiffer stress-strain relationship (27). The simulated passive inflation of each heart with its unique V_o and constitutive law is illustrated in Fig. 5. Although they have a stiffer myocardium, there was little difference in chamber compliance between strains at week 15. In contrast, compliance increased markedly in the 31-wk $MLP^{-/-}$ hearts despite further myocardial stiffening. This demonstrates the concept that myocardial stiffness can increase, but chamber stiffness can decrease, due to the impact of chamber dilatation.

DISCUSSION

The $MLP^{-/-}$ model is known to consistently develop a phenotypic picture of clinical dilated cardiomyopathy with systolic dysfunction (right shifted P-V loops, depressed systolic contractility, increased LV end-diastolic and end-systolic dimensions, and decreased percent fractional shortening) and transition to overt heart failure as an adult (20–28 wk), although at 2 wk of age, mice appear to have systolic function comparable with wild-type controls (7, 21). However, the time course of that progression or its governing factors remain unknown. The aim of this study was thus to characterize this evolution and examine the role of myocardial stiffness and ventricular dilatation in altering chamber compliance and mediating systolic function.

Results of this study have uncovered an abrupt dilatation phase beginning around week 18 and continuing through week 27 in the $MLP^{-/-}$ heart. Although depressed versus control ejection fraction, stroke volume, developed pressure, $dP/dt_{max}/EDP$, and $dP/dt_{min}/EDP$ were all found to increase temporally concurrent with this dilatory phase. Thus there appears to be a compensatory phase during the progression toward heart failure in this model in which systolic function may actually transiently improve in the dilated ventricle.

Computational modeling showed that, despite increasing myocardial stiffness over time, dilatation led to a more compliant ventricle in the 31-wk MLP^{-/-} hearts compared with that in wild-type or younger knockout hearts. These results suggest a shifting balance between the effects of geometry and material properties on chamber compliance. At *week 15*, the slightly stiffer curve may be a function of the increased myocardial stiffness, as reflected in the elevated stress-scaling coefficient, and the lack of any dilatation, resulting in a decreased chamber compliance in MLP^{-/-} hearts. As the ventricle dilates yet continues to stiffen, the balance shifts in favor of geometrical change resulting in a heart with increased compliance.

The factors that impact the nonlinear relationship between diastolic pressure and volume have been well documented. Glantz and Parmley (14) have proposed a mathematical model that illustrates the balance between ventricular geometry and material stiffness on chamber compliance. They incorporate geometry in a wall thickness-to-radius ratio, η , and show that, for a given material stiffness, increasing the ratio shifts the P-V curves leftward, whereas decreasing it has the opposite effect. Mirsky and Parmley (22) echo the importance of geometry on diastolic behavior by including a wall-to-chamber volume ratio as an analog to η . In the canine, Laks et al. (18) have shown that hypertrophy shifts P-V curves leftward, whereas Gaasch et al. (11) have shown that dilatation in human hearts moves flattened curves toward higher pressures. Increasing wall stiffness while holding geometry constant in the Glantz model shifts P-V curves leftward. Smith et al. (29) demonstrate the same effect in humans after myocardial infarction before dilatation dominates the balance. We propose that the balance in this dichotomy plays a prominent role in remodeling and the progression toward overt failure in the MLP^{-/-} hearts.

The striated muscle-specific protein MLP is expressed at high levels in atrial and ventricular myocytes during development and in the adult (1). It is believed that MLP is an essential promoter of proper cardiomyocyte architectural organization within the heart wall, where genetically engineered models lacking this protein present with significant disruption in ultrastructure (2). In addition, MLP has been proposed to play an important role in mechanotransduction and the control of hypertrophic remodeling via the titin/Tcap/MLP/ α -actinin complex (17). In the present study, hypertrophic growth appears to diminish at ~15 wk for unclear reasons. The hypertrophic stimulus may have been removed or is still present, but the heart is no longer able to properly sense the load, possibly due to the mechanotransduction deficiency associated with the gene deletion. Although tissue mass has peaked by *week 15*, anatomical and functional changes continue through *week 31*, suggesting they are not directly related to hypertrophic growth. The temporary improvement in systolic performance during that time is thus likely attributable to other adaptations that are not sustainable as the mice age and transition to failure. It is well known that stroke volume improves with an increased LV cavity size, as may be the case during eccentric hypertrophy and normal physiological growth (5). Dilatation may thus serve as a mechanical pathway to augment stroke volume during this particular phase. However, without an increase in percent wall thickening, dilatation, even with the accompanying increased stroke volume, will result in a decreased ejection fraction that will eventually result in the failure that has classically been associated with a dilating ventricle (6, 28). In the event that both stroke volume and ejection fraction increase with dilatation, one may propose that systolic function must also increase. This premise is supported by measures of dP/dt/EDP and developed pressure. The basis for this improvement may lie in the changes in chamber compliance, which are mediated by dilatation.

A mechanism by which these changes in compliance attempt to maintain systolic performance during this period remains undefined. Subsequent to *week 15*, remodeling no longer seems to be driven by hypertrophic growth but rather may be governed by dilatation and the associated alterations in passive mechanics. At *week 15*, the EDPVR in the MLP^{-/-}

is comparable with control hearts. At that time, EDP is elevated, suggesting that, in the continued absence of geometrical change via dilatation, function is being maintained by the Frank-Starling mechanism where working pressures are higher on the EDPVR curve. As the pressures continue to rise, a physiological limit may be reached where stiffness becomes extremely high and there is little change in volume for a given change in pressure. A phase of dilatation may ensue to serve multiple purposes. First, chamber compliance of the ventricle will increase, aiding in filling. Second, working pressures will decrease back toward normal levels, restoring the ability to again operate on increasing portions of the Frank-Starling curve. These two effects may manifest in the improved or maintained stroke volume and ejection fraction observed during the dilatory phase. These results also suggest that there may be multiple mechanisms of growth and recovery and that change may progress in a cyclical fashion, where the balance in contribution may shift between these preferred pathways depending on the condition of the heart at that time. In the $MLP^{-/-}$ hearts, function may initially be maintained by the Frank-Starling mechanism but subsequently by anatomical restructuring as stiffness increases in the absence of hypertrophic growth. This balance is mediated by changes in chamber compliance, which are in turn mediated by dilatation. A limit to this compensatory mechanism may be reached that marks the threshold to overt failure.

Noninvasive high-field cardiac MRI has proven to be a powerful tool in assessing both anatomy and function in the mouse model (23). To characterize transitional phases within a process, it is essential to noninvasively monitor changes in the same animal as they develop. In this longitudinal study, MR techniques were applied to obtain a high-resolution time course of LV remodeling that spanned an 8-mo time frame from ~2 to 32 wk of age. The dilatory phase revealed is typically thought of as an event marking a transition toward overt heart failure. In this model, there appeared to be a transient remodeling phase in which dilatation is associated with enhanced systolic function. The motivation thus existed to further characterize the hemodynamic state of the $MLP^{-/-}$ heart at various time points throughout this period. Pressure and volume data taken directly from the LV via catheter insertion provided a detailed hemodynamic picture to supplement the findings of the MR study. Ejection fraction and stroke volume were again found to increase during dilatation, in agreement with MR measurements. FE analysis provided a platform for the integration of both experimental techniques in estimating the EDPVR before and after the dilatation phase. By fitting the curve to hemodynamic data, this approach made it possible to assess the effects of geometry and material properties separately. Such time courses may also be valuable in defining targeted time points where the up- and downregulation of genes may modulate the hypertrophic response.

There are several possible sources of error associated with each experimental technique as well as the mouse model. Although the MR images have sufficient spatial resolution in plane, longitudinal resolution was limited to a 1-mm slice thickness, affecting volume calculations. Also, the volume effects associated with bright blood imaging may impact endocardial delineation. The summed effect of both was estimated to be on the order of 3–5% by linear regression to wet weight. Catheter studies are suspected of having sources of error attributed predominantly to the nonlinear electrical properties of the myocardium. The underestimation of absolute volumes compared with MR is a documented deficiency with conductance catheters in the mouse (16). Moreover, volumes are often a function of calibration, contributing to the discrepancy between MR and hemodynamic values, yet relative changes between time points and among strains are not. Care was taken to consistently calibrate the volume measurements, making statistically significant differences in time valid. Volume traces are also particularly sensitive to catheter orientation within the ventricle. Despite its limitations, conductance catheters have been successfully applied to murine cardiac studies (24, 25, 33). Both MR and catheter techniques demonstrate the same

temporal trends in volume-related indexes, lending further credibility to the results presented here. A potential limitation to FE modeling was the reduction of an exponential constitutive law to a single scaling variable. With only one degree of freedom, it was not necessary that the EDPVR would pass through the two hemodynamic points. Although convergence would improve with more degrees of freedom, least-squares optimization for the data presented was within 2% error. The estimated value of C was also relatively insensitive to V_o , which was derived from physiological measurements. Linear regression for the 31-wk MLP^{-/-} group, for example, was $C = V_o (0.11) - 1.8$ with $R^2 = 0.98$. The FE models could be improved with more detailed geometries, such as nonaxi-symmetric models, and with improved constitutive laws that allow for anisotropic materials accounting for laminar sheet structure. With respect to the animal model, MLP^{-/-} mice are certainly undergoing normal growth in addition to pathological hypertrophy during development. This study focuses on a time period where heart mass has stabilized (heart weight, 1.8%; and body weight, 0.7% change from 15 to 31 wk), minimizing the confounding factors that any growth may play. Anatomically, mice are at the extreme of the thickness-to-radius and thickness-to-myocyte diameter ratios (32). They also differ kinematically and metabolically (12), mainly due to the accelerated heart rate. Despite these limitations, mouse models are crucial for understanding human myocardial growth and remodeling.

In summary, the remodeling process in a genetically engineered mouse model of heart failure has been characterized in detail during a phase of major anatomical and functional change. Multiple experimental techniques were integrated to reveal a transitional period that has not been previously described. Systolic parameters, such as ejection fraction, stroke volume, developed pressure, $dP/dt_{\max}/EDP$, and $dP/dt_{\min}/EDP$, all improve temporally concurrent with this phase of rapid end-diastolic dilatation. These findings support the proposition that this improvement may be the result of alterations in chamber compliance, which are mediated by dilatation. In turn, a balance may exist between exploiting the Frank-Starling mechanism and altering chamber compliance that maintains function in the absence of hypertrophic growth. Although initially compensatory, this process will eventually exhaust itself and consequently transition to a maladaptive course.

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Fig. 1.

MRI-derived time courses of remodeling for muscle LIM-only protein-deficient (MLP^{-/-}) vs. 129/Sv hearts. One-hundred forty-two MRI experiments from weekly imaging protocol are summarized at 18-binned time points. Plots are presented as means $\pm$ SE of all mice imaged at nearest 3 time points, i.e., 2–4 wk, 5–7 wk, etc., for each genotype. Note the presence of hypertrophy up to 15 wk, as indicated by a comparable dilatation index among strains (A) but a larger end-diastolic volume (EDV) (B) in MLP^{-/-} hearts, and abrupt dilatation phase (indicated by bracket in A and B) that occurs temporally concurrent with increase in stroke volume (C) and ejection fraction (D). LV, left ventricular; Epi, epicardial. $\dagger P < 0.05$, MLP^{-/-} vs. 129/Sv; $\ddagger P < 0.0001$ 15- vs 31-wk MLP^{-/-}.

Fig. 2. MRI-derived time course of LV myocardial mass for MLP^{-/-} vs. 129/Sv hearts. Plots are presented as binned means $\pm$ SE of all mice imaged at nearest 3 time points for each genotype. Note presence of hypertrophic growth in MLP^{-/-} mice from an early age and its termination at around week 15. There were no statistically significant differences in body weight found between strains at any time point. $\ddagger P < 0.05$, MLP^{-/-} vs. 129/Sv.

Fig. 3. Representative pressure-volume loop data for 22-wk 129/Sv (*A*) vs. $MLP^{-/-}$ (*B*) hearts. Ten consecutive cardiac cycles are shown for each heart. Note elevated chamber volumes in $MLP^{-/-}$ hearts, indicative of LV dilatation, and depression in systolic pressures.

Fig. 4. Measured parameters from hemodynamic analysis for MLP^{-/-} vs. 129/Sv hearts. Forty-eight hemodynamic experiments are summarized. Graphs are presented as means $\pm$ SE for each genotype. Note improvement in systolic function that occurs during dilation phase. Also note decreasing end-diastolic pressure (EDP). $dP/dt_{\max}$ and $dP/dt_{\min}$ maximum and minimum first derivative of pressure, respectively. $\ddagger P < 0.005$, $\ddagger\ddagger P < 0.0001$, MLP^{-/-} vs. 129/Sv; $\dagger P < 0.05$, $\dagger\dagger P < 0.001$, $\dagger\dagger\dagger P < 0.0001$, 15- vs. 31-wk MLP^{-/-}. A: ejection fraction; B: stroke volume; C: EDP; D: developed pressure; E: $dP/dt_{\max}/EDP$; F: $dP/dt_{\min}/EDP$.

Fig. 5.

End-diastolic pressure-volume relationship (PVR) modeled with finite element analysis for MLP^{-/-} vs. 129/Sv hearts. Curves are fitted to volumes numerically estimated from finite element models (FEMs) at two time points for both genotypes. Closed symbols are two hemodynamic points used to fit each end-diastolic PVR, whereas open symbols are calculated unloaded volumes (V_0). SE for all modeled volumes ranged from 0.04 to 1.4 μl . Note increased chamber compliance of hearts at week 31, despite a stiffer myocardium. Also note higher working pressures in week 15 MLP^{-/-} vs. control hearts but comparable pressures in week 31 MLP^{-/-} hearts.

Table 1

Weights of MLP^{-/-} vs. 129/Sv at 8, 15, 22 and 31 wk

	Body Weight, g	Heart/TL, mg/cm	Lung/TL, mg/cm	Liver/TL, g/cm	Heart Wet/Dry Weight
8 wk					
129/Sv	23.21 ±0.79	49.09 ±1.57	64.18±2.18	0.406±0.012	4.009 ±0.470
MLP ^{-/-}	21.71 ±0.68	73.69±1.99	90.74 ±1.40	0.567±0.027	4.194±0.110
<i>P</i> value	NS	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> =0.0002	NS
15 wk					
129/Sv	26.93±0.89	57.34±2.15	70.23 ±1.39	0.493 ±0.023	4.833±0.237
MLP ^{-/-}	29.36±0.94	85.72±2.32	96.69±4.83	0.653±0.025	4.829±0.161
<i>P</i> value	NS	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> =0.0002	NS
22 wk					
129/Sv	28.52±0.93	59.40±2.94	76.23 ±2.58	0.506±0.017	4.901 ±0.434
MLP ^{-/-}	30.19±0.52	84.81 ±1.42	92.27 ±2.90	0.713 ±0.020	5.149±0.313
<i>P</i> value	NS	<i>P</i> <0.0001	<i>P</i> =0.0005	<i>P</i> <0.0001	NS
31 wk					
129/Sv	31.30±0.60	71.74±3.15	79.11±2.92	0.589±0.026	4.710±0.316
MLP ^{-/-}	29.61 ±0.85	82.96±2.15	88.88±1.81	0.704 ±0.024	5.189±0.729
<i>P</i> value	NS	<i>P</i> =0.0056	<i>P</i> =0.0066	<i>P</i> =0.0039	NS

Values are means ± SE. TL, tibial length; MLP^{-/-}, muscle LIM-only protein-deficient mice; NS, not significant.

Table 2

End-diastolic volume and stiffness

	Wall Thickness, mm	Radius, mm	FEM EDV, μl	Millar EDV, μl	V_o FEM, μl	Stress-Scaling Coefficient, kpas
129/Sv						
15 wk	0.71 $\pm$ 0.03	1.96 $\pm$ 0.03	28.02 $\pm$ 0.06	27.77 $\pm$ 0.04	14.7 $\pm$ 0.75	0.74 $\pm$ 0.13
31 wk	0.65 $\pm$ 0.02	1.85 $\pm$ 0.04	28.03 $\pm$ 0.06	28.33 $\pm$ 0.03	14.8 $\pm$ 0.11	0.80 $\pm$ 0.02
MLP ^{-/-}						
15 wk	0.71 $\pm$ 0.01	1.91 $\pm$ 0.04	31.81 $\pm$ 0.06	31.83 $\pm$ 2.59	16.4 $\pm$ 1.41	1.19 $\pm$ 0.07
31 wk	0.70 $\pm$ 0.02	2.37 $\pm$ 0.09	45.16 $\pm$ 0.11	45.02 $\pm$ 1.26	29.2 $\pm$ 0.61	1.52 $\pm$ 0.08

Values are means $\pm$ SE. FEM, finite element model; V_o , unloaded volume; EDV, end-diastolic volume.