Transition From Compensated Hypertrophy to Intrinsic Myocardial Dysfunction During Development of Left Ventricular Pressure-Overload Hypertrophy in Conscious Sheep

Systolic Dysfunction Precedes Diastolic Dysfunction

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Background. Patients with aortic stenosis have a period of compensated left ventricular hypertrophy but may eventually develop congestive heart failure. Previous experimental studies showed either normal myocardial contractility in mild short-term pressure overload or myocardial dysfunction with severe pressure overload. Transition from compensated left ventricular hypertrophy to myocardial dysfunction has not been experimentally demonstrated in an adult large animal. Controversial issues in pressure-overload hypertrophy include whether the left ventricular dysfunction is due to insufficient hypertrophy (afterload mismatch) or to intrinsic myocardial dysfunction and whether diastolic dysfunction precedes systolic dysfunction.

Methods and Results. We induced left ventricular hypertrophy (41% increase in left ventricular to body weight ratio) by gradually tightening a hydraulic constrictor around the ascending aorta in 9 chronically instrumented conscious sheep. Afterload (end-systolic stress) elevation remained constant (approximately 33% greater than baseline) by adjustment of the aortic constrictor over 6 weeks, gradually increasing left ventricular pressure (from 117±6 to 163±5 mm Hg) as hypertrophy developed. Four sets (baseline, 2 weeks, 4 weeks, and 6 weeks) of serial hemodynamic studies were performed in each animal with β-blockade, first with and then without aortic constriction to mechanically match loading conditions. Stepwise methoxamine infusion was performed to obtain load-independent assessment of myocardial contractility. Midwall shortening (P<.05) and shortening rate (P<.05) at mechanically matched loading conditions showed that myocardial dysfunction developed between the fourth and the sixth week. Shortening-preload-afterload (P<.05) and shortening rate-preload-afterload (P<.05) relations, load-independent contractility indices based on the systolic myocardial stiffness concept, also revealed depressed myocardial contractility at the sixth week. Time constant of left ventricular isovolumic relaxation and diastolic myocardial stiffness constant did not change over the 6 weeks.

Conclusions. Transition from normal myocardial contractility to myocardial dysfunction was demonstrated. This transition occurred even when the elevation of afterload remained constant as hypertrophy incompletely adapted to increasing left ventricular pressure. Systolic dysfunction preceded diastolic dysfunction in this model. (Circulation. 1993;88[part 1]:2415-2425.)

Key Words • aorta • stenosis • hypertrophy

Patients with aortic stenosis and hypertension have a long period of compensated left ventricular (LV) hypertrophy but develop congestive heart failure unless the pressure overload is relieved. The transition from stable compensated hypertrophy to myocardial dysfunction is poorly understood, and experimental studies of myocardial function in animals with pressure-overload hypertrophy have yielded different results with different models. Moderate, gradually applied, and short-term pressure stress on young animals induced compensatory hypertrophy with normal myocardial contractility. Severe, abruptly applied, and long-term pressure stress in adult animals has resulted in hypertrophy with depressed myocardial contractility. In this study, we evaluated LV myocardial function serially in chronically instrumented sheep with gradually applied mechanical pressure overload. This model has similarities to clinical aortic stenosis in that pressure overload was gradually and progressively applied on large adult animals. This model provides information regarding the relation between the time course of

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hypertrophy and myocardial dysfunction to distinguish whether the cardiac dysfunction was due to insufficient hypertrophy (afterload mismatch)\textsuperscript{2-4,6-9} or intrinsic myocardial dysfunction\textsuperscript{6-16} and whether diastolic dysfunction preceded systolic dysfunction. The current study was designed to answer these questions by serial assessment of myocardial systolic and diastolic function in each animal using methods independent of loading conditions.

Effects of general anesthesia and recent surgery were avoided by using chronically instrumented animals. Serial evaluation of the same animal during the development of gradually induced hypertrophy minimized interanimal variability.

Most previous studies have used indices that are dependent on preload or afterload, factors known to change with pressure overload and hypertrophy. We used midwall shortening and shortening rate at common loading conditions, using both mechanical matching (hydraulic aortic cuff constrictor) and gradual methoxamine infusion and the myocardial stiffness concept.\textsuperscript{16-18}

**Methods**

**Animal Model**

Studies were performed on nine Suffolk and Hampshire nonpregnant ewes (Earle Parsons and Sons, Inc, Hadley, Mass) at age 2 years (young adult). Before the application of pressure overload, the following instrumentalations were implanted through a left thoracotomy using sterile surgical technique under general anesthesia induced with intramuscular ketamine (10 mg/kg) and maintained with inhalation of halothane and intravenous ketamine (10 mg·kg\textsuperscript{-1}·h\textsuperscript{-1}): a miniature pressure transducer (P3.5, Koningsberg Instruments, Pasadena, Calif) to measure LV pressure and a Tygon catheter (Norton Plastic and Synthetic Division, Akron, Ohio) for in vivo calibration, Tygon catheters in the descending thoracic aorta and right atria, sonomicrometer crystals (SL-5 and WT-5, Triton Technology Inc, San Diego, Calif) to measure internal diameter and wall thickness of the LV, and a hydraulic constrictor (Hazen Everett, Teaneck, NJ) around the ascending aorta (supracoronary) to induce LV pressure-overload hypertrophy and to acutely alter afterload. The thoracotomy incision was closed in layers, and the animals were allowed to recover for 1 to 2 weeks. Penicillin and streptomycin (Combictive, Pfizer) were administered by intramuscular injection before surgery and continued for 5 days thereafter.

**Protocol**

For the week before the first study, the animals were trained to rest quietly, upright in a nylon sling (Thelma Nantz, DeWitt, Iowa). All hemodynamic studies were performed after animals had been acclimated to the laboratory environment without anesthesia or sedative. These study conditions eliminate possible complicating effects on myocardial function of anesthetic and sedative agents or recent surgery.\textsuperscript{19}

One to 2 weeks after the instrumentation, baseline hemodynamics were assessed. The amount of saline required to infuse into the aortic cuff constrictor to produce an LV-aortic pressure gradient of 40 mm Hg was determined by temporary constriction. Then, before the hemodynamic study, all animals were pretreated with propranolol (1 mg/kg) and atropine (0.1 mg/kg) to eliminate sympathetic and parasympathetic influences. Adequacy of \(\beta\)-blockade was demonstrated by elimination of inotropic and chronotropic responses to intravenous bolus isoproterenol (0.2 \(\mu\)g/kg). The end-systolic stress-strain relations were obtained by altering loads with graded infusion of methoxamine (6, 12.5, 25, and 50 \(\mu\)g·kg\textsuperscript{-1}·min\textsuperscript{-1}) through the right atrial line. The methoxamine infusion was increased at 5-minute intervals, when steady-state hemodynamics had been achieved. On the same day, the hemodynamic study was repeated after application of aortic constriction by infusing the predetermined amount of saline into the cuff constrictor.

After the assessment of hemodynamics at baseline, the aortic constrictor was kept inflated for 6 weeks to chronically elevate afterload. As wall thickness increased in response to the pressure overload, the aortic constrictor needed to be tightened further to raise the pressure gradient in order to maintain elevated afterload. This controlled increase in afterload was achieved by maintaining an LV-aortic peak-to-peak systolic pressure gradient in the absence of autonomic blockade of 40 mm Hg as determined above for the first 2 weeks and by incrementing the gradient in the absence of autonomic blockade by an additional 10 mm Hg at 2 and 4 weeks (see Fig 1). Monitoring and increasing the pressure gradient were done without \(\beta\)-blockade. Increases in the pressure gra-
dient were done at the beginning of the third (from 40 to 50 mm Hg) and fifth (from 50 to 60 mm Hg) week without autonomic blockade. It should be noted that the pressure gradient at the time of the hemodynamic studies after autonomic blockade in each animal was not exactly 40, 50, or 60 mm Hg, respectively, and was presumably due to the inotropic and chronotropic effects of propranolol and atropine. Throughout the study, adjustment of the constrictor was performed without β-blockade every 2 to 3 days to maintain the desired pressure gradient. This stepwise increase in LV-aortic pressure gradient resulted in an end-systolic stress that was constantly elevated by approximately one third of baseline (nonconstricted value).

Hemodynamic studies were repeated on each animal at the second, fourth, and sixth weeks during the development of hypertrophy both with and without aortic constriction. Methoxamine was infused to elevate blood pressure both with and without aortic constriction during each hemodynamic study. In this way, loading conditions were mechanically matched, allowing myocardial contractility to be directly compared throughout the development of hypertrophy, using midwall shortening or shortening rate. At the sixth week, after completion of hemodynamic studies, the animals were given an overdose of pentobarbital (100 mg/kg). The position of all instrumentation was confirmed at necropsy. The hearts were excised, separated into atria, LV free wall, intraventricular septum, and right ventricular free wall, lightly blotted, and weighed.

The animals used in this study were maintained in accordance with the guidelines of the Children's Hospital Animal Use Committee and Guidelines for the Care and the Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Council (DHHS publication No. [NIH] 85-23, 1985).

**Hemodynamic Measurement**

Aortic and LV pressures were measured with chronically implanted Tygon catheters and in vitro Statham P23ID strain gauge manometers (Statham Instruments, Oxnard, Calif) calibrated using a mercury manometer. The solid-state LV pressure transducer was calibrated in vivo using the fluid-filled LV catheter. LV internal diameter and wall thickness were measured using a sonomicrometer transit-time dimension gauge (Triton Technology Inc, San Diego, Calif). All analog signals were directed through a central electronic distribution panel (Thomas Patrick, Framingham, Mass) to an eight-channel analog tape recorder (Hewlett-Packard 3968A instrumentation recorder) and displayed on an ink writing oscillograph (Gould-Brush 2800 and 260, Cleveland, Ohio). The first derivative of LV pressure (dp/dt) was recorded from the solid-state catheter using an operational amplifier (Teledyne Philbrick, Dedham, Mass) functioning as a differentiator at 700 Hz. The differentiator was calibrated directly using a triangle wave generator (Hewlett-Packard 3311A function generator). Analog tape recordings were digitized using an IBM PC/AT with an A-D convertor (Cyborg Equipment, Cambridge, Mass) at 2.5-millisecond intervals for 5 seconds. An average beat was constructed from 5 to 15 consecutive beats, depending on heart rate.

**Data Analysis**

**Assessment of wall stress.** Instantaneous average global LV circumferential wall stress, \( \sigma_0 \) (g/cm²), was calculated throughout the cardiac cycle assuming a cylindrical annulus at the site of dimension measurement as

\[
\sigma_0 = 1.36 \times \frac{P}{D/2h}
\]

where P is the instantaneous LV cavity pressure (mm Hg), D is the minor axis diameter, and h is the wall thickness. End-diastolic and end-systolic circumferential wall stresses were used as measures of preload (\( \sigma_{e0} \)) and afterload (\( \sigma_{a0} \)), respectively. Peak systolic stress and mean systolic stress (time-average stress between \( [\text{dp/dt}]_{\text{max}} \) and \( [\text{dp/dt}]_{\text{min}} \)) were also obtained. End diastole was defined as the time of relative minimum pressure preceding an increase in LV pressure with systole. End systole was defined as the time of maximum systolic stiffness as described in the "Appendix." Circumferential stress was used for quantification of preload and afterload since circumferential shortening and shortening rate were used as indices of fiber shortening and shortening velocity, respectively.

**Assessment of myocardial contractility I: Midwall shortening (Sm) and shortening rate (SRm) at constant loads adjusted by the aortic constrictor.** The midwall diameter (Dm) was calculated so that the same fiber, which was at the midpoint of the wall at end diastole, was tracked throughout cardiac cycles and alterations of loads taking into account the thickening gradient from endocardium to epicardium. Based on a cylindrical model of LV geometry and incompressibility of myocardium, we obtained

\[
D_m = \sqrt{D_{es}^2 + h_{es}(2D_{es} + h_{es})(D_{es} + h_{es})/(D_{es} + h_{es})}
\]

where D and h are internal short-axis diameter and wall thickness, and subscripts es and es denote end diastole and end systole, respectively.\(^5\)\(^6\)\(^20\)

Myocardial contractility was evaluated on the basis of midwall shortening (Sm) given by

\[
Sm = 1 - \frac{D_{es}}{D_{es}}
\]

where D_{es} and D_{es} are end-systolic and end-diastolic midwall diameter, respectively. Midwall shortening rate was also obtained as a measure of myocardial contractility as

\[
SRm = Sm/(ST/\sqrt{RR})
\]

where ST (systolic time) is the time from end diastole to end systole and RR is the cycle length.

Both midwall shortening (Sm) and shortening rate (SRm) calculated in this manner are known to be preload and afterload dependent.\(^5\)\(^6\) However, to avoid any potential confounding effects of loading state on the assessment of contractility in this study, Sm and SRm were obtained at common loading conditions throughout the four serial sets of hemodynamic studies by temporarily releasing the aortic constriction. Thus, Sm and SRm directly reflect myocardial contractility.

Alternative shortening parameters derived from end-diastolic and end-ejection diameters were also obtained. End-ejection diameter and wall thickness were determined as diameter and wall thickness at \( [\text{dp/dt}]_{\text{min}} \) (Reference 21) and used to obtain the conventional
shortening and shortening rate (ie, not those based on the systolic myocardial stiffness concept). Although (dP/dt)min may occur after end ejection, dimensions at (dP/dt)min and those at end ejection should be similar because both (dP/dt)min and end ejection occur during the isovolumic phase.

Assessment of myocardial contractility II: Midwall shortening- and shortening rate-preload-afterload (Sm-σe,σa and SRe-σe,σa) relations. We used preload- and afterload-independent assessments of myocardial contractility based on the systolic myocardial stiffness concept. The indices Sm-σe,σa and SRe-σe,σa relations were previously described in detail and were demonstrated to be applicable to the hypertrophied ventricle. The "Appendix" briefly describes equations to obtain the Sm-σe,σa and SRe-σe,σa relations.

Conventional midwall shortening (Sm), endocardial shortening (Se), and endocardial shortening rate (SRe)-afterload (σa) relations were also analyzed. Linear regressions were performed on Sm-σa, Se-σa, and SRe-σa data obtained during the stepwise methoxamine infusion, and the Sm, Se, and SRe corresponding to common afterloads were obtained from these linear equations.

Diastolic properties. The time constant of isovolumic relaxation, τa, was obtained as described below. Pressure-time (P-t) data during the isovolumic relaxation period was modeled as

\[ P = P_0 e^{-\tau a} + P_b \]

where \( P_0 \), T, and \( P_b \) are constants obtained by a nonlinear regression analysis. The time constant τa [defined as the time for pressure at (dP/dt)min to be reduced by the factor (1/e)] is then given by

\[ \tau_a = T \log(e P_0 / (P_0 + P_b - e P_b)) \]

Note that for zero asymptote (\( P_b = 0 \)), τa is equal to T.

Diastolic myocardial stiffness was obtained by curve-fitting the stress (σ) - midwall diameter (Dm) relation in the form of \( \sigma = C D_m^\gamma \) yielding the expression

\[ E_s = K d\sigma / de = K d\sigma (dDm/Dm) = K(\gamma\sigma) = k\sigma \]

where \( de = dDm/Dm \) is the midwall incremental strain, K is a geometric factor (K = 1/4 for a cylinder), \( k = K \gamma \), and \( \sigma \) is stress difference. Thus, k, which is dimensionless, was used as an index of diastolic myocardial elastic properties. As an indirect measurement of LV diastolic filling, \( (dD/dt)_{max} \) (the maximum rate of increase in LV internal diameter during LV early filling) was obtained.

Statistics

Statistical software packages SPSS (SPSS Inc., Chicago) and PRIMER BIOSTATISTICS (McGraw-Hill, New York, NY) were used for statistical analyses. Multiple comparisons within groups were analyzed using a repeated-measures ANOVA and Student-Newman-Keuls test. Linearity of the end-systolic stress-log Dm relation was examined by F test on the significant contribution of the squared term to the correlation coefficient in the quadratic fitting. Results were described as mean±SEM, and \( P < .05 \) was taken as significant.

Results

Time Course of Pressure Overload and Conventional Hemodynamic Parameters

The increasing LV aortic pressure gradient from 40 mm Hg initially to 60 mm Hg 4 weeks later was accompanied by an increase in LV peak systolic pressure and a decrease in aortic peak systolic pressure. At the beginning of the 6-week protocol, the initial aortic constriction elevated LV peak systolic pressure from 117±6 to 143±8 mm Hg and end-systolic stress from 165±14 to 207±17 g/cm². At the end of the 6 weeks, LV peak systolic pressure had increased to 163±5 mm Hg (\( P < .01 \)) by gradually tightening the aortic constriction. This increase in LV peak systolic pressure was accompanied by hypertrophy (increase in the cross-sectional wall area from 22.2±1.7 cm² at baseline to 24.0±2.3, 24.5±2.3, and 25.9±2.1 cm² at the second, fourth, and sixth weeks, respectively, \( P < .01 \)), maintaining relatively constant elevation of afterload (end-systolic stress) (Figs 2 and 3 and Table 1). Peak and mean systolic stresses changed similarly to end-systolic stress; that is, they were constantly elevated for the 6 weeks.
Therefore, compensating but insufficient hypertrophy balanced an increasing LV peak systolic pressure maintaining afterload elevation constant.

The (dP/dt)max did not change in response to pressure-overload hypertrophy. Elevated peak systolic pressure (P<.01) and end-diastolic pressure and end-diastolic stress, although change in the latter was not statistically significant, might have masked a change in (dP/dt)max, which is load dependent. Indeed, (dP/dt)max at 6 weeks during temporary release of the constriction tended to be decreased although statistically not significant. Correction of (dP/dt)max with end-diastolic volume or fiber length would have possibly detected systolic dysfunction.

The postmortem LV weight and heart weight were 170±13 g and 217±18 g, respectively. The LV weight to body weight ratio was 3.51±0.23 g/kg, which was 41% greater than that in 2-year-old control sheep in our previous work.16 No animal had postmortem findings suggesting congestive heart failure such as ascites, pleural effusion, or pulmonary edema.

Myocardial Contractility I: Shortening and Shortening Rate With Mechanical Matching for Loads

To mechanically match loading conditions, hemodynamic measurements were repeated with acute aortic constriction at baseline and after temporary release of chronic aortic constriction at the second, fourth, and sixth weeks. That is, hemodynamic measurements were performed both with and without the aortic constriction each time.

Midwall shortening (Fig 4A) decreased at the sixth week of chronic pressure overload by 32% (P<.05) with aortic constriction and by 33% (P<.05) without aortic constriction compared with the respective baseline values. Midwall shortening rates were 0.57±0.08, 0.54±0.08, 0.48±0.07, and 0.41±0.08 s⁻¹ at baseline, 2, 4, and 6 weeks, respectively, without aortic constriction, and 0.46±0.07, 0.48±0.08, 0.43±0.06, and 0.35±0.06 s⁻¹ at baseline, 2, 4, and 6 weeks, respectively, with aortic constriction (P<.05 vs baseline values). Midwall shortening rate changed qualitatively similar to shortening. Relatively great scatter in shortening compared with shortening rate is presumably due to the former’s heart rate dependency. Endocardial shortening decreased at the sixth week by 16%, which was significant (P<.05) but less than the decrease in midwall shortening (Fig 4B). Although endocardial shortening rate tended to decrease from 1.12±0.12 at baseline to 1.00±0.12 at the sixth week, it was not statistically significant.

<table>
<thead>
<tr>
<th>Table 1. Time Course of Conventional Hemodynamic Parameters</th>
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<tbody>
<tr>
<td><strong>Baseline</strong></td>
</tr>
<tr>
<td>PSP, † mm Hg</td>
</tr>
<tr>
<td>AoP, mm Hg</td>
</tr>
<tr>
<td>LV-Ao, mm Hg</td>
</tr>
<tr>
<td>σes, † g/cm²</td>
</tr>
<tr>
<td>σpeak, † g/cm²</td>
</tr>
<tr>
<td>Wted, † mm</td>
</tr>
<tr>
<td>IDd, mm</td>
</tr>
<tr>
<td>IDd/Wted</td>
</tr>
<tr>
<td>(dP/dt)max, mm Hg/s</td>
</tr>
<tr>
<td>(dP/dt)min, mm Hg/s</td>
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<tr>
<td>(dP/dt)/Pmax, s</td>
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</tbody>
</table>

PSP indicates left ventricular (LV) peak systolic pressure; AoP, aortic peak systolic pressure; LV-Ao, LV to aorta peak-to-peak pressure gradient; σes, σpeak, and σmean, end-systolic, peak-systolic, peak systolic, and mean systolic stresses, respectively; WTed, end-diastolic wall thickness; IDd, end-diastolic internal diameter; (dP/dt)max and (dP/dt)/Pmax, maximum and minimum values of the first derivative of LV pressure, respectively; (dP/dt)/Pmax, maximum value of the first derivative of LV pressure divided by instantaneous LV pressure.

Values are mean±SEM. Data with temporary release of the aortic constrictions are shown in the parentheses. *P<.05 and †P<.01, significant changes over the 6 weeks of pressure overload.
Fig 4. Plot of time course of myocardial contractility I: mechanical correction for preload and afterload. Midwall shortening fraction (panel A) and endocardial shortening (panel B) with (open squares, AS+) and without (closed squares, AS−) the aortic constriction decreased (P<.05, repeated-measures ANOVA) over the 6 weeks. Small open circles and small closed circles are individual data with and without the aortic constriction. Midwall shortening and endocardial shortening were significantly less (*P<.05, Student-Newman-Keuls test) than the baseline value at the sixth week.

Alternative shortening parameters derived from end-diastolic and end-ejection diameters responded similarly to the end-systolic–derived shortening parameters. Midwall (Smₑₑ) and endocardial (Seₑₑ) shortening from end diastole to end ejection with mechanical load matching, respectively, decreased by 27% (P<.05) and 12% (P<.05) at the sixth week (Table 2). Midwall (SRmₑₑ) and endocardial (SReₑₑ) shortening rate, respectively, decreased by 22% (P<.05) and 6.4% (P<.05) at the sixth week (Table 2). The degree of depressions of endocardial indices was less than in midwall indices.

**Myocardial Contractility II: Shortening-Preload-Afterload and Shortening Rate-Preload-Afterload Relations**

The Smₑₑ−σₑₑ−σₑₑ and SRmₑₑ−σₑₑ−σₑₑ relations were obtained on the basis of the linear end-systolic stress-strain relations and diastolic stiffness curve. The Smₑₑ−σₑₑ−σₑₑ and SRmₑₑ−σₑₑ−σₑₑ relations demonstrated qualitatively similar results to those in Sm and SRm with mechanical matching of loads by means of the aortic constrictor described above. Midwall shortening (Fig 5A) and shortening rate (Fig 5B) at common preload and afterload remained normal until the fourth week of the aortic constriction but were depressed at the sixth week. Fig 6 demonstrates how the decrease in Sm at common preload and afterload between the fourth and the sixth week was out of proportion to the increase in the myocardial cross-sectional area. Thus, depression of contractility did not parallel the degree of hypertrophy, indicating the transition from compensated hypertrophy to intrinsic myocardial dysfunction.

Fig 7 demonstrates midwall shortening (Sm−afterload, endocardial shortening (Se−afterload, and endocardial shortening rate (SRe−afterload relations obtained using linear regressions on raw data points during incremental infusion of methoxamine. The midwall shortening (Sm−afterload relation was depressed (P<.05) at the sixth week, whereas downward changes in endocardial shortening (Se−afterload or endocardial shortening rate (SRe−afterload relations did not reach statistical significance.

**Diastolic Function**

The mean left ventricular end-diastolic pressure was 10±2 mm Hg at baseline and 15±3 mm Hg at the sixth week with chronic aortic constriction (the change was not statistically significant). Left ventricular end-diastolic pressure increased (P<.05, Student-Newman-Keuls test) from end diastole to end ejection.

**Table 2.** Time Course of Conventional Shortening and Shortening Rate

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>2 Weeks</th>
<th>4 Weeks</th>
<th>6 Weeks</th>
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<tbody>
<tr>
<td>Smₑₑ* %</td>
<td>15.7±2.6</td>
<td>14.1±2.4</td>
<td>13.3±2.1</td>
<td>11.1±1.7</td>
</tr>
<tr>
<td></td>
<td>(15.1±2.4)</td>
<td>(13.9±2.0)</td>
<td>(11.5±1.6)</td>
<td></td>
</tr>
<tr>
<td>Seₑₑ* %</td>
<td>28.3±3.6</td>
<td>27.1±4.3</td>
<td>26.5±3.4</td>
<td>23.9±3.4</td>
</tr>
<tr>
<td></td>
<td>(28.9±4.2)</td>
<td>(27.5±3.2)</td>
<td>(24.9±3.5)</td>
<td></td>
</tr>
<tr>
<td>SRmₑₑ*</td>
<td>0.495±0.084</td>
<td>0.431±0.082</td>
<td>0.388±0.064</td>
<td>0.333±0.056</td>
</tr>
<tr>
<td></td>
<td>(0.486±0.080)</td>
<td>(0.445±0.068)</td>
<td>(0.384±0.056)</td>
<td></td>
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<tr>
<td>SReₑₑ*</td>
<td>0.935±0.118</td>
<td>0.876±0.150</td>
<td>0.811±0.112</td>
<td>0.764±0.117</td>
</tr>
<tr>
<td></td>
<td>(0.973±0.149)</td>
<td>(0.911±0.109)</td>
<td>(0.875±0.124)</td>
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</tr>
</tbody>
</table>

Smₑₑ and Seₑₑ indicate conventional midwall and endocardial shortening from end diastole to end ejection, respectively; SRmₑₑ and SReₑₑ, conventional midwall and endocardial shortening rate from end diastole to end ejection. Data with temporary release of the aortic constriction are shown in parentheses.

Values are mean±SEM. *P<.05, significant changes over the 6 weeks of pressure overload.
ic pressure without aortic constriction was unchanged during the 6 weeks. The time constant of isovolumic decrease in pressure, tau, did not change during the 6 weeks of pressure overload. Myocardial diastolic stiffness constant, k, did not change during the 6 weeks of the aortic constriction. The maximum rate of increase in LV internal diameter during early diastolic filling, (dD/dt)max, was also unchanged during the course of the study, indicating no changes in LV early diastolic filling rate. Fig 8 demonstrates average diastolic stress–midwall diameter curves, indicating that the pressure-overload hypertrophy did not significantly alter the diastolic myocardial stiffness relation. Thus, no evidence of diastolic myocardial dysfunction was detected at resting heart rates in this model of pressure-overload hypertrophy.

**Discussion**

We demonstrated transition from LV pressure-overload hypertrophy with normal myocardial function to myocardial dysfunction in chronically instrumented conscious sheep with ascending aortic constriction. This study is unique in that gradual but progressive pressure overload was applied in large adult animals combined with serial load-independent assessment of myocardial contractility. Implanting a fixed constriction in an immature animal and allowing it to grow also induces progressive chronic pressure-overload hypertrophy. We previously demonstrated that pressure-overload hypertrophy induced in adult sheep differs from that in growing young lambs in terms of the effects on myocardial function, an effect that may be in part related to sudden versus gradual imposition of the pressure load. The adjustable constrictor not only enabled the gradual imposition of pressure overload but also allowed the animal to recover from surgery without pressure overload, avoiding potential initially acute myocardial damage as a result of simultaneous surgical intervention and initiation of pressure overload. With this model, midwall shortening and shortening rate at matched loads using an adjustable aortic constrictor, and the load-independent indices of contractility Sm-σe-σm and SRm-σe-σm relations showed a significant decrease in myocardial contractility at the sixth week.

An alternative explanation for the depression in midwall shortening and shortening rate observed in this study that should be considered is a failure to keep pace with the increasing severity of aortic stenosis without intrinsic myocardial dysfunction. According to this hypothesis, shortening and shortening rate decrease as a result of elevated afterload in the presence of normal myocardial contractility. In this study, we mechanically maintained a constant elevation of afterload. The initial decrease in shortening and shortening rate was proportional to the rise in afterload, consistent with the afterload mismatch hypothesis. Since the elevation in afterload was constant, the decrease in shortening and shortening rate from baseline values should have been
constant, if intrinsic myocardial contractility remained normal. However, the progressively decreasing shortening and shortening rate at constant afterload indicated deterioration of intrinsic myocardial contractility in addition to afterload mismatch. Furthermore, Sm-σm-σa, and SRm-σm-σa relations theoretically account for both preload and afterload and also indicate the onset of depressed contractility. Thus, elevated afterload cannot fully explain the depressed shortening and shortening rate observed during the development of hypertrophy in this model.

Fig 7. Line plots show time course of conventional midwall shortening (Sm)–, endocardial shortening (Se)–, and endocardial shortening rate (SRe)–afterload relations. Linear regressions were performed on operating raw midwall shortening (Sm)–afterload (σm), endocardial shortening (Se)–afterload (σa), and endocardial shortening rate (SRe)–afterload (σa) data. Sm (panel A), Se (panel B), and SRe (panel C) corresponding to common afterloads (150, 200, 250, and 300 g/cm²) were obtained. Sm was significantly depressed at all afterload levels with downward shift of the Sm–σa relation. In contrast, changes in endocardial indices (Se and SRe) were blunted, and only Se at afterload of 150 g/cm² was significantly decreased.

Diastolic function parameters under resting conditions remained unchanged throughout the 6 weeks of pressure overload (Table 3). Gradual application of the pressure overload in the current study may have contributed to the absence of prolongation of the time constant of relaxation and impairment of diastolic elastic properties. However, we did not perform interventions such as rapid pacing or hypoxia, and there remains the possibility that diastolic function reserve was depressed. We previously demonstrated prolongation of the time constant of relaxation during rapid atrial pacing in a similar model of sheep LV hypertrophy.

Table 3. Time Course of Diastolic Function Parameters

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>2 Weeks</th>
<th>4 Weeks</th>
<th>6 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDP, mm Hg</td>
<td>10±2</td>
<td>12±3</td>
<td>13±1</td>
<td>15±3</td>
</tr>
<tr>
<td>tau, milliseconds</td>
<td>28.0±2.5</td>
<td>24.0±1.2</td>
<td>26.1±1.8</td>
<td>29.4±3.3</td>
</tr>
<tr>
<td>k</td>
<td>27.8±4.5</td>
<td>29.9±6.6</td>
<td>27.1±4.4</td>
<td>24.9±6.4</td>
</tr>
<tr>
<td>(dD/dt)max, mm/s</td>
<td>15.8±1.4</td>
<td>17.8±3.0</td>
<td>16.6±3.6</td>
<td>16.7±2.3</td>
</tr>
</tbody>
</table>

EDP indicates left ventricular (LV) end-diastolic pressure; tau, time constant of LV isovolumic pressure decay; k, diastolic myocardial stiffness constant; (dD/dt)max, maximum rate of early diastolic increase in LV internal diameter. No significant changes during the 6 weeks of the pressure overload were detected in any of the resting diastolic function parameters examined. Data with temporary release of the aortic constrictions are shown in parentheses. Values are mean±SEM.
phy induced by 6 weeks of aortic banding.27 The previous results would indicate that diastolic function reserve may have been depressed in the current study.

Mechanisms responsible for the myocardial dysfunction have yet to be determined. For example, an alteration in calcium availability,27,28 intermittent myocardial ischemia,29 cytoskeletal changes such as microtubule polymerization,30 and decreased volume fraction of cardiocyte myofilibrils31 may be responsible for the progression of myocardial dysfunction. Accumulation of fibrillar collagen within the extracellular matrix and around intramyocardial coronary arteries of hypertrophied ventricles32,33 was shown to be associated with diminished ventricular diastolic distensibility and, as a result, ventricular pump function. However, in the current study, the fibrosis is not likely to be a major factor explaining the depressed myocardial systolic function, since diastolic myocardial stiffness constant remained similar throughout the protocol. A decrease in myofibrillar volume density could certainly yield myocardial dysfunction. We previously observed normal myosin heavy chain mRNA level in LV myocardium of a similar 6-week pressure-overload LV hypertrophy sheep model.28 This would suggest that the relative decrease in myofibrillar volume density is not likely to fully explain the myocardial dysfunction in the current model.

Endocardial contractility indices (Sc and SRe at mechanical unloading and SRe-σa relation) decreased less or only insignificantly in contrast to midwall-derived contractility indices (Sm and SRm at mechanical unloading and Sm-σa, SRm-σa relations), which clearly revealed significant contractile dysfunction at the sixth week. Many previous studies showing normal ventricular contractility in hypertrophy used endocardial ejection indices,7,23,34 which tend to overestimate myocardial function in the hypertrophied ventricle.16 In an experimental right ventricular pressure-overload hypertrophy, Cooper et al11 observed normal ventricular ejection fraction and cardiac output with depressed contractility of papillary muscles from the same right ventricle. The blunted responses of endocardial indices to pressure-overload hypertrophy may be explained by substantial contribution of radial wall thickening in hypertrophied ventricle.16,35 Midwall fibers are predominantly oriented circumferentially, whereas endocardial fibers are mainly oriented in the meridional direction. Because we assess circumferential fiber shortening–circumferential myocardial stress relationships, it is more appropriate to follow the midwall fiber than to follow the endocardial fiber. Hypertrophy of myocytes may not be homogeneous through the ventricular wall, and stress distribution may not be homogeneous. Midwall analyses dealing with a conceptual median fiber may be more representative of transmural myocardial function than endocardial analyses.

Study Limitations
We terminated the study at 6 weeks even though data after longer periods of pressure overload would probably have revealed more substantial myocardial dysfunction and a clearer discrepancy between systolic and diastolic dysfunction. Our primary goal was to detect the transition from compensated hypertrophy to intrinsic myocardial dysfunction. Further exposure to pressure overload after development of myocardial dysfunction at 6 weeks would have probably induced clinical congestive heart failure, as shown previously.4 Systemic heart failure elicits secondary neurohumoral influences, which tend to complicate and obscure the effects of mechanical overload on myocardial function. The 41% increase in LV to body weight ratio represents severe LV hypertrophy for an adult large animal model. Further continuation of pressure overload in this model may or may not induce further hypertrophy and/or result in additional afterload mismatch or acute heart failure. A pilot study in three sheep indicated that extention of pressure overload to 8 weeks resulted in a substantially increased risk of premature death (two of three sheep) caused by congestive heart failure and rupture of the ascending aorta. Higher mortality would result in biased selection of surviving subpopulation.

A potential criticism of this study is the lack of long-axis dimension measurement. Long-axis dimension crystals were not implanted in order to minimize the morbidity and mortality. Large alterations in LV geometry may invalidate the assumption of cylindrical geometry. However, we believe that the changes in geometry induced by hypertrophy, representing a 40% to 50% increase in LV to body weight ratio, was unlikely to change our results. A postmortem study in 11 patients with pressure-overload hypertrophy36 showed homogeneous increase in myocyte size among five different ventricular sites (apical, anterolateral, posterolateral, anteroseptal, and posteroseptal LV). That study in part suggested that the cylindrical assumption and the equatorial shortening–stress analyses can assess and compare myocardial function in normal and hypertrophied ventricles. On the other hand, inclusion of long-axis dimension would have enabled us to assess ventricular contractility indices such as (dP/dt)max–end-diastolic volume relation,37 preload recruitable stroke work,38 and ventricular filling parameters.

We used end-systolic myocardial stress to quantitate afterload as the hemodynamic stimulus inducing hypertrophy. At the myocardial level, we believe myocardial stress rather than LV pressure or LV-aortic pressure gradient is the major determinant of LV hypertrophy. Mean or peak systolic stress may be a more appropriate measure of stimulus to hypertrophy than the end-systolic stress. However, we used the end-systolic stress because of controversy as to the beginning and end of systole, which substantially influences the calculated values of mean systolic stress. Nevertheless, mean and peak stresses had changes that were qualitatively similar.

Patients with aortic valve stenosis respond over years to a gradual increase in pressure overload. Once they develop clinical overt heart failure, prognosis is grave, with average survival of less than 2 years.39 These patients often have much greater hypertrophy7 than animal models and are often reported to have normal function.7,34 Although the stepwise aortic constriction for 6 weeks is considered a gradually applied pressure overload and the 41% increase in LV to body weight ratio is considered moderate to severe hypertrophy in adult large animal models of pressure-overload hypertrophy, the current model still represents acute pressure overload and mild hypertrophy compared with patients with years of aortic stenosis and severe LV hypertrophy. Thus, the current experimental results may not be directly extrapolated to human chronic pressure-over-
Shortening- and Shortening Rate-Preload-Afterload Relations

First, the end-systolic stress-strain relation was obtained as follows. The average stress difference ($\sigma$) is given by

$$\sigma = \sigma_{\text{es}} - \sigma_{\text{a}} = 1.36 \times (\text{PD}/2\text{h}) \times [1 + h/(D + h)]$$

where $\sigma_{\text{es}}$ and $\sigma_{\text{a}}$ are the integrated mean circumferential and radial stresses, respectively. Maximum systolic stiffness (Eav)$_{\text{es}}$ is defined as

$$(\text{Eav})_{\text{es}} = \max[\sigma(4/3)\log(Dm/Dom)]$$

where Dm is midwall diameter and Dom is midwall diameter at zero stress. The parameter Dom is evaluated iteratively in the manner described in the earlier studies. If linearity of the stress vs log Dm$_{\text{es}}$ relation is established, the end-systolic stress-strain ($\sigma_{\text{es}}$, $\epsilon_{\text{es}}$) relation is expressed in the form

$$\sigma_{\text{es}} = \max \text{Eav} \times \epsilon_{\text{es}}$$

where

$$\epsilon_{\text{es}} = (4/3) \log(Dm_{\text{es}}/Dom)$$

Thus max Eav is the slope of this linear relation. Fig 9A demonstrates representative end-systolic stress-strain data.

The $\text{Sm} - \sigma_{\text{es}}$, $\sigma_{\text{es}} - \sigma_{\text{a}}$, and $\text{SRm} - \sigma_{\text{es}} - \sigma_{\text{a}}$ relations represent shortening and shortening rate adjusted for preload and afterload.

This was performed by calculating the midwall shortening (Sm) from the end-diastolic midwall diameter (Dm$_{\text{es}}$) and end-systolic midwall diameter (Dm$_{\text{es}}$), respectively, corresponding to any given preload and afterload as

$$\text{Sm} = (\text{Dm}_{\text{es}} - \text{Dm}_{\text{es}})/\text{Dm}_{\text{es}}$$

The relation between end-diastolic midwall diameter (Dm$_{\text{es}}$) and preload ($\sigma_{\text{es}}$) was determined by curve-fitting the end-diastolic circumferential stress-midwall diameter data over the observed physiological range to a curve of the form

$$\sigma_{\text{es}} = C \text{Dm}_{\text{es}}^\delta$$

so that

$$\text{Dm}_{\text{es}} = (\sigma_{\text{es}}/C)^{1/\delta}$$

where C and $\delta$ are curve-fitting parameters. Fig 9B demonstrates representative diastolic stress-midwall diameter data. The relation between end-systolic midwall diameter (Dm$_{\text{es}}$) and afterload ($\sigma_{\text{a}}$) was determined by combining the linear regression equation between end-systolic stress difference ($\sigma_{\text{es}}$) and afterload ($\sigma_{\text{a}}$) over the physiological range ($\sigma_{\text{es}} = \alpha + \beta \sigma_{\text{a}}$) with the stress-strain relation, to yield

$$\text{Dm}_{\text{es}} = \text{Dom} \times \exp[(\alpha + \beta \sigma_{\text{a}})/(4/3 \max \text{Eav})]$$

Thus, midwall shortening for given preload ($\sigma_{\text{es}}$) and afterload ($\sigma_{\text{a}}$) was calculated as

$$\text{Sm} = 1 - (\text{Dom}/(\sigma_{\text{es}}/C)^{1/\delta}) \times \exp[(\alpha + \beta \sigma_{\text{a}})/(4/3 \max \text{Eav})]$$

The midwall fiber shortening rates (SRm) at given preloads and afterloads were obtained by dividing midwall shortening (Sm) by rate-corrected systolic time (STc) as

$$\text{STc} = \text{ST}/\sqrt{\text{RR}}, \quad \text{SRm} = \text{Sm}/\text{STc}$$

where ST, systolic time, is as defined above; STc and ST are systolic time corrected for heart rate and their average throughout the load alteration, respectively; and RR is a cycle length. The rate-corrected systolic time (STc) did not change in response to stepwise infusions of methoxamine.

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Transition from compensated hypertrophy to intrinsic myocardial dysfunction during development of left ventricular pressure-overload hypertrophy in conscious sheep. Systolic dysfunction precedes diastolic dysfunction.

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