Wall thickness referenced to myocardial volume: a new noninvasive framework for cardiac mechanics

STEWART DENSLOW, SESHADRI BALAJI, AND KENNETH W. HEWETT
The Children’s Heart Center of South Carolina, Medical University of South Carolina, Charleston, South Carolina 29425

Denslow, Stewart, Seshadri Balaji, and Kenneth W. Hewett. Wall thickness referenced to myocardial volume: a new noninvasive framework for cardiac mechanics. J. Appl. Physiol. 87(1): 211–221, 1999.—Dimensional variables measured for study of left ventricular mechanics are subject to errors arising from difficulty in determining zero-stress dimensions for use as a reference. Based on a method validated for measurements within individuals, we have devised an approach that facilitates comparison between individuals while minimizing random scatter. We define an exact mathematical index of strain, ln(h0/h), using wall thickness (h) referenced to extrapolated wall thickness at zero-luminal volume (h0). Noninvasive data from rabbits, pigs, and humans all yielded highly similar myocardial stress, ln(h0/h), and work values. The stress-ln(h0/h) relationship during afterload variation was constant among individual pigs with a twofold variation in ventricular mass. Stress-ln(h0/h) data from our analysis displayed lower scatter than either pressure-volume data normalized to myocardial mass or stress-ln(h0/h) data referenced to end-diastolic dimensions. A Frank-Starling-like curve with high correlation (r² = 0.96) was constructed from single points from different pigs, suggesting a low level of size and intersubject scatter. This method offers high precision for noninvasive characterization of ventricular and myocardial mechanics and for comparisons between subjects and between species.

ventricular function; wall stress; ventricular geometry

Glossary

A Area of a truncated conical section of myocardium, cm²
D Instantaneous endocardial short-axis of left ventricle, cm
Dm Instantaneous midwall short-axis of left ventricle, cm
Dm0 Midwall short-axis of left ventricle at zero-luminal volume, cm
ESPVR End-systolic pressure-volume ratio
FS Fractional shortening of the left ventricle
h Instantaneous measured wall thickness, cm
h0 Wall thickness at zero-luminal volume, cm
L Endocardial long-axis of left ventricle, cm; mean sarcomere length, µm
LV Left ventricle
L0 Mean sarcomere length at zero-luminal volume, µm
P Blood pressure in ventricular lumen, g/cm²
p, q, r, a, b Arbitrary intermediate quantities for solving a cubic equation
T Mean of circumferential and meridional tension, g/cm
V Volume of a truncated conical section of myocardium, cm³
VC Cavity volume of the left ventricle, cm³
V lum Left ventricular luminal volume, cm³
Vm0 Left ventricular myocardial volume, cm³
VT Left ventricular total volume (luminal + myocardial volume), cm³
Vw Left ventricular wall volume, cm³
V0 Extrapolated left ventricular luminal volume at zero pressure, cm³
VCF Velocity of circumferential fiber shortening, circumferences/s

RECENT REPORTS HAVE DESCRIBED a framework for noninvasive analysis of myocardial behavior based on mean wall stress and a strainlike quantity, the natural logarithm of reciprocal of wall thickness, ln(1/h) (21–23). Wall thickness is used as a precise index of myocardial area under the assumption of the approximated constancy of myocardial volume. This approach has been shown to provide accurate estimates of myocardial work per unit volume of tissue (21, 22). Within individuals, it is sensitive to the effects of β-blockers and other inotropes (21). It has the further advantage of being based on easily obtainable echocardiographic measurements.

The above investigations avoided the inclusion of a reference value for wall thickness. When stress-strain analysis is used to investigate constitutive relationships, such a reference measurement is generally made at a point of zero net stress, a point not obtainable under clinical conditions. Thus all analysis in these studies was in terms of increments in the variable ln(1/h) that indexed strain.

The lack of a reference, although practical for determining energetic and geometric changes, prevents direct comparison of values between different individuals. This lack also causes the units of the strainlike variable to be ambiguous. We investigated whether use of a calculated reference would provide a basis for comparison of values between individuals, as well as avoided the use of the ambiguous units of natural logarithm of reciprocal distance. Because myocardial mass has been shown to be effective in normalizing other descriptors of myocardial behavior (4, 15), we specifically examined references calculated as cube-root functions of this quantity to produce a value with units of length rather than volume. The present paper discusses results obtained by using a reference that is the extrapolated value of wall thickness that would occur if the luminal volume could go to zero.
Definition of variables. Two widely used descriptors of material deformation are LaGrangian strain, \((x - x_0)/x_0\), and natural strain, \(\ln(x/x_0)\). The natural strain-like quantity used by Nakano et al. (21–23) is not referenced and thus is not directly comparable in magnitude between different hearts. One logical reference dimension, \(h_0\), would be the wall thickness at zero external load, producing the variable \(\ln(h_0/h)\). However, the roughly tangential approach of the myocardial stress-strain relationship to the zero external stress axis (10, 39) results in large random errors in any measurement of this reference point. Consequently, we sought a reference that was more precisely determinable despite the fact that it would not correspond to zero net stress.

The net effect of a reference thickness taken at a new point is to cause a constant additive shift in all \(\ln(h_0/h)\) values parallel to the \(\ln(h_0/h)\) axis. This is due to the identity \(\ln(h_0/h) = \ln(h_0) - \ln(h)\). A new \(h_0\) is simply a different additive constant that does not change the shape of empirical stress-\(\ln(h_0/h)\) contours.

Because of the demonstrated usefulness of myocardial mass (and the directly proportional myocardial volume) in normalizing descriptors of myocardial mechanics (4, 15), we sought a reference value directly related to this quantity. The line plotted in Fig. 1 is the relationship between wall thickness and luminal dimension based on the approach of Hugenholtz et al. (17), which models the myocardial wall as an ellipsoid of constant wall volume. This assumption has been shown to provide an accurate prediction of wall thickness over the physiological range of ventricular contraction (17). Extrapolation beyond the physiological range to zero-luminal diameter provides a clearly defined calculated value that is precisely representative of the mean of the myocardial volume while having the proper units (length) for use as a reference quantity. Although this value is not obtainable under physiological conditions, we felt that its clear definition and precise relationship to myocardial mass strongly suggested its appropriateness.

Tangential wall-stress relation to radial thickness. Description of the mathematical relationship between (radial) wall thickness and (tangential) wall stress has been published previously by others (21, 22). Briefly, approximation of the myocardium as incompressible allows substitution of a variable (thickness) that lies along the transmural dimension for the mean of the dimension variables (arc lengths or areas) that lie in the plane of the wall. Contraction of the myocardium is described in terms of perimeter tension and area strain of a truncated conical region of the ventricular wall. Work, \(W\), done by this region is the integral of perimeter tension and area strain of a truncated conical region: \(W = \int T \cdot dA\). Dividing by volume, \(V\), of the wall region gives work per unit volume of myocardium: \(W = \int(T/V) \cdot dA\). The volume of the region is the product of the area and the thickness, \(h\); hence the expression can be written as \(W = \int(T/h)(dA/A)\). Tension over thickness is the mean wall stress, \(\sigma\), from equatorial and meridional directions, whereas \(dA/A\) is equal to \(d(\ln A)\). The formula becomes \(W = \int \sigma d(\ln A)\). Area, \(A\), can be replaced by \(V/h\), where \(V\) is again the constant volume of the truncated conical region. Because \(V\) is constant, the differential can be manipulated for its elimination: \(d[\ln(V/h)] = d[\ln(V + \ln(1/h))] = d(\ln(1/h))\). The result is \(W = \int \sigma d(\ln(1/h))\).

For the present extension of this approach, we took the integration between the calculated reference of extrapolated thickness at zero-luminal volume, \(h_0\), and instantaneous thickness, \(h\), measured from M-mode echocardiograms. The general result is \(W = \sigma \ln(h_0/h)\), where \(\ln(h_0/h)\) is an exact mathematical index of natural strain in the circumferential direction, \(\ln\) (length/reference length). In that their product is work, \(\sigma\) and \(\ln(h_0/h)\) form a pair of intensive and extensive mechanical variables analogous to pressure and volume or force and length. However, the work expressed is per unit volume of material rather than total work.

The reference value, \(h_0\), is in the numerator rather than the denominator. When the index of strain is defined this way, its value increases in parallel with luminal diameter and volume, i.e., as the wall becomes thinner. Work calculated by using a more conventional definition, \(\ln(h_0/h)\), would have a minus sign during
ejection of blood. We chose to avoid this potential source of confusion by keeping the inverted form resulting from the above derivation. The zero point of this index of strain corresponds to the zero value of luminal volume, resulting in a qualitative similarity between pressure-volume plots and stress-ln(h/L) plots.

Previous work (21) has demonstrated shifts in data within individuals in response to β-blockers and other inotropes. The addition of a reference dimension does not alter this sensitivity. Because all data from one individual will be referenced to the same value based on the myocardial mass (h_0), all data will be shifted identical distances with the result that prereference shifts will remain. Relative position is only changed between data points obtained from hearts of different mass.

Recent reports provide some separate support for the usefulness of referencing to zero-volume dimensions (2, 3, 10). These studies found that, during ejection, the instantaneous mean sarcomere length, L, is accurately predicted by a function including calculated sarcomere length at zero-luminal volume, L_0, and the ratio of luminal and wall volumes, V_lum and V_w: 

\[ L / L_0 = (1 + (3V_{lum})/V_w)^{1/3} \]

Thus the sarcomere length can be referenced to zero-volume length in a useful predictive equation: 

\[ L / L_0 = (1 + (3V_{lum})/V_w)^{1/3} \]

**METHODS**

Human studies. Patient data were collected retrospectively from records of the Division of Pediatric Cardiology at our institution. Data were selected from patients who underwent 1) successful ablation of substrate of intermittent supraventricular tachycardia (Wolf-Parkinson-White or atrioventricular node reentrant tachycardia) in a heart without shunts, incompetent or stenosed valves, or restrictions to flow in the great vessels; and 2) same- or next-day postcatheterization echocardiographic study. M-mode echocardiographic measurements were taken perpendicular to the long axis of the ventricle at the tops of the papillary muscles with the cursor directed through the septum to a point between the papillary muscles. Measurements taken from the M-mode recordings were end-diastolic wall thickness (mean of posterior wall and intraventricular septum), end-diastolic luminal diameter, and end-systolic luminal diameter. Because blood pressures were not available as part of the echocardiographic studies, systolic and diastolic cuff pressures recorded during catheterization were used. Sixteen patients were included (ages 6–32 yr, mean 14 yr). Calculated ventricular masses (including intraventricular septum; see Calculations below) ranged from 37 to 124 g.

Animal models. All animals were treated and cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Research Council, Washington, DC, 1996).

Swine studies. Ten Yorkshire pigs (25–30 kg) of either sex were part of studies of congestive heart failure in which animals were subjected to rapid atrial pacing. For the present study, data were collected from animals that had yet to receive pacing, after 2 wk of recovery from surgery. The surgical techniques of pacemaker and aortic access port implantation have been described previously (32, 34).

Pigs were presedated with diazepam (10 mg) and brought to the laboratory in a sling. For recording of aortic pressure tracings, a vascular access port was entered by using a 12-gauge Huber needle attached to a saline-filled tube and a Statham 23D (Gould, Oxnard, CA) pressure transducer. Further sedation was achieved with 5- to 10-mg boluses of midazolam given through the vascular access port. Surface electrocardiographic (ECG) electrodes were attached to each limb, and standard ECG leads I, II, and III were displayed on a physiological recorder (EVR-16, Electronics for Medicine, Overland Park, KS). Two-dimensional and M-mode echocardiographic studies of left ventricle (LV) function were performed (2.25-MHz transducer, ATL UltraMark VI, Bothell, WA) from the right parasternal approach. LV dimensions were measured at end systole and end diastole as described for patients. Calculated LV myocardial masses (see Calculations below) ranged from 23 to 64 g.

Six pigs were given variable doses of phenylephrine to increase afterload. Phenylephrine infusion started at a rate of 1.3 µg·kg⁻¹·min⁻¹ and increased in such a manner so as to obtain four to seven different simultaneous M-mode tracings and blood pressures. This allowed determination of four to seven different paired values of afterload and fractional shortening for each pig.

Rabbit studies. Data were obtained from three sham-operated rabbits (New Zealand White, 3.5–4.5 kg, 5 mo of age) that served as controls in a study of pacing-induced congestive heart failure. Procedures for instrumentation in this model have been published previously (33). The animals were administered 4 mg/kg sc of enrofloxacin (Baytril, Miles, Shawnee Mission, KS) twice per day on postoperative days 1 and 2. In 4 wk of time, two-dimensional and M-mode echocardiography (5-MHz transducer, ATL Ulramark VI) were used to image the LV from the right parasternal approach (33). The same ventricular dimension measurements were taken in rabbits as in pigs and patients. Before being brought to the laboratory, rabbits were sedated with 10 mg of intramuscular midazolam (Versed, Hoffman-LaRoche, Nutley, NJ) and placed in a custom sling, and an ECG was established.

Pressure was obtained at the time of the terminal experiment with a fluid-filled catheter advanced into the LV via the right carotid artery. The catheter was connected to a previously balanced and calibrated Statham 23D pressure transducer (Gould). LV masses obtained at autopsy ranged from 3.4 to 4.7 g wet wt.

Calculations. For determination of wall thickness at other than end diastole, we used the approach validated by Hugenholz et al. (17) with luminal volume values determined as in Zile et al. (40). The latter method assumes constant myocardial volume and is based on the modeling of the LV as a half-ellipsoid of circular cross section with a constant ratio of epicardial long and short axes of 1.2 (40). Wall thickness at the apex is assumed to be 0.4 times that at the base (40).

Accordingly, using 1) averaged end-diastolic wall thickness, 2) end-diastolic luminal dimension, and 3) end-diastolic luminal dimension, we calculated end-diastolic (end-ejection) and zero-luminal wall thicknesses (Eqs. A5 and A6, respectively, in the APPENDIX). Start-ejection wall thickness was taken as equal to end-diastolic thickness.

Wall stresses (mean fiber stress) at start and end ejection were calculated based on end-diastolic and end-systolic arterial pressures, respectively, with the use of the formula of Regen (25). End-systolic pressure was estimated as equal to the calculated mean aortic pressure (28, 29).

End-systolic pressure

\[ \text{End-systolic pressure} = \frac{2 \times (\text{end-diastolic pressure}) + \text{peak systolic pressure}}{3} \]
Mean fiber stress, in g/cm², was calculated as

$$\text{Mean fiber stress} = \frac{1.36(\text{end-systolic pressure})}{\ln \left( \frac{\text{total volume}}{\text{cavity volume}} \right)}$$

where total volume is the sum of the luminal volume and myocardial volume (25). Mean fiber stress is the mean of orthogonal fiber stresses (meridional and circumferential) on an element of myocardium (25).

LV luminal and myocardial volumes, including free wall and septum, were calculated based on the model of Hugenholtz et al. (17). We used the Zile geometric model (40) for the determination of the long axis of the ventricle. We recognize that use of this model may have resulted in a bias in determined volume and mass values. However, because we were concerned only with the relative sizes of the ventricles, the use of a consistent formula was felt to be a valid approach.

Myocardial mass was calculated as myocardial volume times a consistent formula was felt to be a valid approach.

The area under ejection trajectories was calculated as mean stress times the difference in ln(h₀/h) values at start and end ejection. The mean stress was calculated as the simple mean of the start-ejection and end-ejection stresses.

Ejection time was measured from M-mode recordings as the time between start of contraction and end of contraction.

Data are presented as means ± SD. Statistical difference between regression lines was tested by using confidence ellipses (24). A Bonferroni correction for multiple tests was used in tests for significance by analysis of variance (35). Spearman's rank correlation was used to test for ordering among these points.

RESULTS

Size independence of stress, ln(h₀/h), and work variables. Figure 2 shows a plot of mean values and 95% confidence intervals of stress and ln(h₀/h) at beginning and end systole obtained from rabbits, pigs, and patients. Estimated ventricular masses had means (and ranges) of 4 (3–5), 40 (23–64), and 78 (37–124) g for rabbits, pigs, and humans, respectively.

End systolic volumes were determined from measurements extracted from cardiac literature. The species studied were rats (18, 19), lambs and sheep (1), and humans (9). These results, although based on data from completely independent studies, agree closely with present results. This pattern suggests that these quantities are insensitive to interobserver variation, and that interobserver variations in these quantities, if present, are small for these species. End-systolic values, which would be expected to vary less with likely differences in loading between experimental populations, show closer similarity.

Fig. 3 shows the same plot as in Fig. 2 with the addition of stress-ln(h₀/h) values that were calculated from measurements extracted from cardiac literature. The species studied were rats (18, 19), lambs and sheep (1), and humans (9). These results, although based on data from completely independent studies, agree closely with present results. This pattern suggests that these quantities are insensitive to interobserver variation, and that interobserver differences in these quantities, if present, are small for these species. End-systolic values, which would be expected to vary less with likely differences in loading between experimental populations, show closer similarity.

By using start- and end-ejection values, ejection work was calculated by approximating the ejection trajectory in the stress-ln(h₀/h) plane as a straight line. Validity of this approximation for normally ejecting ventricles in dogs and humans is supported by published studies of stress-strain loops (22, 37). On the basis of the Laplace law relating stress to pressure and proportional geometry, the similarity in ventricular geometry and pressure waveforms among the species...
Table 1. Ejection work per unit volume of myocardium calculated from mean stress and ln(h0/h) values at start- and end-ejection

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Ejection Work, g·cm</th>
<th>Left Ventricular Mass, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>16</td>
<td>81.4</td>
<td>78 ± 30*</td>
</tr>
<tr>
<td>Rabbits</td>
<td>3</td>
<td>85.4</td>
<td>4 ± 0.7†</td>
</tr>
<tr>
<td>Pigs</td>
<td>9</td>
<td>135.6</td>
<td>40 ± 14*</td>
</tr>
<tr>
<td>Rats (19)</td>
<td>6</td>
<td>76.9</td>
<td>0.98 ± 0.05*</td>
</tr>
<tr>
<td>Rats (18)</td>
<td>10</td>
<td>90.9</td>
<td>1.08 ± 0.19†</td>
</tr>
<tr>
<td>Sheep (1)</td>
<td>7</td>
<td>72.4</td>
<td>132 ± 5.7†</td>
</tr>
<tr>
<td>Lambs (1)</td>
<td>7</td>
<td>55.7</td>
<td>45.7 ± 3.5†</td>
</tr>
<tr>
<td>Human (9)</td>
<td>33</td>
<td>67.2</td>
<td>168 ± 55*</td>
</tr>
</tbody>
</table>

Mass values are mean ± SD. n, No. of subjects. Nos. in parentheses are reference nos. * Masses calculated; † masses measured directly.

Included in this study should result in similar ejection trajectories. The area under this line segment represents the work per unit myocardium during ejection. The values are similar, despite a roughly 150-fold range in myocardial mass (Table 1). Observed variations are on the order of what would be expected within single individuals because of preload and afterload variations. The values are also similar to those reported by Nakano et al. (Ref. 22; 40–96 g·cm⁻¹·cm²) for patients for the full cardiac cycle.

The response of end-systolic stress and ln(h0/h) to afterload variation is graphed in Fig. 4. End-systolic points for each pig fall along straight lines with little scatter (r² = 0.94–0.99). These lines cluster tightly without ordering according to mass (23–40 g, mean 31 g) [Spearman’s rank correlation between mean end-systolic ln(h0/h) and myocardial mass not significantly different from zero]. None of the regression lines shows a statistically significant difference from the others.

Figure 5 combines Fig. 4 with results from Table 1. The response of end-systolic stress and ln(h0/h) to afterload variation from graded doses of phenylephrine is shown in Fig. 4. End-systolic points for each pig fall along straight lines with little scatter (r² = 0.94–0.99). These lines cluster tightly without ordering according to mass (23–40 g, mean 31 g) [Spearman’s rank correlation between mean end-systolic ln(h0/h) and myocardial mass not significantly different from zero]. None of the regression lines shows a statistically significant difference from the others.

The values of both slope and intercept from the recalculated dog results range from below to above those of the pig data. The variation in parameter values is much greater in the recalculated dog data than in the pig data. This variation might be due to a round-off error introduced when published data were reanalyzed, or it may represent real variations in the slopes and intercepts that are characteristic of the species. As would be expected because of the complete overlap of ranges, no statistically significant difference was found between the population means of the canine and swine results. Changes in both mass and species resulted in little or no position change in the stress-ln(h0/h) domain.

Fig. 4. Relationships of end-systolic stress and ln(h0/h) during graded phenylephrine infusion in 6 different pigs. Data points from each pig (from 4 to 7 points/animal) are denoted by a different symbol. Solid lines are regression lines through each data set (n = 4–7; r² = 0.94–0.99). Regression lines displayed no significant differences or ordering according to mass.

Fig. 5. Relationships of end-systolic stress and ln(h0/h) calculated from results obtained by Nakano et al. (23) during variable occlusion of aorta in open-chest dogs. Data from each dog are denoted by a different symbol. Solid lines are regressions through reanalyzed data. Dotted lines are regressions from Fig. 4. There was no significant difference detectable between the two sets of regression lines (unpaired t-tests of slope and intercept with Bonferroni correction).
to the lower range of preloads that is attainable with this type of preparation. The higher preloads observed under afterload variation apparently span only a linear portion of the total curve.

As can be seen in Fig. 6, the stress-ln(h_0/h) curves are almost superimposable, despite the species and size differences. If the data are presented in the ln(1/h) format of Nakano et al. (21–23), i.e., without referencing, the mean x-positions of the data sets are −0.62 and 0.60. After referencing, the mean positions are 0.56 and 0.51.

Precision of stress and ln(h_0/h) variables. As opposed to the direct measurement of volume carried out for pressure-volume results (4, 15), noninvasive approaches require the calculation of volumes from linear measurements. Such calculated volumes will have a magnified level of scatter (lower statistical precision or sensitivity) because of the cubing of measured values. To demonstrate this effect, the stress-ln(h_0/h) values from one of the phenylephrine infusion experiments in Fig. 4 is reproduced in Fig. 7A, whereas Fig. 7B displays values of pressure and luminal volume over mass that have been calculated from the same raw data. The value of the coefficient of determination, r^2, is the fraction of total variance in the data that is accounted for by the regression relationship (24) and thus is a direct indicator of the precision of the data. The r^2 value was higher for the present analysis (0.95 vs. 0.72), indicating a lower level of scatter. As shown in Table 2, this pattern of r^2 values was true for data from all six pigs from the phenylephrine-infusion experiments (Fig. 4).

Other noninvasive methods of ventricular performance assessment, such as FS (7), velocity of circumferential fiber shortening (VCF) (5), and corrected VCF (VCFc) (8), use end diastole as a reference state. This is also true for some invasive methods (20). However, natural variation in end-diastolic distension from beat to beat would be expected to result in additional random scatter not accounted for by an underlying

Fig. 6. Reanalyzed data from rabbit (■) and dog (○) isolated, cross-circulated hearts (15, 39). End-systolic data from isovolumic contractions with varying preload were used to generate results shown here. Calculated end-systolic stress-ln(h_0/h) relationships are nearly superimposable.

Fig. 7. Plots of alternate descriptors calculated from same data as those in Fig. 4. Displayed data were taken from animal with greatest no. of points recorded (n = 8) during graded phenylephrine infusion. A: plot of stress-ln(h_0/h) data calculated by presently proposed method. B: plot of pressure vs. volume/mass values calculated from same data as used for A. C: plot of stress vs. ln(h_0/h) referenced to end-diastolic dimension calculated from same data as used for A. Pattern of lower r^2 values for alternate approaches was observed in all 6 pigs displayed in Fig. 4 (see Table 2). h_wd and h_res. End-diastolic and end-systolic wall thickness, respectively.
Tables 2. Values of slope, intercept, and $r^2$ for regressions of three pairs of variables calculated from raw data used to produce Fig. 4

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Stress vs. ln(h/h)</th>
<th>Pressure vs. EDV/Mass</th>
<th>Stress vs. ln(h/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope</td>
<td>Intercept</td>
<td>$r^2$</td>
</tr>
<tr>
<td>1</td>
<td>697</td>
<td>-329</td>
<td>0.97</td>
</tr>
<tr>
<td>2</td>
<td>566</td>
<td>-214</td>
<td>0.99</td>
</tr>
<tr>
<td>3</td>
<td>695</td>
<td>-308</td>
<td>0.95</td>
</tr>
<tr>
<td>4</td>
<td>709</td>
<td>-319</td>
<td>0.93</td>
</tr>
<tr>
<td>5</td>
<td>660</td>
<td>-238</td>
<td>0.98</td>
</tr>
<tr>
<td>6</td>
<td>775</td>
<td>-341</td>
<td>0.98</td>
</tr>
</tbody>
</table>

EDV, end-diastolic luminal volume; ED, end diastole; mass, myocardial mass.

stress-$\ln(h/h)$ relationship. Figure 7C displays values of stress paired with $\ln(h/h)$, calculated from the same raw data set as Fig. 7A but by using end-diastolic thickness as a reference rather than thickness at zero-luminal volume. As expected, the value of $r^2$ was higher (0.95 vs. 0.87) when the reference was the zero-luminal thickness. Again, this pattern was true for all six pigs from the phenylephrine-infusion experiments.

Thus in all six sets of regression results, the present approach produced the highest $r^2$ value and highest precision. With the use of a sign test (31), this result was tested against a null hypothesis of the $r^2$ value of the present approach not being the highest (i.e., one or both of the other approaches having equal or higher $r^2$). The test showed significance with a $P$ value of 0.016.

Ejection energetics and end-diastolic $\ln(h/h)$. If indeed the tested method has a high precision, it should allow the discernment of underlying patterns that are obscured by random scatter in other methods. Accordingly, a final area of investigation was the relationship of ejection work per unit volume of myocardium to end-diastolic $\ln(h/h)$. This relationship is analogous to a Frank-Starling curve relating stroke volume to end-diastolic luminal volume. Single-point data, each based on M-mode and pressure measurements from a single time point, from 10 healthy control pigs of similar age are plotted in Fig. 8. Linear regression analysis demonstrated a high correlation ($r^2 = 0.96$) between ejection work and end-diastolic $\ln(h/h)$. The probability of this relationship of points occurring by chance, when no actual relationship exists, is $<0.0001$ ($P$ value of the regression). As would be expected based on the Frank-Starling relationship, work increased with diastolic distension (high values of $\ln(h/h)$). Remarkably, the present result has been obtained by using independent baseline measurements from 10 different animals. Although the contractilities, and hence Frank-Starling curves, of these animals might be expected to be similar, it would be predicted that measurements from separate animals would be confounded by individual variations in size and physiology, thus lowering correlation.

Regression with the use of end-systolic points, points not expected to follow a Frank-Starling relationship, showed no correlation ($r^2 = 0.02, P < 0.71$), suggesting that the end-diastolic correlation was not due simply to correlation between work and overall ventricular mass. Regression was also performed by using data obtained from the same animals after 3 wk of rapid atrial pacing used to induce heart failure. The heart-failure animals would be expected to have a large range of (reduced) contractilities (32) and thus would not be expected to share similar Frank-Starling relationships. The regression failed to show any correlation ($r^2 = 0.05, P < 0.56$).

We also investigated the behavior of calculated ejection power (ejection work/ejection time) with end-diastolic $\ln(h/h)$ in the same set of control pigs (Fig. 9). We again found a high correlation ($r^2 = 0.89$) with end-diastolic $\ln(h/h)$, but not end-systolic $\ln(h/h)$ ($r^2 = 0.01, P < 0.78$) or end-diastolic $\ln(h)$, after induction of heart failure by rapid atrial pacing ($r^2 = 0.01, P < 0.76$).

**DISCUSSION**

The method of Nakano et al. (21–23) offers the advantages of being noninvasive and physics based. It has been demonstrated to provide accurate estimates of myocardial work and to be sensitive to myocardial inotropic state. The present study has demonstrated the additional precision achieved by referencing the index of strain, $\ln(1/h)$ to calculated thickness at zero-luminal volume, $h_0$. Measured values of stress, $\ln(h_0/h)$,

![Fig. 8. Plot of ejection work per unit volume of myocardium against start-ejection $\ln(h/h)$. Each point represents single baseline measurement on a separate pig (n = 10). Solid line is regression line through points, and dotted lines are two SEs above and below the line. SEE, SE of estimate.](http://jap.physiology.org/Downloaded from http://jap.physiology.org/)

```latex
\begin{table}
\centering
\begin{tabular}{|c|c|c|}
\hline
Animal No. & Stress vs. ln(h/h) & Pressure vs. EDV/Mass & Stress vs. ln(h/h) \\
\hline
1 & 697 & -329 & 0.97 \\
2 & 566 & -214 & 0.99 \\
3 & 695 & -308 & 0.95 \\
4 & 709 & -319 & 0.93 \\
5 & 660 & -238 & 0.98 \\
6 & 775 & -341 & 0.98 \\
\hline
\end{tabular}
\end{table}
```
and work per unit volume of myocardium from differently sized individuals of one species are very similar when zero-volume referencing is used. Furthermore, the similarity of myocardial energetic and mechanical properties among different mammalian species, widely demonstrated at tissue and biochemical levels, can be directly observed in vivo by using this noninvasive approach.

Despite the noninvasive nature of the method, patterns of stress and ln(h₀/ℎ₀) under varying afterload were found to have high correlations and remarkable similarity among ventricles of differing size in pigs. Position shifts in plots of stress vs. strain index, observed in previous studies employing the unreferenced strain index ln(1/ℎ₀) (21), thus have significance for absolute as well as relative value. The absolute location of a stress-ln(ℎ₀/ℎ₀) curve could potentially indicate an abnormal myocardial state without reference to a control value within the same animal. Initially variant or abnormal individuals could be identified and analyzed accordingly.

Results obtained from published data in a different species (dog), collected completely independently from the present work, also demonstrated the increased clustering of results within a species that is achieved by the referencing to ℎ₀. The mean positions of the clustered data points were similar between the species as were the mean slopes and intercepts of regression lines. Again, this allows analysis in terms of absolute as well as relative position of the stress vs. strain index curves. Whereas the dog curves showed a greater variation in parameters (mean abcissa, slope, and intercept), the means of these parameters were not different from those of the swine data. This difference in variance of the location parameter would not be detectable under systems in which location is not adjusted according to myocardial mass, such as when ln(1/ℎ₀) is used.

We have demonstrated the lower random scatter (higher precision) that results from the present approach, compared with approaches based on calculated volumes or on end-diastolic references. Random scatter was also low enough to allow the construction of a Frank-Starling-like curve by using single points from different animals. The known underlying relationship was discernible despite variations between individuals.

Referencing to zero-volume thickness may seem problematic because such dimensions are not physiological. This thickness, however, is a characterization, or parameter, of the basic geometry of the ventricle rather than a physiological measurement. It would be mathematically equivalent to our present method to use a dimension occurring at a different luminal volume, as long as that luminal volume were a constant proportion of myocardial volume. For example, calculating a reference thickness at luminal volume equal to myocardial volume, rather than at zero lumen, would lower all presented ln(h₀/ℎ₀) values by a constant value, 1.056. Whereas most values would shift to negative, all correlations among the data and similarities between data sets would remain unchanged. Although a choice among mathematically equivalent reference points cannot avoid being somewhat arbitrary, we feel that the choice of a point at zero volume avoids possible confusion engendered by negative values of ln(h₀/ℎ₀) as well as resulting in the coincidence of zero ln(h₀/ℎ₀) and zero-luminal volume.

Comparison to mass-based normalizations. Normalization of ventricular measurements to the myocardial mass has been used to standardize pressure-volume relations (4, 15). The present method is analogous to such an approach because wall thickness at zero-luminal volume is directly related to the total myocardial volume. However, normalizations that are based on mass utilize a ratio involving two different quantities (luminal volume and wall mass) in contrast to the ratio of two values of the same quantity (wall thickness) as in the present approach. The combination of values from different geometric structures cannot be used as an index of strain in analysis, thus complicating possible comparisons to stress-strain behavior (curve shapes) in other situations, such as isolated myocardial strips and trabeculae (6, 14).

Because, due to its noninvasiveness, the present method relies on distance measurements rather than direct measurement of volume, only calculated volumes can be obtained. The present approach avoids this demonstrated source of scatter (Fig. 7) by measuring and calculating linear quantities only.

Results comparable with the present study are theoretically obtainable when midwall measurements are normalized or referenced to calculated values at zero-luminal volume rather than end-diastolic dimensions, as have been used previously (12, 13, 20, 27, 30). The mathematical relationship between wall-thickness ratios and midwall–diameter ratios is not linear, however (see the APPENDIX); therefore, the shapes of the relationships with stress, work, and power may also be different. The use of wall thickness and luminal diameter in the present investigation is based on the ease of obtaining these measurements through echocardiographic imaging.
Physical meaning of variables. The present study investigated stress, ln(h_y/h), work, and power. These variables are fundamental physical quantities expressed in fundamental physical units. Accordingly, these noninvasively determined values may be directly compared with values obtained from experiments that used other fundamental quantities, such as force and distance, pressure and volume, or generated heat. This situation contrasts with that of other noninvasively obtained quantities, such as FS (7), VCF (5), and VCF_c (8), that can only be compared with other measurements of the same indexes.

The work of Nakano et al. (21) using ln(1/h) suggests that the relationship of that variable to mean tangential stress can be an index of contractility. Comparison of the present work with their results would suggest that this group relationship could be used in a similar fashion. We did not explicitly manipulate contractility, however, and, therefore, have not addressed this question. Accordingly, our main conclusion is that the confounding effects of size and species on measurements of mechanical and energetic variables appear to be substantially reduced or eliminated by our approach.

Limitations of the present work. We chose to use a particular geometric model of the LV that, as with any geometric model, contains some inaccuracy. The consistent use of this model for all calculations should result in equal bias for all results and thus allow the valid comparison of values.

Because it is a material property, ln(h_y/h) should be only slightly affected by the shape of the chosen geometric model. Furthermore, because the stress variable used here is the mean of equatorial and meridional stress, changes in shape have little effect on its final value (2). We tested these predictions and found <1% change in calculated values of stress or ln(h_y/h) when the geometric model was varied in the ratio of the long axis to the short axis from 1 to 1.4. Thus our particular choice of long-axis/short-axis ratio should have a negligible effect on the accuracy of the results.

There may be a varying degree of inaccuracy of the geometric model, depending on the size and, especially, species origin of the heart. This potential source of error cannot be eliminated from the present study. Considering the similarity of values among the species investigated in the present work, it would seem that this error is not of great magnitude.

There may be a high percentage of error in measurements of small values of wall thickness. Despite the fact that many of the data points were single measurements of wall thickness, little scatter was observable in plots of end-systolic stress and ln(h_y/h) under varying afterload. The high correlations obtained in these experiments would suggest that this source of measurement error had little effect on the results.

The systolic trajectory in the stress-ln(h_y/h) relationship was approximated as a straight line, as suggested for results in dogs and humans. It is not known how consistently this pattern is found within these species or in the other species that are used in the present study. The trajectory may instead be concave up or down. Thus it must be stated that the work and power estimates made in this study may contain an unknown amount of error or bias. As stated in RESULTS, we have assumed that the similarity in ventricular geometry and pressure waveforms among the species included in this study would result in similar ejection trajectories because of the law of Laplace. Analysis in terms of this principle suggested that changes in pressure within a physiologically realistic range (for normal individuals) during ejection would produce only small departures from linearity of this trajectory. These changes would result in <20% increase in the ejection work estimate.

The present stress-ln(h_y/h) data could not be used directly to derive a constitutive or phenomenological (predictive) equation. The advantages gained in ease and precision of measurement would seem to make this limitation an acceptable trade-off.

It can be argued that the stress values used here are hypothetical because they are not measured directly (2). Pressure waves within the lumen and anisotropy within the wall certainly cause variations in stress among different depths and regions of the myocardium that are beyond the analysis provided by the present approach.

The stress values are not hypothetical, however, when the mean fiber stress over the full thickness of the myocardium in the geometric model employed here is discussed. The overall balance of mean pressure and mean stress in ellipsoidal shapes has been demonstrated to be almost completely independent of the ratios of diameters (2, 26). This fact and the fundamental law of balance of stresses in a plane (38) require that measured pressures and wall-luminal geometries uniquely and accurately predict values of mean fiber stress.

In summary, we have developed and tested a formalism for LV chamber and myocardial mechanics that includes referencing of wall-thickness measurements to calculated thickness at zero-luminal volume. This approach allows increased direct comparison between mechanical and energetic measurements by 1) reducing scatter, 2) eliminating size dependence, and 3) employing variables that are expressed in basic physical units. Underlying relationships are less obscured by random variations in measurements. The fact that the approach involves only noninvasive measurements makes it practical for use in longitudinal studies and clinical applications.

APPENDIX

A cubic equation was used to calculate wall-thickness values from the measured values of 1) end-diastolic values of wall thickness and luminal diameter and 2) end-systolic luminal diameter. It was based on the geometric model used by Zile et al. (40) and the approach used by Hugenholtz et al. (17). LV geometry was assumed to be a half-ellipsoidal shell. The proportionality of the epicardial long and short axes of this solid of revolution was assumed to be 1.2, and the wall thickness at the apex was assumed to be 0.4 times that at the base. In the following, L is the endocardial long axis, D is the endocardial short-axis, h is wall thickness at the base, V_T is
total ventricular volume (luminal + wall), and \( V_c \) is cavity (luminal) volume. The constant myocardial volume is \( V_{myo} \).

The basic equations describing this model are

\[
\frac{L + 0.4h}{D + 2h} = 1.2; \quad V_T = \frac{\pi}{6} (L + 0.4h)(D + 2h)^3; \tag{A1}
\]

\[
V_c = \frac{\pi}{6} D^2 L; \quad V_{myo} = V_T - V_c
\]

After \( V_{myo} \) is evaluated by using end-diastolic dimensions, the four equations are reduced to one

\[
V_{myo} = \frac{\pi}{5} (D + 2h)^3 - \frac{\pi}{6} D^2 (1.2D + 2h) \tag{A2}
\]

This cubic equation is solved analytically by using classic methods. Rearranging produces

\[
h^3 + \frac{3}{2} Dh^2 + \frac{13}{24} D^3 h - \frac{5V_{myo}}{8\pi} = 0 \tag{A3}
\]

The following intermediate quantities are evaluated

\[
p = \frac{3}{2} D; \quad q = \frac{13}{24} D^2; \quad r = -\frac{5V_{myo}}{8\pi}; \quad a = -\frac{p^2}{3}; \quad b = \left(\frac{1}{27}\right)(2p^3 - 9pq + 27r)
\]

The solution for \( h \) is then

\[
h = \sqrt[3]{\frac{b^2}{2} + \sqrt{\frac{b^2}{4} + \frac{a^3}{27}}} + \sqrt[3]{\frac{b^2}{2} - \sqrt{\frac{b^2}{4} + \frac{a^3}{27}} - \frac{p}{3}} \tag{A5}
\]

To find \( h_0 \), Eq. A3 is solved for the case when \( D = 0 \)

\[
h_0 = \sqrt[3]{\frac{5V_{myo}}{8\pi}} \tag{A6}
\]

For purposes of simplicity, a spherical ventricular geometry will be used to illustrate the relationship between \( h/\alpha \) and the ratio of midwall dimensions. For a hemiellipsoidal geometry, such as the model of Zile et al. (40) used in this study, the result is qualitatively identical with only coefficients changed.

An end-diastolic midwall encloses some proportion, \( \alpha \), of the total myocardial mass, \( V_{myo} \). The diameter ensoising this fraction of mass at an arbitrary point in contraction is \( D_{m0} \), whereas the diameter of the lumen is \( D \). At zero-luminal volume, this fraction of mass would form a sphere with diameter \( D_{m0} \).

The total volume of the lumen plus myocardium can be expressed in two ways

\[
\frac{\pi}{6} (D + 2h)^3 = \frac{\pi}{6} D^3 + V_{myo} \tag{A7}
\]

This equation can be reduced to a quadratic in \( D \)

\[
D^2 + 2hD + \frac{1}{2}h^2 - \frac{V_{myo}}{\pi h} = 0 \tag{A8}
\]

Using the quadratic formula gives an expression for \( D \) in terms of \( h \) and \( V_{myo} \)

\[
D = h \left( -1 + \sqrt{\frac{V_{myo}}{\pi h^3} - \frac{1}{3}} \right) \tag{A9}
\]

The volume enclosed in diameter \( D_m \) is the sum of the luminal and a fixed proportion, \( \alpha \), of the myocardial volume

\[
\frac{\pi}{6} D_m^3 = \frac{\pi}{6} D^3 + \alpha V_{myo} \tag{A10}
\]

which gives an expression for \( D_m \)

\[
D_m = \sqrt[3]{\frac{3}{D^3} + \frac{6\alpha V_{myo}}{\pi}} \tag{A11}
\]

Substitution for \( D \) using Eq. A9 gives

\[
D_m = h \left( -1 + \sqrt{\frac{V_{myo}}{\pi h^3} - \frac{1}{3}} \right) + \frac{6\alpha V_{myo}}{\pi h^3} \tag{A12}
\]

or equivalently

\[
D_m = h \left[ -1 + \sqrt{\frac{V_{myo}}{\pi h^3} - \frac{1}{3}} + \frac{6\alpha V_{myo}}{\pi h^3} \right] \tag{A13}
\]

At zero-luminal volume, the midwall diameter still encloses the same proportion, \( \alpha \), of myocardium, but no lumen, whereas the wall thickness encloses the full myocardial volume

\[
\frac{\pi}{6} D_{m0}^3 = \alpha V_{myo}; \quad V_{myo} = \frac{4\pi}{3} h_0^3 \tag{A14}
\]

These expressions are combined to give an expression for \( D_{m0} \) in terms of \( h_0 \)

\[
\frac{\pi}{6} D_{m0}^3 = \alpha V_{myo}; \quad V_{myo} = \frac{4\pi}{3} h_0^3 \tag{A15}
\]

The ratio of \( D_{m0} \) to \( D_m \) gives the following nonlinear expression

\[
\frac{D_{m0}}{D_m} = h_0 \left[ -1 + \sqrt{\frac{4h_0^3}{3h^3} - \frac{1}{3}} + \frac{8h_0^3}{h^3} \right] \tag{A16}
\]

The quotient on the right equals unity when \( h \) equals \( h_0 \) and decreases as the chamber expands. As a result of this behavior, the relationship between mean fiber stress and \( \ln(h_0/h) \) would differ in shape from the relationship between mean fiber stress and \( \ln(D_{m0}/D_m) \).

Address for reprint requests and other correspondence: S. Denslow, The Children’s Heart Center of South Carolina, 165 Ashley Ave., PO Box 250915, Charleston, SC 29425 (E-mail: denslow@musc.edu). Received 8 September 1998; accepted in final form 18 March 1999.

REFERENCES


WALL THICKNESS REFERENCED TO MYOCARDIAL VOLUME


