Contribution of mitral annular dynamics to LV diastolic filling with alteration in preload and inotropic state

C. Carlhäll, K. Kindberg, L. Wigström, G. T. Daughters, D. C. Miller, M. Karlsson, and N. B. Ingels

Departments of Cardiothoracic Surgery and Radiology, Stanford University School of Medicine, Stanford, and Laboratory of Cardiovascular Physiology and Biophysics, Research Institute, Palo Alto Medical Foundation, Palo Alto, California; and Department of Clinical Physiology, Linköping University Hospital, and Department of Biomedical Engineering, Linköping University, Linköping, Sweden

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The helical myocardial fiber architecture of the LV produces both long- and short-axis motion as well as torsional deformation (15, 17, 28). The longitudinal excursion of the mitral annular (MA) plane is an important component of LV filling and ejection (2–5, 20, 26, 33). This excursion is a clinically useful variable, related to both systolic and diastolic LV function (13, 14). The longitudinal excursion of the MA toward the cardiac apex during ventricular systole has the effect of increasing the capacity of the left atrium. This atrial volume increase and atrial pressure fall facilitate a rapid filling from the pulmonary veins. Subsequently, during diastole, the reverse excursion of the MA toward the atrium contributes to the transition of atrial volume into ventricular volume (14, 20). Thus the longitudinal excursion of the annulus encompasses a volume that is part of the total LV filling volume, such that altered MA dynamics could affect LV filling (3, 27). The impact of a change in preload or inotropic state on the contribution of MA excursion and area variation to total LV filling has not been characterized. Therefore, we sought to test the hypothesis that a change in LV preload and inotropic state would not alter the contribution of MA dynamic motion to LV diastolic filling.

METHODS

All animals received humane care in compliance with the “Principles of Laboratory Animal Care,” formulated by the National Society for Medical Research, and the Guide for Care and Use of Laboratory Animals, prepared by the National Academy of Sciences and published by the National Institutes of Health (Publication No. 85–23, Revised 1996). This study was approved by the Stanford Medical Center Laboratory Research Animal Review Committee and conducted according to Stanford University policy.

Surgical Preparation

Six healthy adult castrated male sheep (64 ± 10 kg) were premedicated with ketamine (27 mg/kg im) and atropine sulfate (0.05 mg/kg iv), anesthetized with thiopental sodium (6.8 mg/kg iv), intubated, and mechanically ventilated (Servo Anesthesia Ventilator, Siemens-Elema, Solna, Sweden). General anesthesia was maintained with inhalational isoflurane 1% to 2.2% and supplemental oxygen. Each animal received a single preoperative dose of cefazolin sodium (1 g iv) and gentamicin sulfate (80 mg iv), and these antibiotics were continued throughout the postoperative period. By use of sterile technique, an incision was made in the left neck, exposing the jugular
vein and carotid artery for catheterization. A micromanometer-tipped pressure transducer (SPC-500, Millar Instruments, Houston, TX) was zeroed in a water bath and inserted into the carotid artery to monitor systemic arterial pressure. A left thoracotomy was performed, and pneumatic occluders (In Vivo Metric Systems, Ukiah, CA) were placed around the superior and inferior vena cavae to provide a means for transient preload reduction (8). The heart was suspended in a pericardial cradle, and nine miniature tantalum radiopaque helixes (inner diameter, 0.8 mm; outer diameter, 1.3 mm; and length, 1.5 to 3.0 mm, some having different small extensions or “tails” to facilitate subsequent radiographic identification) were inserted into the LV wall epicardium and septum (Fig. 1) (9).

On cardiopulmonary bypass with the heart arrested, the left atrium was opened. Eight miniature tantalum radiopaque markers were sutured to the atrial side of the MA at equal distances around its circumference (Fig. 1), one near each commissure (markers 16 and 20) and three along the perimeters of the anterior (markers 15, 21, and 22) and posterior (markers 17, 18, and 19) leaflets. An implantable micromanometer (PA4.5-X6, Konigsberg Instruments, Pasadena, CA) was placed via the LV apex for LV chamber pressure monitoring. The animal was weaned from cardiopulmonary bypass, and an intercostal block (30 ml of 0.25% bupivicaine) was performed at the fourth, fifth, and sixth intercostal spaces to minimize immediate postoperative pain. Catheters were placed in the left jugular vein and carotid artery and brought out through the skin in the posterior neck, providing indwelling central venous and arterial lines. The chest and neck incisions were closed, hydromorphone hydrochloride (0.03 mg/kg iv, Dilaudid, Knoll Pharmaceuticals, Whippany, NJ) was given as needed to minimize incisional discomfort, and the animals recovered in the experimental ovine cardiac surgical intensive care unit.

Experimental Protocol

After 9 ± 2 days, each animal was taken to the experimental animal cardiac catheterization laboratory for hemodynamic and biplane videofluorographic data acquisition. The animals were premedicated with ketamine (27 mg/kg iv), intubated, and mechanically ventilated (Veterinary Anesthesia Ventilator 2000, Hallowell EMC, Pittsfield, MA) with 100% oxygen. Sedation was maintained with ketamine (1 to 4 mg·kg⁻¹·h⁻¹ iv) and supplemental diazepam (5 mg iv), administered as needed. A micromanometer-tipped catheter (Millar SPC-500) was calibrated and advanced via a left femoral artery cut down into the descending aorta for aortic pressure monitoring. UL-FS49 (Boehringer-Ingelheim, Ridgefield, CT), a highly specific negative chronotropic agent that does not change the QT interval, inotropic state, or systolic or diastolic blood pressure (25), was administered (8 mg iv, single dose) to lower heart rate. Such heart rate reduction facilitated subsequent cinefluoroscopic visualization and tracking of marker motion.

For all data acquisition runs, hearts were in normal sinus rhythm, ventilation was arrested briefly at end expiration, and data were obtained during steady-state conditions and over a physiological range of peak LV systolic pressures during vena caval occlusion (VCO). The animals were allowed to stabilize for 3 to 5 min between all data acquisition sequences. Any data sets containing premature ventricular contractions were discarded.

Data Acquisition

Images were acquired with the animal in the right lateral decubitus position using a Philips Optimus 2000 biplane Lateral ARC 2/Poly DIAGNOST C2 system (Philips Medical Systems, North America Company, Pleasanton, CA) with the image intensifiers in the 9-in. cinefluoroscopic mode. Data from the two radiographic views were digitized and merged to yield three-dimensional (3-D) coordinates for each of the radiopaque markers every 16.7 ms using custom-designed software (6). The accuracy of these 3-D reconstructions from biplane videograms of length measurements, compared with known marker-to-marker 3-D lengths, was previously shown to be 0.1 ± 0.3 mm (6).
LV pressure (LVP) and ECG signals were digitized and recorded simultaneously during image acquisition. Data were acquired under 1) control conditions [VCO control (VCOC)], 2) transient preload reduction induced by VCO [3 beats: early, mid, and late [VCO(1-3)] during LVP reduction to ~50% of peak systolic LVP], 3) control conditions (calcium control), 4) inotropic augmentation (15 mg/kg iv bolus) with calcium chloride, 5) control conditions (nitroprusside control), and 6) pre (and after)-load reduction induced by intravenous infusion (0.5–8 µg·kg⁻¹·min⁻¹) of sodium nitroprusside.

Data Analysis

Data from two consecutive steady-state beats were averaged and analyzed for the calcium and nitroprusside runs and their respectively controls. Data from one beat were analyzed for the VCO runs [VCO(1-3)] and their control.

Cardiac cycle timing. Onset of LV filling (t = 0) was defined as the videographic frame, during isovolumic relaxation, immediately following the time that LVP had fallen to 10% of total LVP range (18). End of filling (t_max) was defined as the videographic frame at peak of the second derivative of LVP (d²P/dt²) with respect to time (Fig. 2, A and B).

MA area. MA area was calculated without assumption of planar or circular geometry. The centroid of the eight annular markers was determined, and the annular area was divided into eight triangular segments. The MA area was then derived as the sum of the individual areas of the segments. The MA mean area during LV filling was computed as the average area of the time frames from t = 0 to t = t_max.

MA excursion. The distance from the centroid of the annular markers to a point at the epicardial apex (apical marker, fixed position at the first time frame of each run) was calculated throughout the cardiac cycle. Total annular excursion during LV filling was defined as the change in this distance from t = 0 to t = t_max.
LV volume. Instantaneous LV volume (LVV) was calculated using 16.7 ms frame-time 3-D coordinates of the nine LV and eight MA markers (Fig. 1) using a space-filling multiple tetrahedral volume method (21). The LV filling volume from any frame (at time $t$) is denoted as $LVV_{fill}(t)$. The LV filling volume from any frame (at time $t$) is defined as $LVV_{fill}(t)$, which is schematically illustrated in Fig. 3(B). This volume, defined as LVV fill, represents an incremental volume change during this time interval. The sum of all eight incremental volume changes constituted the LVV fill from any frame (at time $t$) to $t = t_{max}$, denoted $LVV_{fill}(t)$.

**MA excursion volume.** During diastole the MA excursion encompasses a blood volume comprising a portion of total LV filling (Fig. 3A). This volume, defined as MA excursion volume (MAEV), is based on MA excursion and MA area variation (Fig. 3B) and can be estimated using a method similar to that described by Carthall et al. (3). Figures 4 and 5 illustrate the principles of the MAEV. From any time $t$ to time $t + 1$, the markers for each one of the eight annular segments (centroid and 2 annular markers) define a triangular prism, representing an incremental volume change during this time interval (Fig. 4). The average change in distance of the three segmental markers to a fixed apical reference point (apical marker, at the first time frame of each run) was used to define the height of this triangular prism. The incremental volume change for each segment was subsequently calculated as the height multiplied by the average area of the triangular segment in the two time frames. The sum of all eight of these incremental volume changes constituted the MAEV from any time $t$ to $t + 1$ (MAEV$_{t_{max}}$), which is schematically illustrated in two-dimensions (although the actual computations were made using the full 3-D data set) in Fig. 5 (shaded gray). By adding the contributions from each time frame from $t = 0$ to $t = t_{max}$, the total volume encompassed by the excursion of the annulus (MAEV$_{t_{max}}$) was estimated.

**Statistical Analysis**

Data are presented as group means ± SD, unless otherwise stated. Statistical analyses were performed using the Statistica software (release 7.1, StatSoft). Student’s t-test for paired observations was used to detect whether there were significant differences between the calcium run and the calcium control run and between the nitroprusside run and the nitroprusside control run. Repeated measures of analysis of variance and a post hoc test (Tukey’s honestly significant difference test) were used to detect whether there were significant differences between the three VCO runs and their control. Statistical significance was set at $P < 0.05$.

**RESULTS**

Postmortem examination of the excised hearts revealed all eight MA markers to be within 1 mm of the MA (as defined by the visible leaflet left atrial endocardial junction) and all nine LV epicardial markers to be within 1 mm of the epicardial surface.

**Hemodynamics**

Hemodynamic data for the calcium and nitroprusside runs are summarized in Table 1. Relative to control, calcium increased the maximum positive rate of change of LVP (+dP/dt; $P < 0.01$), indicating increased LV contractility, whereas LV end-systolic volume (+dP/dt, maximum positive rate of change of LV pressure; −dP/dt, maximum negative rate of change of LV pressure). Even though MA excursion increased ($P < 0.01$) and area

<table>
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<tr>
<th>Hemodynamic data from the VCO runs</th>
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<tr>
<td>VCO</td>
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<tr>
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<tr>
<td>HR, beats/min</td>
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<tr>
<td>LVP$_{max}$, mmHg</td>
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<tr>
<td>LVP$_{min}$, mmHg</td>
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<tr>
<td>EDV, ml</td>
</tr>
<tr>
<td>ESV, ml</td>
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<tr>
<td>+dP/dt, mmHg/s</td>
</tr>
<tr>
<td>−dP/dt, mmHg/s</td>
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</table>

Values are means ± SD. VCC, venous occlusion (VCO) control; VCO1, VCO beat 1; VCO2, VCO beat 2; VCO3, VCO beat 3; LVV$_{max}$, minimum LV pressure. $^{*}P < 0.01$ and $^{+}P < 0.001$ vs. VCO1; $^{†}P < 0.01$ and $^{‡}P < 0.001$ vs. VCO2.
decreased (P < 0.01), the absolute or relative contribution of MA dynamics to LV filling was also similar (both P = NS).

Nitroprusside. The contribution of MA dynamics to total LV filling with nitroprusside versus control is shown in Table 3. Total LV filling decreased (P < 0.05) without change in the absolute or relative contribution of MA dynamics to LV filling (both P = NS), although MA mean area decreased (P < 0.05).

VCO. The contribution of MA dynamics to total LV filling during VCO versus control is shown in Table 4. MAEV decreased at VCO1 relative to control (P < 0.001) but without further decrease between the subsequent occlusions, whereas total LV filling fell between VCOOC and VCO1 (P < 0.001) and also between VCO1 and VCO2 (P < 0.05), providing an increase in the relative contribution of MA dynamics to LV filling at VCO2 compared with VCOOC (P < 0.001) and with VCO1 (P < 0.01). The reduction in MA mean area appeared to be greater than the reduction in MA excursion during the VCO process. Total LV filling, the absolute contribution of MA dynamics to LV filling, MA mean area (all P < 0.001), and MA excursion (P < 0.01) were all lower, whereas the relative contribution of MA dynamics to LV filling was higher (P < 0.001) at occlusion beat 3 relative to control (Table 4).

DISCUSSION

The principal findings of this study were that, although MA excursion and mean area changed with pharmacologically induced preload reduction and inotropic augmentation, the contribution of MA dynamic motion to LV filling was constant and had substantial magnitude, accounting for approximately one-fifth of total LV filling. Furthermore, with abrupt mechanically induced preload reduction, the relative contribution of MA dynamics to LV filling increased twofold, thereby accounting for more than two-fifths of total diastolic filling.

The observation that nearly one-fifth (18 ± 4%) of total LV filling was due to MAEV during control conditions in sheep hearts is consistent with the recent study of Carlhäll et al. (3) who observed a 19 ± 3% contribution in healthy humans, using a similar algorithm when computing the MAEV from 3-D echocardiographic data. Earlier, Toumanidis et al. (29) estimated using two-dimensional transthoracic echocardiography the MAEV as the volume of a truncated cone. When we corrected the equation for the volume of the truncated cone by adding the denominator, the contribution of MA dynamics to total LV filling was also ~18%, i.e., in the same range as later findings. Tibayan et al. (27) used a slightly different method, which besides MA dynamics also accounted for the dynamic motion of the basal part of the LV myocardium in assessing regional LV filling. They documented a contribution of ~25% in ovine hearts, which closely corresponds to our current findings.

Pharmacological Alterations in Inotropic State and Preload Condition

With inotropic augmentation (calcium), both the relative and absolute contributions of MA dynamics to total LV filling were constant, although MA excursion increased and MA mean area decreased. This increase in MA excursion is concordant with earlier documentation in animals and humans showing a strong correlation between LV systolic function/endocardial and LV longitudinal motion (13, 24). Furthermore, it is also known that enhanced inotropy reduces both systolic and diastolic MA areas, possibly facilitated by the insertion of myocardial fibers from the ventricle and the atrium into the MA (10, 30, 32).

Moreover, it appears that calcium administration is also accompanied by preload reduction (11, 24), which might be a reason for the smaller MA area (7). The mechanism behind this preload reduction is not clear, but it is possible that filling from the pulmonary circulation is transiently limited in relation to the rapidly increased demand caused by the enhanced contractility. With calcium-associated lusitropic augmentation, an increase in total LV filling would be expected, but this increase was absent, possibly due to such preload reduction.

During pharmacologically induced preload reduction (nitroprusside), both the relative and absolute contributions of MA dynamics to total LV filling as well as the MA excursion were decreased (both P < 0.01).

Table 3. Contribution of MA dynamics to total LV volume increase during filling, from the Ca and N runs

<table>
<thead>
<tr>
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<th>CaC</th>
<th>Ca</th>
<th>NC</th>
<th>N</th>
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<tbody>
<tr>
<td>Total LVFV, ml</td>
<td>16.9±4.7</td>
<td>16.4±5.9</td>
<td>17.9±5.5</td>
<td>15.4±4.6*</td>
</tr>
<tr>
<td>MAEV, ml</td>
<td>3.1±0.9</td>
<td>3.2±0.9</td>
<td>3.4±1.1</td>
<td>3.0±0.7</td>
</tr>
<tr>
<td>MAEV/total LVFV, %</td>
<td>18.5±3.2</td>
<td>20.6±4.5</td>
<td>19.1±3.4</td>
<td>20.5±3.6</td>
</tr>
<tr>
<td>MA, cm</td>
<td>0.43±0.10</td>
<td>0.50±0.11†</td>
<td>0.47±0.11</td>
<td>0.47±0.09</td>
</tr>
<tr>
<td>MA mean area, cm²</td>
<td>7.4±0.7</td>
<td>6.4±0.7†</td>
<td>7.3±0.8</td>
<td>6.5±0.6*</td>
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</tbody>
</table>

Values are means ± SD. *P < 0.05 and †P < 0.01 vs. respective control.

Table 4. Contribution of MA dynamics to total LV volume increase during filling, from the VCO runs

<table>
<thead>
<tr>
<th></th>
<th>VCOC</th>
<th>VCO1</th>
<th>VCO2</th>
<th>VCO3</th>
</tr>
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<tbody>
<tr>
<td>Total LVFV, ml</td>
<td>15.9±4.0</td>
<td>8.3±2.1c</td>
<td>5.4±1.7e</td>
<td>4.5±1.2c</td>
</tr>
<tr>
<td>MAEV, ml</td>
<td>2.8±0.7</td>
<td>2.0±0.7c</td>
<td>2.0±0.5c</td>
<td>1.9±0.4c</td>
</tr>
<tr>
<td>MAEV/total LVFV, %</td>
<td>18.2±3.8</td>
<td>25.0±10.0</td>
<td>39.7±13.1e</td>
<td>44.7±13.3c</td>
</tr>
<tr>
<td>MA, cm</td>
<td>0.40±0.1</td>
<td>0.31±0.12c</td>
<td>0.34±0.09e</td>
<td>0.33±0.10p</td>
</tr>
<tr>
<td>MA mean area, cm²</td>
<td>7.3±0.6</td>
<td>6.2±0.5c</td>
<td>5.5±0.5e</td>
<td>5.2±0.5c</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.05, †P < 0.01, and ‡P < 0.001 vs. VCOC; 4P < 0.05, 5P < 0.01, and 6P < 0.001 vs. VCO1.
constant, although MA mean area decreased. In this case, the preload reduction could also be the reason for the decreased MA mean area (7).

**Mechanical Alterations in Preload Condition**

The mechanically induced preload reduction (VCO) appeared to be more extensive than the pharmacologically induced preload reduction, as indicated by the lower LV end-diastolic volumes. During this process the decrease in total LV filling was more prominent than the decrease in MAEV, resulting in a marked increase in the relative contribution of MA dynamics to LV filling (Fig. 6). Furthermore, mainly due to the preload reduction, a decrease in end-systolic volume with a development of accentuating negative early diastolic LVP was observed (16). Thus, in this preload-depleted state, MA dynamic motion and LV suction appeared to be important mechanisms for LV diastolic filling.

**MA Excursion Coupling to LVV Increase**

The present study suggests that the MAEV is not only the result of immediate LV recoil after isovolumic relaxation but rather appears as a more gradual process throughout diastole. Therefore, it seems unlikely that this contribution to LV filling is based on merely a release of energy stored in the myocardium from the previous systole. It appears more likely that the MAEV results from a relaxation process in combination with geometric alterations brought about by LVV increase due to filling itself, although the design of this study does not allow a resolution of this cause/effect issue.

**Clinical Implications**

Mitrail valve repair with ring annuloplasty is still the procedure of choice for surgical treatment of mitral regurgitation; however, this technique has been associated with transmural pressure gradients and other diastolic filling abnormalities (1, 23). These changes may be due to restricted dynamic motion of the MA and base of the heart (27, 31). Assessment of the contribution of MA dynamics to total LV diastolic filling may prove to be a useful tool in the further development of mitral reparative techniques in the context of optimizing regional LV filling, which may or may not pivot on the use of an annuloplasty ring.

**Limitations**

The data acquisition was performed 7–10 days after the instrumentation in closed-chest, sedated animals, which may have influenced the hemodynamic conditions to some extent. Although the total LVV was computed based on epicardially located markers and thus includes myocardial volume, we have previously shown that changes in this volume accurately reflect changes in LV chamber volume (21).

In the calculation of the MA area, triangles are used to measure a curved area. Thus there is an estimated error of ~10% in MA area and excursion volume. However, this error does not importantly affect the results because we used the same technique when calculating and comparing baseline data with respective provocation data, which was the primary purpose of the study.

**Conclusion**

The findings of this study show that, although the MA excursion and mean area are different with moderate preload reduction and inotropic augmentation, the contribution of MA dynamic motion to LV filling is constant and has substantial magnitude, accounting for approximately one-fifth of total LV filling. With marked preload reduction, the relative contribution of MA dynamics to LV filling increased extensively, accounting for more than two-fifths of total diastolic filling.

**GRANTS**

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