Dynamic deformation characteristics of porcine aortic valve leaflet under normal and hypertensive conditions

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Yap CH, Kim H, Balachandran K, Weiler M, Haj-Ali R, Yoganathan AP. Dynamic deformation characteristics of porcine aortic valve leaflet under normal and hypertensive conditions. Am J Physiol Heart Circ Physiol 298: H395–H405, 2010. First published November 13, 2009; doi:10.1152/ajpheart.00040.2009.—Calcific aortic valve (AV) disease has a high prevalence in the United States, and hypertension is correlated to early onset of the disease. The cause of the disease is poorly understood, although biological and remodeling responses to mechanical forces, such as membrane tension, have been hypothesized to play a role. The mechanical behavior of the native AV has, therefore, been the focus of many recent studies. In the present study, the dynamic deformation characteristics of the AV leaflet and the effects of hypertension on leaflet deformation are quantified. Whole porcine aortic roots were trimmed and mounted in an in vitro pulsatile flow loop and subjected to normal (80/120 mmHg), hypertensive (120/160 mmHg), or severe hypertensive (150/190 mmHg) conditions. Local valve leaflet deformations were calculated with dual-camera photogrammetry method: by tracking the motion of markers placed on the AV leaflets in three dimensions and calculating their spatial deformations. The results demonstrate that, first, during diastole, high transvalvular pressure induces a stretch waveform which plateaus over the diastolic duration in both circumferential and radial directions. During systole, the leaflet stretches in the radial direction due to forward flow drag forces but compresses in the circumferential direction in a manner in agreement with Poisson’s effect. Second, average diastolic and systolic stretch ratios were quantified in the radial and circumferential directions in the base and belly region of the leaflet, and diastolic stretch was found to increase with increasing pressure conditions.

dynamic stretch; dynamic strain; hypertension; dual-camera photogrammetry

Calcific Aortic Valve (AV) disease has a high prevalence, especially among the elderly (16). In the United States alone, AV disease is the third most common cardiovascular pathology and is a strong risk factor for other cardiac-related deaths (17, 18, 21). Every year, nearly 9,50,000 procedures are performed on the AV, making AV surgeries second only to the coronary bypass procedure (13, 27). The number of patients requiring AV surgery is expected to triple by 2050 (34). A calcified AV has increased thickness, collagen fiber disarray, and deposition of calcium, and thus has a drastically reduced leaflet flexibility, disabling native valve kinematics and function, resulting in AV stenosis and/or regurgitation and heart failure (19).

The majority of AV calcification has an idiopathic etiology. Recent studies have shown that AV sclerosis is an active process akin to atherosclerosis, involving lipoprotein deposition, chronic inflammation, and active leaflet calcification mediated by cell differentiation (11). Furthermore, in a manner similar to the correlation between adverse hemodynamic/mechanical environment and atherosclerosis formation (9, 10), data in the literature suggest that adverse mechanical forces may elicit pathological responses of AV leaflets. Isolated mechanical forces, including stretch, pressure, and fluid shear, have been shown to affect the remodeling activity in AV tissues (3, 32, 33). Butcher et al. (6) compared transcriptional profiles of porcine AV endothelial cell cultures in vitro and found that exposure to shear stress was protective against inflammatory and oxidative gene expressions, compared with static culture. Warnock et al. (31) reported that elevated cyclic pressure significantly downregulated osteopontin and upregulated VCAM-1 in porcine AVs, indicating a proinflammatory response. Balachandran et al. (4) exposed fresh porcine AV leaflets to a range of cyclic stretch magnitudes and found that excessive stretch resulted in adverse remodeling and cell turn-over events. These studies suggest that a certain combination of adverse mechanical forces can result in active AV cellular processes that lead to degenerative valve disease.

The relationship between the mechanical environment of the AV and its biological response can explain the relationship between hypertension and AV disease. Epidemiological studies have demonstrated a correlation between hypertension and the occurrence of AV disease. In a review of existing epidemiology studies, Rabkin (20) reported that three population-based cross-sectional studies, with total sample size of 6,450 individuals, showed a consistent and significant relationship between hypertension and AV sclerosis, with an odds ratio of 1.23–1.74, indicating that hypertensive individuals are more likely to develop AV sclerosis. A possible mechanism to explain this correlation is as such: hypertension imparts excessive pressure on valve leaflets to cause additional tension and stretch in the AV leaflets, which, on a chronic basis, may elicit active cellular processes that promote calcific AV disease. Unfortunately, there is insufficient data on the dynamic strain characteristics of the AV under hypertensive conditions to allow us to put this hypothesis to test.

Investigation on the solid mechanics of the AV has been ongoing. Adamczyk and Vesely (1) and Sun et al. (28) performed quasi-static measurements of the strain response of the AV leaflets to varying pressures. Billiar et al. (5), Stella et al. (25, 26), and Vesely and Noseworthy (30) excited leaflets of the AV and performed biaxial mechanical tests to characterize the leaflet mechanical properties. A number of these studies were performed on bioprosthetic valves, such as the work of

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MATERIAL AND METHODS

Valve model. The valve model is shown in Fig. 1. Fresh porcine AVs were harvested from healthy young adult sows between 12 and 24 mo old from a local slaughterhouse, and the aortic roots were excised and trimmed of excess tissues. These AVs show no sign of calcification or fibrosis. The coronaries were then ligated with sutures, and the aortic root was firmly sutured onto two circular metal rings with a 25-mm inner diameter. Only valves with aorta sizes between 22 and 23 mm were chosen to match the size of the mounting rings. Valves were chosen to be slightly smaller in root diameter than the rings to introduce a slight circumferential stretch when sutured. This was done to prevent the valve from being restrained from expansion under applied physiological pressures. The AV leaflets were 0.5 ± 0.1 mm thick. The metal rings were connected to each other by bolting to four threaded rods, thus creating a rigid frame for the aortic root. The bolts were adjusted to maintain a distance between the rings, such that a slight axial tension was maintained in the aortic root. This axial tension allowed the AV commissures to provide structural support to the leaflets and thus allowed proper coaptation geometry. Additional rubber rings were added to the system to prevent perivalvular leakage. The valve, together with the AV frame, was then inserted into tight-fitting cylindrical chamber and mounted onto the flow loop. In our experiments, the active contractile motions of the valve annulus were not reproduced, and their effects were not considered. Being sutured to a rigid ring at the base, the valve annulus was somewhat restricted in its passive radial expansion and relaxation motion under varying pressures within the root. To minimize this, we sutured the valves such that there was 10–15 mm of myocardium between the base ring and the valve annulus, ensuring that some degree of freedom of valve annulus motion is retained.

Flow loop. The AV and the chamber were mounted onto the aortic position of the GT Left Heart Simulator (15), which was a pulsatile flow loop capable of simulating physiologically normal and pathological hemodynamic conditions (Fig. 2). The flow loop consisted of a bulb pump driven by compressed air, which simulated the pumping of the left ventricle. A bi-leaflet mechanical valve upstream of the bulb pump acted as the mitral valve. The porcine AV was placed downstream of the bulb pump, and a series of compliance chambers and resistance clamps simulated the compliance and resistances of the cardiovascular system. Saline (0.9%) was used as the flow loop fluid to keep the valve hydrated and to avoid damage to the valve tissues, which may lead to the alteration of the mechanical properties of the valve. The flow loop was tuned to a heart rate of 70 beats/min, and a cardiac output of 5 l/min, which corresponded to conditions of a normal resting adult heart. Three pressure conditions were simulated on the flow loop: normotensive (Norm), hypertensive (Hyp), and severe Hyp (SHyp) conditions. Aortic pressures were 120/80 mmHg for Norm condition, 160/120 mmHg for Hyp condition, and 190/150 mmHg for SHyp condition. An ultrasonic flow probe (T108, Transonic System, Ithaca, NY) was used to measure bulk flow rate directly downstream of the AV, while pressure gauges were used to measure pressures directly upstream and downstream of the valve. These pressure gauges were no further than 8 cm away from the valve leaflets. The timing of the acquisition of pressure and flow data was controlled by a pulse programmer, and sampling rate was 500 data points/s.

Dual-camera photogrammetry. Dual-camera photogrammetry was employed to measure the dynamic strain. This technique has been previously used to characterize the kinematics of the mitral valve.

Fig. 1. The porcine aortic valve model used for dynamic deformation measurements. Porcine aortic root is excised from a fresh heart, trimmed, ligated at the coronaries, sutured onto metal rings, and given an axial stretch by adjusting the bolts on the metal rings.
leaflet in vitro (14, 22). Using a black tissue marking dye (Black Tissue Marking Dye, Thermoelectron, Pittsburgh, PA), a regular array of markers was placed on the ventricular surface of the AV leaflets. Two cameras (Basler camera A504K, Basler) of 1280 × 1023 pixels in resolution were positioned at an angle to each other on the ventricular side of the AV. Together with an image-grabbing system (EPIX CL3SD, Buffalo Grove, IL), the cameras were used to capture the dynamic motion of the AV leaflets at 500 frames/s simultaneously. Image acquisition was gated to the acquisition of hemodynamic data through the pulse programmer. At the end of dynamic image acquisition, without draining fluid from the loop, both the ventricular and aortic chambers were exposed to atmospheric pressure, and the valve was allowed to open and to attain equilibrium. Images of the AV leaflets exposed to this zero transvalvular pressure were captured, and the corresponding leaflet geometry was used as the unloaded reference.

The deformation metric, stretch ratio, was computed at two locations, the belly region, which was the center of the leaflet, and the base region, which was on the center line of the leaflet and near to the annulus (Fig. 3). Square arrays of markers were tracked at these locations for deformation computations. The two-dimensional (2D) coordinates of the markers, as viewed from the two cameras, were automatically tracked with a custom Matlab program. These two sets of 2D marker coordinates were converted to the true three-dimensional (3D) coordinates of the markers by direct linear transformation. To resolve the relative angle between the two cameras, a metal cube of known dimensions was inserted into the chamber at the location of the AV annulus, and images of the cube were captured with the two cameras. Coordinates of the seven visible vertexes of the cube were used for computing the differences in camera angle and position.

The 3D marker coordinates over relevant time points were then analyzed for leaflet deformation. Shell-based 2D isoparametric finite-element shape functions were used to fit the surface geometry and cover the different groups of markers on the leaflets for interpolation. Two types of shape functions were used: the C-1 continuous four-node Hermite shape function was used for interpolation at locations where sufficient markers were visible throughout the intended duration; otherwise, the C-0 continuous nine-node Lagrangian shape functions were used for interpolation. Coordinates of an array of 9 markers (3 × 3) were used for the fit to the Lagrangian shape function, and coordinates of an array of 16 markers (4 × 4) were used to derive the Hermite shape function. Both the Lagrangian and Hermite elements had biquadratic functions. The nine-node Lagrangian polynomial has one term defined for each node: the coordinate term, while the four-node Hermite polynomial has four terms defined for each node, the coordinate term and the terms for the first, second, and cross derivative of the coordinate with respect to the element local coordinate, \( \xi \) and \( \eta \). Coordinates at any point within the elements can be obtained by interpolating the shape function terms defined at the nodes, as follows (the summation notation applies) (24):

\[
R_p = \sum_{i=1}^{9} N_i^p(\xi_p, \eta_p) R_i^p
\]

where \( i = 1, 9 \) and \( j, k = 0 \) for 9 node Lagrangian shape function, and \( i = 1, 4; j, k = 0, 1, j + k \leq 1 \), for four-node Hermite shape function; \( N_i \) is the shape function for each node; \( R_i \) is the known coordinates at the nodes; and \( R_p \) is the interpolated coordinates at the specific

Fig. 2. Schematic of the pulsatile flow loop (The Georgia Tech Left Heart Simulator) used for dynamic deformation measurements. The flow loop is driven by a compressed air bulb pump. Two high speed cameras are placed at substantial angle with each other and record images of the valve from the ventricular view. A data acquisition system records the ventricular and aortic pressures and aortic flow. Image acquisitions and hemodynamic measurements were synchronized with a trigger signal from a pulse generator.

Fig. 3. Marker array on the ventricular surface of the valve leaflets, as viewed from the two cameras. A sequence of images from both cameras covering the entire cardiac cycle were recorded, digitized, and analyzed to determine principal stretches on both the belly and base region of the right coronary leaflet. The superimposed dark shaded spot shows the location of the belly region, whereas the light shaded spot shows the location of the base region.
location defined by $\xi$ and $\eta$. $j$ and $k$ are differential indexes with respect to $\xi$ and $\eta$. Derivative of coordinates are required for the Hermitian element and were obtained by fitting a 9-node ($3 \times 3$) Lagrangian shape functions to subsets of the 16-marker arrays, centered at the subject node, and calculating the coordinate derivatives.

The shape functions were subsequently used to compute stretch ratios and strains at relevant time points in the cardiac cycle. Principal Almansi strains ($e$), Principal Green strains ($E$), and principal stretch ratios ($\lambda$) were calculated as follows:

$$e_{ab} = \frac{1}{2}(g_{ab}^* - g_{ab})$$

$$E_{ab} = \frac{1}{2}(G_{ab}^* - G_{ab})$$

$$\lambda_{ab} = \sqrt{2e_{ab} + I}$$

Where $g_{ab}$ and $G_{ab}$ are components of the metric tensor in the deformed configuration and in the unloaded reference configuration, respectively; and $I$ is identity matrix. The methodology for the computation of stretch ratio and strains was outlined by Smith et al. (24).

From the calculated stretch ratios, we obtained the stretch rate by discrete differentiation of stretch ratio with respect to time. Peak stretch rate was computed and compared over the different conditions simulated. As a measure of the deformation trauma experienced by the valve leaflet, the pressure-stretch energy parameter was calculated.

This was a substitution for the strain energy density of the valve leaflets, which could not be obtained with the method described in this study, because in-plane stresses of the valve leaflet could not be obtained. In the computation of the pressure-stretch energy ($E$), in-plane stress is substituted by transmembrane pressure:

$$E = \int \Delta P \cdot d\lambda$$

where $\Delta P$ is the transvalvular pressure gradient, and $\lambda$ is the areal stretch ratio.

**Statistical analysis.** All data were first analyzed for normality with the Anderson Darling Test. If data exhibited normality, paired $t$-test was applied to analyze differences in the data; otherwise, Wilcoxon sign rank test was applied. Significance at $P$ value of 5% and 10% are reported.

**RESULTS**

**Leaflet kinematics.** The valve motion was visually observed at various important time points, such as those during the opening and closing phases, and their relationship with pressure and flow was reported. Transvalvular pressure was defined as the subtraction of ventricular pressure from the aortic pressure.

Figure 4A shows a typical pressure and flow curve. Figure 4B shows the images of the valve at these time points of interest. The same time points are indicated in Fig. 5.

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**Fig. 4. A:** typical hemodynamic waveforms measured from the flow loop. The condition simulated is normotensive (Norm) condition. Points $a$–$h$ are time points of interest; $a$–$c$ are time points during valve closing phase; $d$ is mid diastole; $e$ and $f$ are time points during the valve opening phase; and point $h$ is the peak flow time point. $B$: images of the valve at these time points of interest. The same time points are indicated in Fig. 5.
points corresponding to those labeled in Fig. 4A. Points a, b, and c were time points during late systole and early diastole, when the valve closed under adverse transvalvular pressure and flow reversal. About 25 ms before point a, the valve started to move toward the closed position, and, at the same time, transvalvular pressure started increasing. At point a, adverse transvalvular pressure gradient started to build up rapidly, and flow first reversed. At this point, the valve had still not coapted, but was rapidly moving toward coaptation. At point b, the valve had coapted, but continued to stretch under the pressure gradient. Reverse flow was at its maximum at this point, and transvalvular pressure was still rapidly increasing. At point c, transvalvular pressure gradient peaks, and the valve was visibly observed to be at its peak stretch. Thereafter, while pressure gradient oscillated slightly around its diastolic plateau value, the valve was observed to move back and forth at the same frequency. The valve then remained motionless for the remaining of diastole, while transvalvular pressure gradient plateaued until the end of diastole. The image of the valve at point d is shown here to provide an illustration of the valve in its fully closed state.

Points e, f, g, and h were time points during late diastole and early systole, when the valve opened. At point e, as transvalvular pressure gradient began a rapid decrease, the valve was visibly observed to start to relax and move forward. During this time, a small amount of forward flow was recorded. At point f, pressure gradient reversed, prompting the valve to increase the speed of its opening motion. However, the valve had not yet opened at this point. The valve only opened at point g, when a visible orifice could be observed. At point h, ~30 ms later, the valve became fully open. Substantial flow was already moving through the valve at this point. Point i was within the peak flow phase, and the image of the valve at this point is shown here to provide an illustration of the valve in its fully open state. From point h onward, the valve leaflets exhibit minor fluttering until the closing phase.

Qualitative dynamic deformation characteristics of AV leaflet. Dynamic stretch ratio at the base region of the leaflet was measured for the entire duration of the cardiac cycle, while that for the belly region was measured for the duration of diastole. This was because, from the ventricular view, the markers at the belly region were visible only during diastole, while those at the base region were visible throughout the cardiac cycle. However, due to difficulty discerning sufficient markers in the base region, we were restricted to using the 9-node Lagrangian method for the base region, instead of the 4-node Hermitian method, since the 4-node Hermitian method requires 16 markers, while the 9-node Lagrangian method only requires 9. We found that the major and minor principal stretch axes aligned with the radial and circumferential directions, respectively.

Figure 5 shows the stretch characteristics at the base region of the leaflet of a typical valve over the cardiac cycle for the Norm condition. Diastole was demarcated as the period between point b and point f, while systole was demarcated as the rest of the cardiac cycle. Generally, radial stretch ratio increased rapidly during early diastole and plateaued at the peak value before unloading at the end of diastole. Shortly after unloading, radial stretch ratio increased again to a systolic peak before decreasing again at the end of systole. Circumferential stretch ratio exhibited a similar diastolic behavior, rapidly increasing during early diastole, remaining constant for most of diastole, and rapidly decreasing during end diastole. However, during systole, circumferential stretch ratio decreased to less than 1 and exhibited a contraction plateau.

The period between points a and c in Fig. 5 can be termed as the “loading phase,” since it was when both circumferential and radial stretch ratios increased rapidly. The region between points f and h can be termed as the “unloading phase,” since it was when the stretch ratios decreased rapidly.

Dynamic stretch characteristics of the valve were closely controlled by the hemodynamic parameters. At various time points, significant events on the pressure, flow, and stretch waveforms coincided with one another. For example, point a corresponded to the minima in the radial stretch ratio, just before the rapid increase in the loading phase, as well as the point where circumferential stretch ratio crossed 1, and the leaflet switched from a compressed state to a stretched state. At the same time, it corresponded to the point of flow reversal, when flow crosses 0, as well as to the point where transvalvular pressure experiences a sudden increase in its rate of change. As described earlier, visual observation of images of the valves indicated that, at point a, the valve had not yet coapted, but was undergoing rapid closure. Coaptation occurred ~25 ms later, at point b.

Point c corresponded to the peak of both circumferential and radial stretch ratios at the end of the loading phase. It also corresponded to the point where transvalvular pressure peaks, and the second point when flow crosses 0, at the end of the flow reversal period between a and c. Visually, the valve leaflets suffered the most stretch and were at the peak of their displacement toward the ventricle at c.

After peaking, both radial and circumferential stretch ratios were in a stable plateau. The stretches exhibited minor oscillations, which corresponded to the oscillations in pressure. Although pressure oscillations were significant, the magnitudes of oscillations in stretch ratios were small. Over the duration of diastole, the transvalvular pressure gradually relaxed, but this resulted in small changes in the stretch ratios. Average diastolic stretch ratio values were tabulated in a later section.

The unloading phase between points e and g witnessed both circumferential and radial stretch ratios decreasing and coincided with the period where transvalvular pressure rapidly decreased during late diastole. Both radial and circumferential stretch ratios decreased slowly at first, but the rate of relaxation gradually became rapid. The rapid decline of stretch ratio in the radial direction was observed earlier than that in the circumferential direction. The decline began at point e for the radial direction, but at point f for the circumferential direction. As noted earlier, the valve leaflets commenced moving toward the aorta at point e, when transvalvular pressure began to decline rapidly, but was not observed to open until point g. Point f corresponded with the point at which transvalvular pressure dropped to 0, and also the point when forward flow began rapid acceleration. At this point, radial stretch ratio had decreased from diastolic plateau to a minima and was about to increase again toward systolic peak. At the same time point, circumferential stretch ratio had decreased from diastolic plateau to 1, was about to switch from a stretched state to a compressed state, and was about to continue declining toward the systolic compression plateau. As reported earlier, visual observation
showed that the valve had not yet opened at this point, but opened at point g, \( \sim 25 \) ms later.

During systole, circumferential stretch ratio exhibited a compression plateau, while radial stretch ratio experienced a stretch peak, which had a lower maximum stretch magnitude as the diastolic plateau. Average systolic stretch ratio values were tabulated in a later section. For most cases, the shape and timing of the systolic radial stretch ratio curve matched that of the corresponding flow curve, suggesting a relationship between systolic radial stretch and flow, as demonstrated in Fig. 5 for the Norm condition. For example, for the Norm condition, radial stretch ratio increases with flow between point g and point i. Beyond point i, both stretch and flow experience steady decreases.

Figure 6 shows the stretch characteristic of the belly region of the valve leaflet across diastole. General characteristics of the stretch ratio curve at the belly region of the leaflet were the same as that in the base region of the valve, including the rapid increase in stretch ratio at end systole, the stretch plateau during diastole, and the timing coincidence between flow, pressure, and deformation waveform.

It is noteworthy that, at both the base and belly region, radial stretch ratio was always above unity: implying that the valve was always stretched in the radial axis throughout the cardiac cycle. At the base region, radial stretch reduced transiently during the transitions between systole and diastole, but it did not fully relax before entering the next phase and increasing again.

Quantitative dynamic deformation measurements and effects of hypertension on stretch. Across different pressure conditions, stretch ratios were measured at the same location, using the same markers on the valve leaflet. Diastolic stretch ratios were averaged over the duration of diastole for different pressure conditions and are shown in Fig. 7A for both the belly and base region. This time-averaged stretch ratio and the corresponding Almansi and Green strains are shown in Table 1. Changes in diastolic stretch ratios from one condition to a higher pressure condition are shown in Fig. 7B for both the belly and base region. Systolic stretch ratios averaged over the duration of systole are shown in 7C for the base region.
For diastolic stretch ratio, radial stretch ratio was always substantially higher than circumferential stretch ratio, and stretch ratios increased with increasing pressure conditions in both radial and circumferential directions. We observed substantial variations in the stretch ratios and strain values across different valves, but for the same valve, there was a consistent trend of increase in stretch ratio when pressure was increased, as illustrated in Fig. 7B. Thus the increase in pressure induced a small but significant change in stretch ratios in both directions at both the belly region of the leaflet. The trend for systolic stretch ratio was less obvious. Comparing stretch ratios at the base and belly region of the leaflet revealed that there were no significant differences in stretch between the base and the belly region.

Loading and unloading strain rate and strain energy. Figure 8 shows a typical plot of the stretch rates over diastole. Stretch rate was stable at 0 most of the time and exhibit spikes during the loading and unloading phases. The most rapid change in stretch ratios occurred during the loading phase, when transvalvular pressure built up. Deformation rate reached values as high as 18,000%/s radially and 10,000%/s circumferentially, even under the Norm condition. Peak loading and unloading phase stretch rates for the base region are plotted in Fig. 9. Although a weak trend of increasing peak loading and unloading phase stretch rate with increasing pressure condition was observed, statistical analysis failed to show significance in these differences.

Figure 10 shows the comparison of the pressure-stretch energy parameter for the loading phase between the different pressure conditions for the base region. Pressure-stretch energy is shown only for the loading phase, because the stretch rates and pressures were the highest during this period. During loading phase, there was a trend for increasing loading phase pressure-stretch energy with higher pressure condition, and pressure-stretch energy for SHyp and Hyp was higher than that for the Norm condition. We note that this pressure-energy data were presented for healthy AV leaflet tissues without fibrosis or calcification.

DISCUSSION

We have presented the dynamic deformation behavior of the porcine AV leaflet under normal and two Hyp conditions at localized positions on the valve leaflet. The results showed that the general shape of the stretch ratio waveforms was in the form of diastolic plateaus and systolic peaks, with rapid change in the stretch ratio value during the transition between diastolic and systolic phases.

Diastolic stretch characteristic. During diastole, the valve was stretched both radially and circumferentially, suggesting the influence of a uniform surface pressure. Furthermore, the timing of significant events in the stretch ratio waveform matches that of the pressure waveform closely during this time, such as the matching of peak pressure and peak stretch at point c in Fig. 5, as explained in the RESULTS section, and the timing of oscillations in both the pressure and stretch ratio waveform after the loading phase. All of these suggest that diastolic stretch is primarily a result of transvalvular pressure.

The results showed that diastolic stretch ratio waveform was a very flat plateau, with little variation, despite movements in diastolic transvalvular pressure. Since the valve leaflets are predominantly composed of collagen, its mechanical properties are expected to be similar to that of collagen fibers: stiffness of the leaflet should be very low at low stresses and very high above a threshold stress. At diastolic pressures, valve leaflets were in the high stiffness zone and thus exhibited little changes in stretch, despite changes in pressure.

The AV is naturally stiffer in the circumferential direction than in the radial direction. The fibrosa layer of the leaflets houses trunk collagen bundles oriented in the circumferential direction, which imparts additional stiffness in the direction. In the present study, the ratio of stretch in the radial to that in the circumferential direction during diastole was 3.6 for Norm condition, 3.5 for Hyp condition, and 3.3 for SHyp condition, and the ratio of the Green strain in the radial direction to that in the circumferential direction was 4.2 for Norm condition, 4.2 for Hyp condition, and 4.0 for SHyp condition. These results had a good match to those from other studies of mechanical properties of the porcine AV leaflet, despite differences in experimental methods. Billiar and Sacks (5) reported a similar value in their biaxial tests of valve leaflets: at an equibiaxial tension of 50 N/m, the radial Green strain was −4.4 times the circumferential Green strain. Using a similar biaxial testing device, Stella and Sacks (26) reported that, at 60
N/m equibiaxial tension, the ratio of stretch in the radial to circumferential direction was \( \frac{3.2}{1} \) times.

In terms of stretch ratio averaged over diastole, a monotonic increase in stretch ratio moving from the Norm condition to Hyp and SHyp conditions was observed. This implies that hypertension imparted more stresses on the leaflet and led to increases in both the radial and circumferential direction. As described earlier, substantial variations in the valve-to-valve stretch values at the same condition were observed. This variation can be explained by the variability in the leaflet thickness, and the variability in the leaflet structure at the stretch ratio measurement location, such as whether collagen trunk fibers were present on the directly opposite aortic surface of the measurement location. However, for any one valve, the effect of increasing pressure condition on stretch ratio was very consistent. Higher pressure condition always increased stretch ratio at the same location, as can be observed from Fig. 7B.

**Systolic stretch characteristic.** Systolic stretch took the form of radial elongation and circumferential compression. The timing of the start and end of the systolic stretch matched the start and end timing of the flow curve, and, in some cases, the shape of the radial systolic stretch ratio plateau resembled that of the flow curve. Furthermore, while diastolic stretch ratio magnitude correlated well with increasing pressure conditions, the trend in systolic stretch ratio was much less significant, suggesting that pressure had small effects on systolic stretch ratio. All of these suggest that the systolic deformation was heavily influenced by forward flow instead of transvalvular pressure. We speculate that this interaction of flow with leaflet stretch came in the form of frictional drag force on the leaflet imparted by flow, which stretched the leaflets in the radial direction. This drag force depended on the flow rate, the rate of flow acceleration, the amount of leaflet flutter, and the geometry of the valve (whether the valve poses a nozzle-like constriction on flow). For a normal valve without stenosis, drag force will be small in magnitude. However, small drag forces are sufficient to cause substantial radial stretch, because the mechanical property of the native AV leaflet is such that it has low stiffness at low stresses (5).

The phenomenon of compression in the circumferential direction can be explained by the influence of systolic flow on leaflet stretch. When an AV is open during systole, the valve is slightly crimped up in the circumferential direction, and there is very low circumferential stress. Forward flow, however, induces radial stresses through drag forces. Consequently, the valve stretches in the radial direction, which, in turn, induces circumferential compression through an effect similar to Poisson’s effect. The close match in timing between the point when circumferential stretch crossed 1 and the point when radial stretch was at its minima indicates that circumferential stretch occurred only when systolic radial stretch commenced and supports the notion that Poisson’s effect was observed. Observation of systolic circumferential compression, in turn, supports the notion that forward flow was the primary influence on stretch during systole.

From the above, it can be concluded that stretch ratio characteristics were influenced more heavily by forward flow during systole, but were influenced more heavily by transvalvular pressure during diastole. During the transition between systole and diastole, valve leaflets relaxed as both of the driving influences on stretch ratio, transvalvular pressure gradient and forward flow, were low in magnitude. These transition periods, however, were insufficient in duration for the valve to entirely relax. This explains why the radial stretch was always above 1.

**Dynamic characteristics of leaflet stretch.** The initiation of diastolic stretch commenced even before the valve leaflets were fully coapted, at point \( a \) in Fig. 5, at which time flow...
reverses. Diastolic stretch was most likely initiated by this reverse flow, which produced transvalvular pressure as fluid moved backwards and pushed on the aortic surface of the leaflets. Upon coaptation at point b, leaflet stretch came under the influence of the rising transvalvular pressure. We further observe that deformation characteristics were slightly delayed from pressure reversal events. Transvalvular pressure gradient first reversed in direction 30 ms before point a in Fig. 5; however, the valve did not commence diastolic stretch until point a. Similarly, during the opening phase, the leaflets were still slightly stretched in both radial and circumferential direction when transvalvular pressure changed direction at point f in Fig. 5, and it was not until point g 25 ms later that the valve reached its relaxed state. This observation can be explained by the existence of virtual mass of the AV leaflets: a valve leaflet can only change its deformation state when the surrounding fluid moves, and thus the system of leaflets with fluid in the immediate vicinity requires larger forces to accelerate, resulting in an apparent delay between driving force events and leaflet motion. This indicates that dynamic effects are present and are an important influence on the deformation characteristics of the valve leaflets during the transition between systolic and diastolic phases. These dynamic effects were not due to viscoelasticity, since AV leaflets exhibit minimal viscoelastic behaviors under physiological stretch rates (25).

**Loading and unloading stretch rates.** The loading and unloading rates are important metrics in terms of the trauma experienced by the valve leaflet, since this is when the valve leaflets experienced the most significant deformations. Statistical analysis of the data failed to show any difference in deformation rates between the different pressure conditions. However, a mild trend of increasing deformation rate with increasing pressure conditions during the loading and unloading phases can be observed from the plot in Fig. 9. Statistical significance may be observed with a substantial increase in sample size, but is beyond the scope of this study.

**Unloaded reference state.** The use of a proper unloaded reference state of the leaflets is undoubtedly important in

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**Table 1. Average diastolic and systolic stretch, Green strain, and Almansi strains at the belly and base regions of the aortic valve leaflet, over the different pressure conditions**

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<th>Minor Axis</th>
<th>Major Axis</th>
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<td><strong>Belly region diastolic deformation</strong></td>
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<tr>
<td>Stretch ratio</td>
<td>1.66±0.06†‡</td>
<td>1.60±0.06†</td>
<td>1.54±0.05</td>
<td>1.20±0.03†‡</td>
</tr>
<tr>
<td>Green strain</td>
<td>0.312±0.012†‡</td>
<td>0.298±0.013†</td>
<td>0.282±0.014</td>
<td>0.147±0.016†‡</td>
</tr>
<tr>
<td>Almansi strain</td>
<td>0.889±0.099†‡</td>
<td>0.793±0.092†</td>
<td>0.695±0.085</td>
<td>0.224±0.035†‡</td>
</tr>
<tr>
<td><strong>Base region diastolic deformation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stretch ratio</td>
<td>1.59±0.10†‡</td>
<td>1.55±0.07†</td>
<td>1.45±0.03</td>
<td>1.28±0.08†‡</td>
</tr>
<tr>
<td>Green strain</td>
<td>0.290±0.022†‡</td>
<td>0.282±0.019†</td>
<td>0.259±0.015</td>
<td>0.181±0.029†‡</td>
</tr>
<tr>
<td>Almansi strain</td>
<td>0.799±0.186†‡</td>
<td>0.716±0.130†</td>
<td>0.558±0.063</td>
<td>0.342±0.112†‡</td>
</tr>
<tr>
<td><strong>Base region systolic deformation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stretch ratio</td>
<td>1.21±0.05†‡</td>
<td>1.19±0.05</td>
<td>1.18±0.09</td>
<td>0.94±0.02†‡</td>
</tr>
<tr>
<td>Green strain</td>
<td>0.148±0.025†‡</td>
<td>0.140±0.023</td>
<td>0.134±0.020</td>
<td>0.076±0.029†‡</td>
</tr>
<tr>
<td>Almansi strain</td>
<td>0.236±0.053†‡</td>
<td>0.218±0.047</td>
<td>0.341±0.0131</td>
<td>-0.060±0.019†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10 for data at the belly region, and n = 7 for data at the base region. Norm, normotensive; Hyp, hypertensive, SHyp, severe hypertensive. Significant difference from *Hyp condition at P < 0.10, †Hyp condition at P < 0.05, ‡Norm condition at P < 0.05.
quantifying the stretch in the AV leaflet. Billiar and Sacks (5) demonstrated that the strain values calculated by taking the unloaded reference state as the configuration with a small preload, as opposed to taking the unloaded reference state as the entirely unconstrained configuration, differs substantially, by factors of ~3.7 and 2.8 in the radial and circumferential directions, respectively. This behavior of the AV leaflet can be explained by its nonlinear mechanical properties, where stiffness is low at low stresses, but increases substantially at high stresses. Small, initial preloads can significantly stretch the valve leaflet from the unconstrained state. Sacks et al. (23) investigated AV fiber architecture with small-angle light-scattering measurements and found that collagen fibers are haphazardly aligned at 0-mmHg transmembrane pressure, but are aligned in the circumferential direction at pressures as low as 1 mmHg. This similarly suggested that small, initial loads affect the deformation characteristics of valve leaflets significantly. Thus care must be taken to ensure that the unloaded reference state is one where valve leaflets are exposed to minimal forces. In the present study, the unloaded reference state was chosen to be the one where there was zero transvalvular pressure, and the valve was open, stationary, and free floating in the flow loop fluid: the unconstrained state. This minimized variability in the calculated stretch values and ensured higher quality measurements.

Significance of study. The understanding of stretch characteristics of the AV under various hemodynamic conditions is important to study the link between the mechanical behavior of the AV mechanical behavior and its biology. Dynamic stretch of the AV leaflet represents continuous and repeated trauma to the valve leaflet and could be a key contributor to idiopathic calcific AV disease. The strong correlation between hypertension and early onset of calcific AV disease can be explained by this hypothesis: higher blood pressure can result in greater cyclic stretch and higher stretch rates in the valve leaflet, as demonstrated in the present study, which can result in adverse biological responses, such as inflammation and increased cell turnover, and which, on the chronic basis, may result in AV disease. The dynamic stretch characteristics of the AV leaflet quantified in the present study can be used as a basis for mechanical force inputs in ex vivo culture studies, investigating effects of stretch on AV leaflets or cells in culture, which can be used to test the above hypothesis. The adoption of measured stretch levels instead of using idealized stretch waveforms makes ex vivo studies more robust. Recent ex vivo culture experiments subjecting porcine AV leaflet samples from the belly region to cyclic stretch for 48 h had demonstrated that, at 20% of cyclic stretch, which corresponds to the SHyp condition, there was significantly higher apoptosis and cell proliferation than fresh tissues (4), implying that stretch imposed by a Hyp environment can lead to adverse biological responses.

The understanding of native AV stretch characteristics can also assist in the tissue engineering of a replacement AV, since exposure to mechanical forces may be important for healthy development of engineered prosthetic valves. Investigators have shown that, in ex vivo culture studies, exposure to mechanical forces closer to native mechanical force characteristics leads to beneficial biological responses (3, 6, 32).

Limitation. The primary limitation of the study is its in vitro nature, which entails the idealization of the ventricle and aorta geometry, rigidity of the ventricular chamber, the use of a bulb pump connected to the ventricular chamber instead of moving walls as the driving force for flow, and the semirigid manner of valve mounting. There will, therefore, be inevitable deviations from native hemodynamic conditions. The rising arm of the simulated ventricular pressure had a rate of increase that was 20% gentler than that seen in vivo (2). Consequently, the loading phase occurred ~20 ms earlier, and the rate of decrease of stretch during the unloading phase was slower than in vivo. Furthermore, ventricular pressure had an unnatural “double-bump” nature during mid systole. This, however, had not translated to abnormal systolic pressure gradient or forward flow and should have had a minimal effect on leaflet stretch. After valve closure, we observed high-frequency oscillations in pressures and flow curves due to rigidity of the flow loop chambers. Pressures and flow, however, oscillated about the desired mean values, and thus the time-averaged diastolic stretch values were not compromised.

Furthermore, the rigid ring mounting of the valve posed some restriction on radial motion of the valve over the cardiac cycle, and the valve annulus did not contract with systolic contractions as a live aortic root should. There were geometric differences, such as the distinction between the arch shape of native aorta and the straight in vitro aorta tube. Also, saline was used for the flow loop fluid instead of blood analogs, because blood analog fluids are aggressive towards biological tissues. There was thus a mismatch in viscosity between the flow loop fluid and blood. An additional limitation of our study is in the fact that normal and Hyp conditions are imposed on healthy tissue without calcified conditions.

Conclusion. In conclusion, we characterized the AV leaflet dynamic stretch at the center and base regions of the porcine AV leaflet, under both physiologically normal and Hyp conditions. Fine details of dynamic deformation characteristics of the AV leaflet and the effects of hypertension were elucidated.

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DISCLOSURES

No conflicts of interest are declared by the author(s).
REFERENCES


