Echocardiographic Left Ventricular Mass and Function in the Hypertensive Baboon

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SUMMARY Nonhuman primates with chronic systemic hypertension provide an ideal model for studying structural and functional alterations associated with compensatory cardiac hypertrophy. Since noninvasive techniques are useful for the longitudinal evaluation of these animals, we sought to critically assess the M-mode echocardiographic estimation of left ventricular mass in the baboon and to characterize estimates of left ventricular size and function in baboons with chronic renal hypertensive. In 23 baboons (12 normotensive, 11 chronic hypertensive), M-mode echocardiography-determined left ventricular mass was 73 ± 13 (SE) g as compared with the necropsy weight of 69 ± 11 g (p = NS), and the correlation was excellent (r = 0.94). When 30 chronically hypertensive baboons being observed longitudinally were compared with 10 normotensive control animals studied under identical conditions, several differences were noted in measures derived from echocardiography and high fidelity pressure measurements. Left ventricular systolic pressure was considerably higher in the hypertensive baboons (113 ± 23 vs 90 ± 11 mm Hg; p<0.001), as was left ventricular mass (148 ± 60 vs 103 ± 38 g; p<0.03). However, since the ratio of posterior wall thickness to cavity dimension was larger in the hypertensive baboons (0.52 ± 0.17 vs 0.43 ± 0.07; p<0.05), this concentric hypertrophy maintained values for left ventricular meridional stress at the same level as in the control animals. Despite matched heart rate and left ventricular stress, the rates of change in left ventricular dimensions and wall thickness in systole and diastole were all approximately 25% less in the hypertrophied baboons. Therefore, M-mode echocardiography is an accurate technique for estimating left ventricular mass in the baboon and can be used for longitudinal assessment of quantitative left ventricular performance in a nonhuman primate model of pressure-overload hypertrophy.

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KEY WORDS • left ventricular hypertrophy • left ventricular mass • left ventricular function • nonhuman primate • hypertension • echocardiography

ANIMAL models of chronic systemic hypertension are essential and widely used for studying the biochemical basis of structural and functional alterations associated with compensatory cardiac hypertrophy. Nonhuman primates may be particularly useful for such investigations for several reasons. First, they are close phylogenetically to humans and consequently may have many biochemical similar-

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and to characterize, by echocardiography and high fidelity pressure measurements, estimates of LV size and performance in normal and chronically hypertensive baboons.

Materials and Methods

Study Population

The study population of baboons (Papio anubis and cynocephalus) consisted of two groups: a group studied immediately before necropsy for the purpose of directly determining LV mass and a group that was preserved for longitudinal studies. The necropsy group consisted of 23 animals, of which 12 were normotensive controls and 11 had LV hypertrophy caused by chronic systemic hypertension. The longitudinal group consisted of 30 baboons with hypertension and LV hypertrophy and 10 normotensive control animals matched for age, sex, and weight. All hypertensive animals were selected from a cohort of 59 baboons modeled for chronic hypertension of gradual onset produced by either the two-kidney, one clip Goldblatt procedure or bilateral cellophane wrap perinephritis. These animals were selected for the greatest extent and duration of hypertension. Their conscious mean arterial pressures, measured using a standard tethering system developed at the Southwest Foundation for Biomedical Research, averaged 135 ± 30 (SD) mm Hg, and they had been hypertensive for a mean of 4.6 ± 0.1 years.4,5

Experimental Procedures

As baboons cannot be studied in the conscious state, the animals were sedated using ketamine HCl, 15 mg/kg i.m. The normal and hypertensive animals undergoing invasive and noninvasive study immediately before death for LV mass validation received no other anesthesia. However, these animals were killed by intravenously administering an overdose of pentobarbital followed by a saturated solution of potassium chloride. The 30 hypertensive animals and the 10 normotensive control animals that were used for comparative echocardiographic LV size and performance studies were subsequently intubated and ventilated spontaneously with 100% supplemental oxygen. Light inhalational anesthesia with 0.8 to 1.5% halothane was employed. Preliminary studies in our laboratory demonstrated that this anesthetic concentration is associated with normotal arterial blood gas values and pH determinations in this model. Autonomic blockade was accomplished with propranolol (0.15 mg/kg i.v.) and atropine (0.03 mg/kg i.v.) to minimize reflex changes in heart rate, blood pressure, or inotropic state during the experiments.

For cardiac catheterization a 7F Millar micromanometer-tipped catheter (Millar Instruments, Houston, TX, USA), with a fluid lumen for high-speed injection was placed retrograde into the LV cavity from the femoral artery. Biplane cine left ventriculography was obtained at a cine-frame rate of 60 frames/sec. Meglumine diatrizoate (Renografin 76, 1 ml/kg), was power-injected over 3 seconds into the LV cavity. LV pressure was measured simultaneously with the Millar micromanometer-tipped transducer, which was calibrated against a mercury manometer at body temperature in a water bath to eliminate thermal drift and equilibrated by imposition of the high fidelity signal with the fluid lumen recordings referenced to the mid-chest level before the ventriculogram.

Echocardiograms were obtained on an M-mode ultrasonoscope using a 2.25-mHz, 10-mm piezoelectric transducer with a repetition rate of 1000 impulses/sec and were continuously recorded at a paper speed of 100 mm/sec. An electrocardiogram was simultaneously recorded. For the echocardiographic recordings, the baboons were placed first in a partial left lateral decubitus position. The transducer was held in the third or fourth left sternal interspace, and the beam was directed slightly laterally and inferiorly to obtain images from the endocardial surface of the left ventricle just below the level of the mitral valve leaflets at the chordal position. If this approach was unsuccessful, a right parasternal approach was used. No animals were rejected because of an inability to record adequate echoes of the left ventricle. However, the right side of the interventricular septum was not obtainable in all animals because of their midline heart position. Figure 1 shows representative echocardiograms from normotensive and hypertensive animals.

Echocardiographic Data Analysis

Cavity dimensions were measured from the LV surface of the interventricular septum to the endocardial surface of the posterior wall of the left ventricle.6 LV posterior wall thickness was measured as the distance between the endocardium of the posterior wall and origin of the epicardial echo obtained during electrical damping of the ultrasonic signal.7 The end-diastolic point was measured at the peak of the R wave, and end systole was defined as the minimum cavity dimension. In the 13 normotensive animals studied before death, all echocardiographic measurements were performed by two observers who were blinded to the status of the animal. One of the two observers performed the same measurements twice.

To obtain the M-mode echocardiographic peak rate of change in the LV internal dimension and posterior wall thickness during systole and diastole, one representative beat was traced manually, using a magnified crosswire cursor, over one cardiac cycle between R waves on the electrocardiogram and digitized using an inductance-digitizing surface at a sampling frequency of 500 to 1000 Hz. The digitized data were processed in real-time using an International Business Machine XT microprocessor (Austin, TX, USA) and software developed in our laboratory. After obtaining a single three-point running average of the digitized data, peak rates of change in LV cavity dimension with time and LV posterior wall thickness were calculated using the method of Gibbons and co-workers.8,9 To facilitate comparison of ventricles of different sizes, the values for rate of change in LV cavity dimension with time...
and in LV posterior wall thickness were normalized using the LV cavity dimension and posterior wall thickness, respectively, which occurred at the peak rate of change (Figure 2).

Echocardiographic LV cross-sectional area and mass were determined using the end-diastolic posterior wall thickness by the method of Troy et al. and were corrected by the technique of Devereux et al., since we used the newer American Society of Echocardiography convention for measuring wall thickness. Echocardiographic wall stress was calculated using the method of Grossman et al. by the following equation: stress = \( P D^2 h (D + h) \), where \( P \) is the LV high fidelity pressure, \( D \) is the echocardiographic LV diameter, and \( h \) is the posterior wall thickness. This formula expresses the average meridional stress, which is the force per unit area acting at the equational plane of the ventricle in the direction of the apex to base axis. This approach to wall stress derivation has the advantage that the value is independent of the long axis of the chamber. End-diastolic wall stress was derived using pressure and dimension measurements taken at the peak of the R wave of the simultaneously recorded electrocardiogram. Peak systolic meridional stress was assessed using the end-diastolic echocardiographic dimension data and the maximal LV pressure since this usually occurs 40 to 60 milliseconds before aortic valve opening prior to any substantial reduction in LV cavity size.

**Biplane Cineangiographic Data Analysis**

Biplane anteroposterior and lateral LV cineangiographic end-diastolic and end-systolic volumes were derived using a modified Simpson's rule algorithm and necropsy-cast water displacement regression equations previously developed in our laboratory. Briefly, LV silhouettes were outlined with a sonic digitizer mounted on a cineprojector, and the digitized data were processed with an online minicomputer (IBM-XT). Correction for magnification and pincushion distortion was accomplished by filming precalibrated grids at the center of the LV mass in both planes. Angiographic
LV mass was calculated using the method of Rackley et al.\textsuperscript{13}

**Determination of Left Ventricular Mass at Autopsy**

LV mass was determined at necropsy as wet weight immediately after death using a standard protocol developed at the Southwest Foundation for Biomedical Research.\textsuperscript{3} This protocol has been used for 86 consecutive baboon necropsies performed because of spontaneous or experimental death in animals not known to have conditions associated with LV hypertrophy. LV mass averaged 72.0 ± 3.1 (SE) g for all 86 animals. The LV mass per body weight ratio was 3.1 ± 0.1 g/kg.

**Statistical Techniques**

Comparisons between the invasive and the noninvasive determinations of mass and the autopsy mass were made by paired Student's \(t\) tests and linear regression analysis. Comparisons of noninvasive values obtained in the normal and hypertensive animals were obtained by unpaired \(t\) tests. All values were reported as means ± SD, and a \(p\) value below 0.05 was considered statistically significant.

**Results**

**Validation of Left Ventricular Mass**

LV mass determined by echocardiography was 73 ± 41 g (mass/body weight = 4.2 ± 1.7 g/kg) and was not significantly different than that determined by autopsy in the same 13 animals (69 ± 39 g; mass/body weight = 4.0 ± 1.8 g/kg). Also, the correlation between echocardiography and autopsy was excellent (\(r = 0.94, \text{ SEE} = 21.5 \text{ g; uncorrected data; Figure 3.}\) The mass determined by cineventriculography was less but was not significantly different from that determined in these 12 animals by autopsy (64 ± 32 vs 78 ± 31 g), and the correlation between the two methods was good (\(r = 0.91, \text{ SEE} = 12.1 \text{ g; Figure 4.}\) Reproducibility of echocardiographic LV mass estimation was assessed in six normal and six hypertensive baboons. Intraobserver variation was 3%, and interobserver variation was 5%. Reproducibility of angiographic LV mass was performed in nine animals and showed that intraobserver variation was 4% and interobserver variation was 5%.

**Comparison of Normotensive with Hypertensive Baboons**

Several echocardiographic indices of LV structure and function were significantly different when the normotensive and hypertensive animals studied under the same conditions of anesthesia were compared (Table 1). Body weight and heart rate were not significantly different between the two groups, but peak systolic LV pressure was greater in the hypertensive animals (113 ± 23 vs 90 ± 11 mm Hg; \(p < 0.001\)). Not surprisingly, end-diastolic LV posterior wall thickness was 30% greater in the hypertensive baboons and end-systolic thickness was 22% greater (\(p < 0.005\)). Consequently, LV cross-sectional area and calculated mass were 44% larger in the hypertensive animals, despite similar LV cavity dimensions. Since the ratio of posterior wall thickness to cavity radius was greater in the hypertensive animals, this concentric hypertrophy maintained the values for LV meridional stress at the same level as in the control baboons. The rates of LV dimension change and wall thickness change in systole and diastole, both raw and normalized, were all approximately 25% less in the hypertensive hypertrophied group in comparison with the normotensive control group, despite matched heart rate and LV stress. However, the overall percentage of change in cavity dimension and wall thickness during systole was not significantly altered in the hypertensive animals.

**Discussion**

**Methodology**

Our results demonstrate the feasibility of using echocardiography for noninvasive assessment of LV mass.
size and performance in the baboon. Echocardiographic LV mass accurately estimated postmortem LV weight, and the echocardiographic measurement of mass was highly reproducible. The technique clearly separated normal animals from those with chronic hypertension and LV hypertrophy, and there was an excellent linear correlation over a wide range of LV weights. LV mass estimates by LV cineangiography also closely approximated the autopsy weight and correlated well over a wide range. Both M-mode echocardiography and biplane cineangiography demonstrate low interobserver and intraobserver variability. These data indicate that echocardiography is a useful technique for LV mass assessment in longitudinal studies of baboons. This finding is particularly important since it may minimize the risk associated with more invasive, less widely available techniques in this valuable model.

In studies by Devereux et al. and Salcedo et al., measurements of LV mass by M-mode echocardiography in animals and humans overestimated postmortem LV weight, and these investigators implicated the measurement of wall thickness by M-mode echocardiography as the problem. Salcedo et al. suggested measuring wall thickness in several sites about the midwall circumference of the left ventricle on two-dimensional echocardiography and using the average value to calculate mass. Devereux et al. found that the overestimation of mass was consistent and suggested that if the American Society of Echocardiography convention was used to measure wall thickness, the values could be corrected using a regression formula. Their study in humans involved patients without major wall motion abnormalities whose LV mass was often in the upper normal range. Since our animals had no evidence of segmental wall motion abnormalities on biplane cineangiography and since LV mass ranged from normal to moderately increased, we thought that their correction factor was appropriate to apply to our data. Also, the amount of overestimation of mass noted in their uncorrected data was similar to the difference between our corrected and uncorrected data.

Recently, Feneley and Hickie suggested that the major source of overestimation of LV mass by the M-mode technique is the inability to measure the long axis of the ventricular cavity, rather than errors in wall thickness estimations. The M-mode algorithm models the ventricle to be a prolate ellipse with a long axis that is twice the measured short axis. This may not always be true, especially in diseased ventricles with a more spherical shape. Also, in smaller ventricles the ratio of long to short axis can approach 3.0. Thus, the cavity size of a small ventricle will be underestimated and the myocardial volume will be overestimated. Two-dimensional echocardiography theoretically could reduce this problem by allowing the measurement of the long axis of the left ventricle. However, the temporal resolution of two-dimensional echocardiography is

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**Table 1.** Echocardiographic Left Ventricular Structure and Performance in Halothane-Anesthetized Normal and Hypertensive Baboons

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotensive (n=10)</th>
<th>Hypertensive (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>26.5±8.7</td>
<td>25.3±6.2</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>83±15</td>
<td>79±12</td>
</tr>
<tr>
<td>LVP (min Hg)</td>
<td>103±28</td>
<td>113±23*</td>
</tr>
<tr>
<td>Peak systolic</td>
<td>22±6</td>
<td>17±7†</td>
</tr>
<tr>
<td>End-diastolic</td>
<td>3.7±0.6</td>
<td>3.8±0.6</td>
</tr>
<tr>
<td>End-systolic</td>
<td>2.6±0.5</td>
<td>2.7±0.7</td>
</tr>
<tr>
<td>LVM</td>
<td>0.77±0.12</td>
<td>1.00±0.24‡</td>
</tr>
<tr>
<td>LVM/weight (g/kg)</td>
<td>3.9±0.8</td>
<td>5.8±1.6‡</td>
</tr>
<tr>
<td>dD/dt (cm/sec)</td>
<td>9.3±2.0</td>
<td>6.7±2.1‡</td>
</tr>
<tr>
<td>Diastolic</td>
<td>0.3±0.1</td>
<td>0.23±0.09§</td>
</tr>
<tr>
<td>N diastolic</td>
<td>7.0±2.3</td>
<td>5.3±1.24</td>
</tr>
<tr>
<td>Systolic</td>
<td>0.2±0.1</td>
<td>0.17±0.06§</td>
</tr>
<tr>
<td>N systolic</td>
<td>4.5±1.5</td>
<td>3.3±1.6†</td>
</tr>
<tr>
<td>Systolic</td>
<td>2.9±0.7</td>
<td>2.2±0.74</td>
</tr>
<tr>
<td>h/r</td>
<td>0.43±0.07</td>
<td>0.52±0.17†</td>
</tr>
<tr>
<td>Meridional stress</td>
<td>22±9</td>
<td>13±7</td>
</tr>
<tr>
<td>End-diastolic (g/cm²)</td>
<td>85±20</td>
<td>85±25</td>
</tr>
<tr>
<td>Peak systolic (g/cm²)</td>
<td>30.2±4.6</td>
<td>28.3±8.5</td>
</tr>
<tr>
<td>ΔP (%)</td>
<td>42±21</td>
<td>33±17</td>
</tr>
</tbody>
</table>

Values are means ± SD. LVP = left ventricular pressure; LVD = LV diameter; LVM = LV wall thickness; LVCSA = LV cross-sectional area; LVM = LV mass; dD/dt = rate of change in D; N = normalized dD/dt; dh/dt = rate of change in h; r = LV radius; ∆ = change.

* p<0.001, † p<0.03, § p<0.005, || p<0.01, ‡ p<0.05, compared with values in normotensive baboons.
considerably less than that of M-mode echocardiography (sampling frequency, 33 vs 1 msec) with conventional equipment. The low-frequency content of two-dimensional echocardiography signals invalidates the determination of time-dependent derivative functions, such as rates of LV cavity or wall thickness change.19 Thus, for the purposes of our study M-mode echocardiography is preferable for estimating serial changes in mass and quantitative LV performance in this nonhuman primate model of pressure-overload hypertrophy.

Hypertensive Model with Left Ventricular Hypertrophy

The duration (4.6 ± 0.1 years) and magnitude (mean conscious arterial blood pressure, 135 ± 30 mm Hg) of renal hypertension produced substantial concentric LV hypertrophy in this primate model. Echocardiographic LV mass (+44%), cross-sectional area (+44%), the ratio of LV mass to body weight (+49%), and the ratio of LV wall thickness to LV radius (+21%) were each significantly elevated in the hypertensive baboons compared with normotensive controls matched for sex, age, and weight. These alterations in LV mass and geometry were associated with significant reductions in the rates of chamber emptying and filling in the hypertensive baboons despite normal overall fractional shortening (see Table 1). This discrepancy between total fractional shortening and the rate of LV dimensional change during systole has been observed by others and is consistent with the concept that velocity measures may be better indices of contractility.20 It is unlikely that the diminished velocity of LV ejection and filling is a consequence of altered loading conditions since both end-diastolic and peak-systolic stress were equivalent between the control and hypertensive animals during halothane anesthesia. In fact, it is likely that anesthesia effects were more profound in the newer control animals, since their LV end-diastolic pressure was higher than that of the hypertensive baboons. Thus, factors intrinsic to the hypertrophy process, such as altered myocardial contractility, relaxation, or material properties, may account for the abnormal LV chamber performance in this model. Human studies have demonstrated variable effects of pressure-overload hypertrophy due to systemic hypertension on LV performance. Whether these variations result from difficulties in dissociating the effects of altered loading and concomitant pharmacotherapy from intrinsic changes in myocardial function in the clinical setting is still open to question.21

The depressed rates of LV chamber emptying and filling that we observed in hypertrophied baboons are consistent with a recent study of experimental gradual-onset renal hypertension by Capasso et al.22 They observed a significant prolongation of isometric time to peak tension and to half-relaxation, but normal levels of peak tension in papillary muscles extracted from rats with Goldblatt hypertension and marked LV hypertrophy (+50% in LV weight). This group subsequently demonstrated that these alterations in contractile behavior of papillary muscles from hypertrophied hearts were associated with depressed calcium-activated myosin adenosine triphosphatase (ATPase) activity and altered myosin isozyme patterns in their model.23 These functional changes in lower animals, such as the rat and rabbit, are believed to be effected in part by the differential expression and repression of at least two genes that encode myosin heavy chains and result in a transition from a myosin isozyme with high to one with low ATPase activity.24, 25 By contrast, higher mammals, such as pigs and humans, appear to have a single myosin isozyme in normal and hypertrophied ventricular myocardium associated with no change or reduced myosin ATPase activity, despite similar alterations in cardiac performance.1, 26-28 Thus, the biochemical basis for these load-independent changes in LV function consequent to hypertensive pressure-overload hypertrophy in higher mammals remains unclear and is a current focus of investigation in our laboratory.

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