Regional differences in the force-frequency relation of human left ventricular myocardium in mitral regurgitation: implications for ventricular shape

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Submitted 22 September 2003; accepted in final form 3 January 2005

Mulieri, Louis A., Marc D. Tischler, Barbara J. Martin, Bruce J. Leavitt, Frank P. Ittleman, Norman R. Alpert, and Martin M. LeWinter. Regional differences in the force-frequency relation of human left ventricular myocardium in mitral regurgitation: implications for ventricular shape. Am J Physiol Heart Circ Physiol 288: H2185–H2191, 2005. First published January 6, 2005; doi:10.1152/ajpheart.00905.2003.—Sphericalization of the left ventricular (LV) chamber shape in patients with mitral regurgitation (MR) contributes to increased LV wall stress and energy consumption. On the basis of previous observations, we hypothesized the existence of regional differences in the force-frequency relation (FFR) within the LV that may contribute to its shape. Accordingly, in the present study, we assessed regional variation in the FFR in patients undergoing surgery for chronic, nonischemic MR with class II–III heart failure symptoms and related our findings to the in vivo LV shape. FFRs (steady-state isometric twitches, 0.2–3.4 Hz, 37°C) were evaluated in MR myocardium from the LV subepicardial free wall (MR-FW) and papillary muscle (MR-PM) and from the subepicardial free wall in coronary artery bypass graft patients with normal LV contraction patterns [nonfailing (NF)]. Ascending slope, optimal stimulation frequency, and maximal twitch tension of the FFR were depressed in MR-FW and MR-PM compared with NF (P < 0.05). FFR depression was greater in MR-PM than in MR-FW. Between 107 and 134 beats/min, twitch tension became weaker in MR-PM, whereas it increased in MR-FW. Elevation of intracellular CAMP with forskolin eliminated FFR depression in MR-FW but not in MR-PM. MR-PM also had a 35% lower myosin heavy chain content and slowed twitch kinetics. In MR patients, the echocardiographic end-diastolic LV shape (end-diastolic eccentricity index = long axis/short axis) correlated with the ratio of ascending FFR slopes such that the end-diastolic eccentricity index increased 10% per 15% increase in slope ratio (r = 0.88, P = 0.01). These regional differences in the frequency dependence of contractility between the free wall and papillary myocardium may contribute to changes in LV shape in MR as well as during exercise.

human myocardium; ventricular function; regional contractility

LEFT VENTRICULAR (LV) chamber shape is an important determinant of the ventricular wall stress needed to support a given chamber pressure. In dilated cardiomyopathies, structural remodeling results in marked chamber sphericalization. This increases wall stress and oxygen demand (2, 3, 7, 10), which may predispose to myocardial hypoxia and/or ischemia (11, 13). Besides structural remodeling, the LV shape changes dynamically. Chamber ellipticalization occurs during the course of each systole (31) and during the transition from resting to exercise conditions (32). Studies in dogs have attributed systolic ellipticalization to a “buttressing effect” on the chamber walls from radially directed components of force developed by the papillary muscles (PMs) (25). However, mechanisms of exercise-related ellipticalization of the LV shape have not been investigated. One possibility is that exercise-related ellipticalization results, in part, from tachycardia-induced changes in the dynamic balance of force development in the free wall (FW) compared with PM. A change in this balance would occur during tachycardia if there were regional differences in the slope of the myocardial force-frequency relation (FFR).

Although direct measurements of regional FFRs within the same heart have not previously been made, comparisons of studies we have published using PMs from patients with mitral regurgitation (MR) (18) with studies using FW myocardium from MR patients (22) suggested that such differences do exist. Furthermore, reports of a transmural gradient in the effect of tachycardia on action potential shape and duration (14) also suggest accompanying FFR differences. Exercise-related chamber ellipticalization diminishes or even reverses as exercise capacity decreases in patients with LV dysfunction and reduced ejection fraction (EF) (32), and prior studies reporting an absence of regional differences in the FFR in end-stage failing hearts (8, 20) may indicate that end-stage remodeling reduces or eliminates regional twitch and FFR differences. Because exercise-related LV chamber ellipticalization is present (although blunted) in less severely failing hearts [as in patients with MR (31)], we studied this question in myocardium from patients undergoing mitral valve surgery for chronic, nonischemic MR. To improve the resolution of differences in the twitch myogram and FFRs between FW and PM myocardium, we made direct FFR comparisons within each heart. The results show that significant regional differences in twitch myogram and FFRs exist in the LV of MR patients and could contribute to dynamic shape changes. An additional, preliminary correlative study suggests that these in vitro regional differences in FFR are also associated with differences in end-diastolic LV chamber shape in MR. A brief report of the present study has been previously presented (24).

MATERIALS AND METHODS

Myocardial biopsy and strip preparation. Subepicardial FW and PM tissue was obtained from 13 patients with New York Heart Association class II–III heart failure symptoms [7 men and 6 women, the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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age 55 ± 3 yr (means ± SE) due to chronic, severe MR who had no other significant valvular abnormalities. All patients underwent mitral valve replacement. None had significant (>50%) coronary stenoses based on coronary angiography. The EF ranged from 0.33 to 0.70 (mean 0.52 ± 0.04). Medications included digoxin (n = 5), verapamil (n = 1), isosorbide dinitrate (n = 1), captopril (n = 4), furosemide (n = 5), coumadin (n = 6), losinopril (n = 1), terfenadine (n = 1), and enalapril (n = 4). PM tissue was also obtained from four patients (65–73 years old) with rheumatic mitral stenosis (MS) and no other significant valvular abnormalities. Control (nonfailing [NF]) subepicardial FW tissue was obtained from five coronary artery bypass surgery patients [age 62 ± 4 yr, P = not significant (NS) vs. MR] with normal LV EF (0.72 ± 0.02, P = 0.01 vs. MR), normal regional wall motion, and no history of myocardial infarction, diabetes mellitus, or hypertension. Patients gave informed written consent before participating, as approved by the Committee on Human Research of The University of Vermont. There were no complications resulting from the biopsy procedure in any patient. Biopsies (~1.5 × 1.5 × 10 mm) were obtained during surgery after the establishment of cardioplectic arrest (21). BWF tissues were obtained from the anterior surface of the LV, at a site one-half to two-thirds of the way from the base to apex. PM biopsies were obtained from the region adjacent to the myotenodinous junction. All biopsies were immediately submerged in 2,3-butanedione monoxime (BDM) protective solution (19). Each biopsy was dissected into one to three thin strips (~0.2-mm diameter, 63 total) (20, 21).

Apparatus and measurements. Isometric twitch tensions were measured at the peak of the tension-length relation (Lmax) using an apparatus, methods, and protocols described previously (20). Steady-state FFRs were obtained at 37°C after 5 min of stimulation at each frequency (0.2–3.4 Hz). Peak twitch tension and timing parameters were measured by digital readout. Values from all FW or PM strips were averaged at each frequency for group comparisons. Optimum stimulation frequency (f0) for each strip was defined as the frequency at which maximal peak twitch tension occurred. Contractile reserve (R) was quantified as the slope of the ascending limb of the FFR by taking the ratio of twitch tension at 120 beats/min divided by twitch tension at 60 beats/min. R was averaged in FW and PM of each MR heart for paired comparisons. All measurements were subsequently repeated 45 min after the addition of forskolin to the muscle bath. Cross-sectional strip areas were calculated by dividing the blotted weight (0.46 ± 0.05 mg) of the active portion of each strip by its length at Lmax (3.45 ± 0.19 mm). Cross-sectional areas did not differ between groups. Strips were subsequently frozen by immersion in liquid nitrogen and stored at −71°C for later measurement of myosin heavy chain (MHC) content.

Myosin determination. Myosin content was determined by quantitative SDS-PAGE as follows (22). Frozen-thawed strip preparations were dehydrated in chloroform-methanol [2:1 (vol/vol)] at 0°C and vacuum dried. Each dried sample was flattened to a 30-μm-thick wafer by compression between the polished jaws of a micrometer caliper. Wafers were placed in gel dissociation buffer (62.6 mM Tris-base, 3% SDS, 20% glycerol, 6 mg/ml DTT; 1 μl/0.75 mg dry tissue wt) and extracted at 100°C for 5 min followed by 60 min at 22°C. After overnight storage (4°C), trace bromophenol blue and fresh DTT (2 μl of 250 mmol/l DTT per 100 μl sample) were added. The filtrate (Gelman Z-spin filter, 0.45-μm pore size) was incubated at 100°C (2 min), vortexed, cooled, and sedimented at 13,000 g. The supernatant was loaded (26 μg dry wt/lane) on 5% SDS-polyacrylamide gels (28) and stained overnight with 0.1% Coomassie blue in 25% isopropanol and 10% acetic acid and destained in 30% methanol-10% acetic acid. MHC content was quantified by densitometric gel scanning (PD I/SUN with "Quantity One" software) using skeletal muscle myosin as a standard. Myosin concentration in the standards was determined by absorbance (E280 = 5.0).

Solutions. All FFR measurements were made in Krebs-Ringer solution as described previously (19). BDM protective solution for dissection consisted of Krebs-Ringer solution plus 30 mmol/l BDM. Forskolin was dissolved in 95% ethanol and introduced into the muscle baths at a concentration of 0.5 μmol/l and <20 μmol/l ethanol. The latter did not produce any changes in the twitch response when introduced separately in preliminary experiments. BDM and forskolin were obtained from Sigma. Glass-distilled water was used for all solutions.

Echocardiography. Routine, clinically indicated preoperative twodimensional echocardiograms were used to derive a LV ellipticity index (EI) in seven of the MR patients. (In the remaining patients, either the preoperative echocardiogram was not thought to be technically adequate to assess LV shape or it was not available to us for analysis.) EI was calculated by taking the ratio of (long axis)/(short axis) at end diastole (EDEI) and end systole (ESEI) (32).

Statistical analysis. Peak twitch tension, f0, and R values (see RESULTS) were compared between NF and MR-FW and between MR-FW and MR-PM. Comparisons were made using ANOVA followed by a Duncan test at three stimulation frequencies corresponding to the mean optimum stimulation frequency in each of the three groups (see Table 1). Additional comparisons were made between NF and forskolin-treated MR myocardium (MR-FW + forskolin or MR-PM + forskolin). A paired t-test was used to compare twitch and FFR parameters (+forskolin) of MR-FW with MR-PM. The in vitro regional FFR was related to the in vivo LV chamber shape by performing a linear regression of the ratio of FW R (RFW) to PM R (RPM) values on ESEI and EDEI (see RESULTS). A P value of 0.05 or less was considered statistically significant. All values are expressed as means ± SE.

RESULTS

FFR in subepicardial FW myocardium from NF patients. Figure 1 shows the average FFR measured in 14 subepicardial strips prepared from the 5 NF hearts. Maximal peak isometric twitch tension (26.4 ± 1.20 mN/mm²) occurred at an f0 of

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Values are means ± SE. NF, nonfailing; MR, mitral regurgitation; FW, free wall; PM, papillary muscle. *P ≤ 0.05 compared with NF-FW; †P ≤ 0.05 compared with MR-FW (paired t-test).
170 ± 4.2 beats/min (2.83 ± 0.07 Hz). R averaged 1.84 ± 0.21 in NF myocardium (Table 1).

**FFR in subepicardial FW and PM from MR patients.** Figure 2 shows averaged FW and PM FFR curves obtained from MR myocardium. Data from 2 of 13 patients were excluded because their maximal peak twitch tensions were lower than the mean by more than 2SD. Average maximal tension in MR-FW strips was 11.3 ± 2.14 mN/mm², and f₀ was 134 ± 2.6 beats/min (see Table 1). This maximal tension was 44% lower (P = 0.05) than NF myocardium at 134 beats/min. At this same frequency, tension developed by MR-PM myocardium averaged 6.56 ± 1.58 mN/mm², 42% lower than MR-FW myocardium (paired t-test, P = 0.03). The difference in optimal frequency between NF and MR-FW was also significant (Table 1). Maximal peak tension in MR-PM occurred at f₀ = 107 ± 10 beats/min, significantly lower than in either MR-FW or NF myocardium (Table 1). At this frequency, the maximal tension in MR-PM averaged 6.91 ± 1.72 mN/mm², significantly lower than the average twitch tension in MR-FW at the same frequency (paired t-test, P = 0.02). Twitch tension in MR-PM declined as stimulation frequency increased from 107 to 134 beats/min, whereas it continued rising in MR-FW (Fig. 2). At 134 beats/min, MR-PM myocardium developed only 63 ± 13% as much twitch tension as that developed by MR-FW (paired t-test, P = 0.03). R was 1.26 ± 0.04 in MR-FW (P = 0.03 with respect to the NF average of 1.84 ± 0.21), whereas in MR-PM it was only 1.05 ± 0.12 (paired t-test, P = 0.05 with respect to MR-FW). This marked regional difference in the ascending limb of the FFR in MR myocardium is quantified in Fig. 3 as the ratio of twitch tensions in FW versus PM as a function of stimulation frequency. MR-FW twitch tension was 30% greater than MR-PM myocardium at 84 beats/min and 72% greater at 144 beats/min (P = 0.01).

**Time course of contraction and relaxation in FW and PM.** There were also differences in the time course of isometric twitches in MR compared with NF myocardium (Fig. 4). Time to peak twitch tension was 30 to 50 ms greater in MR-PM than NF-FW at all stimulation frequencies (P = 0.05). In contrast, time to peak FW tension was very similar in MR-FW and NF. Half-relaxation time in both MR-FW and MR-PM was 10 to 15 ms greater (P = 0.05) than in NF-FW myocardium at 120 and 180 beats/min (Fig. 5). This difference was similar in MR-FW at 60 beats/min but was even greater (30 to 40 ms) at 60 beats/min in MR-PM myocardium (P = 0.02).

**Effects of forskolin on FFR.** In MR-FW, 0.5 μmol/l forskolin increased maximal twitch tension 2.6 ± 0.3-fold (paired t-test, P = 0.003) and f₀ by 1.3 ± 0.04-fold (paired t-test, P = 0.001). The potentiated values of maximal twitch tension and f₀ were similar to those measured in NF myocardium in the absence of forskolin (Fig. 6 and Table 1). The potentiated value of R (1.6 ± 0.09) in MR-FW was not significantly different than the value in NF myocardium in the absence of forskolin (1.84 ± 0.21, P = 0.31). In MR-PM myocardium, forskolin increased maximal twitch tension 1.7 ± 0.2-fold (paired t-test, P = 0.04), to a value not significantly different than the tension developed by NF myocardium in the absence of forskolin at the same frequency (106 beats/min). However, unlike MR-FW, f₀ in MR-PM (106 ± 12.3 beats/min) remained 38% lower than in NF myocardium (P = 0.003) and R remained 40% below the NF value (P = 0.01; Table 1). Use of higher or lower

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**Fig. 1.** Average steady-state isometric twitch tension (means ± SE) versus stimulation frequency [in beats/min (bpm)] in myocardium from the left ventricular (LV) freewall of 5 nonfailing (NF) hearts. Data from 2 or 3 strips from each heart were averaged before averaging across hearts.

**Fig. 2.** Average steady-state isometric twitch tension (means ± SE) versus stimulation frequency in free wall and papillary muscle myocardium from 11 patients with mitral regurgitation (MR). Data from 1 to 3 strips from each region of each heart were averaged before averaging across all hearts.

**Fig. 3.** Stimulus frequency dependence of the ratio of free wall to papillary muscle twitch tension in MR myocardium. Average values were calculated from the same strips as in Fig. 2.
forskolin concentrations did not improve R or \( f_o \) in MR-PM, nor was there improvement when membrane permeant dibutyryl-cAMP (DB-cAMP; 90 µmol/l, 45-min equilibration) was used to potentiate twitch tension. We did not quantitate the effects of forskolin on NF myocardium because at 0.5 µmol/l it caused the ascending limb of the FFR to prematurely rise to its maximum at a very low stimulation frequency (60 beats/min) with no further rise and sometimes a fall in twitch tension above 60 beats/min.

Forskolin shortened time to peak twitch tension by 23–27% at all frequencies in both MR-FW and MR-PM, but it remained longer in MR-PM than in MR-FW (by 24 ± 1%, \( P = 0.001 \)). Forskolin reduced the half-relaxation time by 36±2% in MR-FW and by 27±2% in MR-PM at all frequencies. Values with forskolin were not significantly different from each other, and they were 15–30% shorter than in untreated NF myocardium [and see Mulieri et al. (23)].

Myosin content in FW and PM. Because maximal twitch tension in forskolin-potentiated MR-PM strips (i.e., at 107 beats/min) remained 55% below the maximal value in NF strips (Table 1; 170 beats/min), we tested for reduced myosin concentration in PMs. The myosin concentration was 39.5±5.2 nM/g (blotted weight) in MR-PM. This was 33–38% lower than in MR-FW (63.7±2.96 nmol/g) or NF-FW myocardium (59.3±2.65 nmol/g, \( P = 0.005 \)). In PM from patients with MS, myosin concentration (51.5±9.2 nmol/g, \( n = 4 \)) also tended to be lower than NF or MR-FW values, suggesting that myosin concentration may normally be lower in PM than in FW independent of disease.

Relation of regional FFR to LV shape. The slopes of the linear regressions of \( R_{FW}/R_{PM} \) on EDEI and ESEI were virtually identical (0.66±0.16 and 0.67±0.34, respectively), but only the EDEI correlation was significant [EDEI = 0.66(R_{FW}/R_{PM}) + 0.76, \( r = 0.88, P = 0.01 \); Fig. 7].

DISCUSSION

Our results demonstrate the novel finding that both the slope of the ascending limb of the FFR and its optimum stimulation frequency differ between LV FW and PM in patients with MR. Thus there was a 20% less increase in twitch tension in PM than in FW as contraction frequency increased between 60 and 120 beats/min (Table 1) and \( f_o \) in PM was 27 beats/min lower than in FW (Table 1). We also found that MR-PM is refractory to the FFR restoration response to forskolin seen in MR-FW. Consistent with our previous report (22), the slope of the ascending limb and \( f_o \) of the FFR were depressed in MR-FW compared with NF-FW myocardium. Although we found that the time course of contraction and relaxation was slower in MR compared with NF myocardium, these differences were not markedly frequency dependent. Most significantly in regard to the possibility that changes in heart rate may cause changes in LV shape because of frequency-dependent changes in regional contractility, we found that PM twitch tension falls, whereas FW tension continues to rise, in the physiological range of 107–134 beats/min.
Intracellular contributors to regional differences in twitch tension and FFR in MR myocardium. In MR-PM, maximal twitch tension was 39% lower than in MR-FW (paired t-test, \( P = 0.03 \)), and it remained 55% below the NF value after maximal forskolin potentiation. To test whether the 35% lower myosin concentration in MR-PM contributes to this deficit in twitch tension, we expressed maximal twitch force in each preparation as the average force generated per myosin cross-bridge head using the following formulas:

\[
F_{CB} = F_{tw}/\#MHC
\]

\[
\#MHC = (W_{hs})/([MHC])(A)
\]

\[
W_{hs} = (W_{strip})(L_{hs}/L_{strip})
\]

where \( F_{CB} \) is the average force per myosin molecule (in pN), \( F_{tw} \) is the maximal twitch force in the muscle strip (in N), \( \#MHC \) is the number of MHC molecules in a half-sarcomere, \( W_{hs} \) is the blotted weight of a half-sarcomere (in g), \([MHC]\) is the MHC concentration (in nmol/g blotted weight), \( A \) is Avogadro’s number, \( W_{strip} \) is the blotted weight of muscle strip preparation (in g), \( L_{hs} \) is the length of one half-sarcomere at \( L_{aux} \) (1.15 \( \mu \)m), and \( L_{strip} \) is the length of the muscle strip preparation (in m) at the peak of the tension-length relation. This calculation yields an average maximal cross-bridge force at the peak of the twitch of \( F_{CB} = 0.59 \pm 0.18 \) pN/MHC in MR-PM and \( 0.43 \pm 0.09 \) pN/MHC in MR-FW (Fig. 8). These values are not significantly different from each other or from the value of \( 0.78 \pm 0.06 \) pN/MHC in NF myocardium. (Note that because \( F_{CB} \) is calculated assuming all cross-bridges are recruited at peak isometric twitch tension, maximal force per cross-bridge may be underestimated by as much as 50–70%).

With maximal twitch potentiation by forskolin, force per MHC in MR-FW (0.97 \( \pm \) 0.10 pN/MHC) and MR-PM (0.94 \( \pm \) 0.20 pN/MHC) approximately doubled, to values similar to those in nonpotentiated NF (Fig. 8). This suggests that the deficits in maximal twitch tension in the absence of forskolin potentiation in MR myocardium (24% in PM and 46% in FW; Fig. 8) result from reduced activation and is consistent with our previous myothermal studies showing a 45% depression in the amount of \( \text{Ca}^{2+} \) cycled per twitch in MR myocardium (9). In MS-PM myocardium, although MHC content tended to be lower than in NF, neither twitch tension per cross-sectional area (30.1 \( \pm \) 8.22 mN/mm\(^2\)) nor calculated maximal twitch force per myosin heavy chain (0.81 \( \pm \) 0.18 pN/MHC) were different than in NF. This suggests that the reduced twitch activation in MR-PM is related to MR rather than to normal regional differences per se.

Besides depression of frequency dependence of twitch activation in PM and FW myocardium in MR hearts, other aspects of excitation-contraction coupling also differed from NF myocardium. The prolongation of the isometric twitch in MR myocardium (Figs. 4 and 5) is likely caused by prolongation of the action potential, as observed in heart failure due to idiopathic dilated cardiomyopathy (1, 5). Greater prolongation of the twitch in MR-PM compared with MR-FW may be due to the normal epicardial-endocardial gradient of action potential duration (14). Diminution in half-relaxation time with increasing frequency is greater in PM (26% between 60 and 120 beats/min) than in either NF or MR-FW myocardium (15% and 13%, respectively). This difference is similar to epicardial-endocardial differences in frequency-dependent abbreviation of the action potential (25% and 13% reduction in action potential duration at 50% repolarization, respectively) observed in tissue from explanted, failing hearts (14). It is possible that prolongation of the action potential also results in the lower values of \( R \) and \( f_0 \) in MR-PM compared with MR-FW. Action potential prolongation would cause the frequency-dependent potentiation of L-type sarcolemmal calcium current to saturate at a lower than normal stimulation frequency (26, 27). This could also account for the inability of cAMP elevation by forskolin or DB-cAMP to raise \( R \) and \( f_0 \) in MR-PM myocardium (Table 1).

Possible influence of regional differences in FFR on ventricular shape. Between contraction frequencies of 80 and 145 beats/min, contractile tension in MR-FW myocardium increases 30% more than it does in MR-PM myocardium (Fig. 3). This includes the region between 107 and 134 beats/min.
where contractile force actually weakens in PM as it simultaneously increases in FW myocardium (Fig. 2). These findings suggest that significant changes in the balance of contractile forces between FW and PM myocardium occur in vivo as heart rate increases over this physiological range. Although the determinants of the shape of the LV are exceedingly complex, differences in the frequency dependence of contractility of one region compared with another could contribute to exercise-associated changes in LV shape. Specifically, based on our FFR results, we hypothesize that changes in the balance of contractile forces between FW and PM contribute to dynamic control of the LV systolic shape because of geometric factors (Fig. 9). The force axes of the PMs are oriented predominantly along the major (long) axis of the LV chamber. Hence, PM contraction tends to shorten the major axis and lengthen the minor (short) axis (29), i.e., it has a sphericizing effect during systole.1 Conversely, FW contractile force is predominantly directed circumferentially causing the minor axis to shorten and the major axis to lengthen (15). This produces a more elliptical chamber shape during systole. Because of the opposite effect of PM and FW contraction on LV shape, we expect tachycardia-induced strengthening of FW contractile force relative to PM contractile force to result in ellipticalization of the LV chamber during systole. Hearts in which there is a greater disparity in the slope of the FFR relation in the FW compared with PM, i.e., a larger \( \frac{R_{FW}}{R_{PM}} \), would be expected to have greater increases in chamber ellipticity with tachycardia and/or exercise and therefore greater ellipticity at any heart rate in the 60- to 120-beats/min range.

The significant correlation we found between EDEI and \( \frac{R_{FW}}{R_{PM}} \) suggests the novel possibility that there is also a connection between regional variation in the FFR and a "static," end-diastolic measure of LV shape that reflects the remodeling that occurs during chronic volume overload. The correlation indicates that for each 15% increase in slope of the FW FFR above the slope of the PM FFR, there is an accompanying 10% increase in LV chamber EDEI. Although the correlation between ESEI and \( \frac{R_{FW}}{R_{PM}} \) did not reach statistical significance at \( P < 0.05 \), its slope was virtually identical to that between EDEI and \( \frac{R_{FW}}{R_{PM}} \). The similarity of the slopes suggests that \( \frac{R_{FW}}{R_{PM}} \) is also a determinant of systolic shape in MR. [We suspect that the ESEI correlation (\( P = 0.10 \)) did not reach significance because of a larger potential for errors in the echocardiographic long- and short-axis measurements at end systole compared with end diastole due to interference from contracting trabeculae, which tends to impair visualization of the endocardial surface.] Further studies in a larger and more diverse patient population are needed to confirm these preliminary findings.

**Study limitations.** We did not measure PM FFRs in NF myocardium. Hence, we cannot be certain if our regional FFR results and their implications for LV shape are applicable to normal hearts. However, as noted earlier, MR hearts do retain the exercise-associated ellipticalization observed in normal hearts. It seems unlikely that the mechanism of this shape change would be qualitatively different in MR and normal hearts.

Our FW biopsies were obtained from the subepicardium, whose fibers are oriented at ~45° to the direction of the LV long axis (30). For these data to be applicable to the mechanism proposed whereby regional FFR variation determines dynamic LV shape, we must assume that the subepicardial FFR is similar to that in the more circumferentially oriented midwall fibers that are most responsible for decreases in the minor axis during contraction. Moreover, to more directly test whether there is a relation between regional FFR variation and dynamic LV shape, we would like to have correlated \( \frac{R_{FW}}{R_{PM}} \) with changes in shape as a function of heart rate, for example, from rest to exercise. Unfortunately, echocardiograms were only available under basal conditions.

Finally, our in vitro studies in myocardial strips did not include the augmentation of the FFR produced by concomitant increases in adrenergic stimulation that occur as heart rate increases during exercise (12). It is possible that in vivo such augmentation could add an additional component to LV shape modulation and that this component might change with disease. However, we believe this is likely to be a relatively modest effect. In studies in dogs, adrenergic stimulation independent of increases in heart rate accounted for only about one-third of the augmentation in contractility occurring during

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1 Observations of ventricular sphericalization after complete section of PM chordae tendinae (21) are more likely related to the passive rather than active properties of the PMs. Diastolic "buttressing" of the FW by the radially directed, passive stiffness of the PMs is lost with chordal section. This causes the LV to take on a more spherical shape due to Laplace law effects during build up of LV pressure.
exercise (16). Moreover, studies of ventricular function responses to increased heart rate (atrial pacing) in normal human subjects (4, 6) reveal augmentation in contractility that is quantitatively very similar to what we observe in vitro over comparable stimulation frequencies.

In conclusion, our results demonstrate the presence of regional differences in the heart rate dependence of contractility in LV tissue obtained from human hearts with chronic MR. Although there are numerous studies indicating that integrity of the PMs and chordae tendineae play an important role in determining LV shape in general, the present study provides the first evidence that differences in the heart rate dependence of contractile strength of the PMs compared with FWs may underlie the dynamic changes in LV shape that occur with exercise. Measurements of myosin concentration and the degree of twitch potentiation by forskolin suggest that contractility in both FW and PM myocardium is depressed in MR due to reduced activation of contractile proteins. The lower myosin concentration is a further cause of tension deficit in PM myocardium. Finally, regional FFR variation may also have a relationship with diastolic shape changes associated with remodeling during chronic MR.

**ACKNOWLEDGMENTS**

We thank Richard R. Lachapelle for design, construction, and maintenance of apparatus used in this study.

**GRANTS**

This study was supported by National Heart, Lung, and Blood Institute Grants R01-HL-55641 (to N. R. Alpert) and R01-HL-61556 (to M. M. LeWinter).

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