Mechanisms of cardiac hypertrophy in canine volume overload

TAKESHI MATSUO, BLASE A. CARABELLO, YOSHITATSU NAGATOMO, MASAAKI KOIDE, MASAYOSHI HAMAWAKI, MICHAEL R. ZILE, AND PAUL J. McDERMOTT
Departments of Medicine, Physiology, and Cell Biology and Anatomy, Gazes Cardiac Research Institute, and Veterans Affairs Affairs Medical Center, Charleston, South Carolina 29403

Matsuo, Takeshi, Blase A. Carabello, Yoshitatsu NagATOMO, Masaaki Koide, Masayoshi Hamawaki, Michael R. Zile, and Paul J. McDermott. Mechanisms of cardiac hypertrophy in canine volume overload. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H65–H74, 1998.—This study tested whether the modest hypertrophy that develops in dogs in response to mitral regurgitation is due to a relatively small change in the rate of protein synthesis or, alternatively, is due to a decreased rate of protein degradation. After 3 mo of severe experimental mitral regurgitation, the left ventricular (LV) mass-to-body weight ratio increased by 23% compared with baseline values. This increase in LV mass occurred with a small, but not statistically significant, increase in the fractional rate of myosin heavy chain (MHC) synthesis (Ks), as measured using continuous infusion with [3H]leucine in dogs at 2 wk, 4 wk, and 3 mo after creation of severe mitral regurgitation. Translational efficiency was unaffected by mitral regurgitation as measured by the distribution of MHC mRNA in polysome gradients. Furthermore, there was no detectable increase in translational capacity as measured by either total RNA content or the rate of ribosome formation. These data indicate that translational mechanisms that accelerate the rate of cardiac protein synthesis are not responsive to the stimulus of mitral regurgitation. Most of the growth after mitral regurgitation was accounted for by a decrease in the fractional rate of protein degradation, calculated by subtracting fractional rates of protein accumulation at each time point from the corresponding Ks values. We conclude that 1) volume overload produced by severe mitral regurgitation does not trigger substantial increases in the rate of protein synthesis and 2) the modest increase in LV mass results primarily from a decrease in the rate of protein degradation.

heart; myosin; mitral regurgitation; protein synthesis

MYOCARDIAL HYPERTROPHY is one of the primary mechanisms by which the heart compensates for a hemodynamic overload. In pressure overload, it is postulated that systolic stress triggers the production of new sarcomeres assembled in parallel, thereby increasing wall thickness (concentric hypertrophy) (11). Because wall stress = (pressure × radius)/(2 × thickness), an increase in thickness in the denominator offsets the increase in pressure in the numerator, maintaining normal systolic stress (afterload). Because afterload is a key determinant of ejection performance, maintenance of normal afterload helps to maintain normal ejection and cardiac output (13). In the volume overload of valvular regurgitation, it is postulated that an increase in diastolic stress causes replication of sarcomeres in series, thereby increasing myocyte length. In this form of compensation, increased myocyte length leads to increased chamber size (eccentric hypertrophy), allowing the ventricle to increase its total stroke volume to compensate for the volume that is regurgitated.

In many types of volume overload, the excess volume is delivered into the aorta, where the increased stroke volume increases pulse pressure and systolic pressure. In this situation, volume overload actually coexists with pressure overload. For example, in aortic regurgitation, aortic systolic pressure is increased, in turn increasing systolic wall stress (30). This form of combined pressure and volume overload results in both concentric and eccentric hypertrophy (10). In distinction, mitral regurgitation, in which the excess volume is ejected into the relatively low-pressure zone of the left atrium, constitutes a relatively “pure” volume overload, in which systolic wall stress is not increased (30). This type of volume overload produces only eccentric hypertrophy (4) and thus is well-suited for studying the mechanisms that cause eccentric as opposed to combined eccentric and concentric hypertrophy.

Comparison of hemodynamic factors that regulate myocardial mass between pressure and volume overload is difficult because these overloads are hemodynamically so dissimilar. However, we recently compared pressure and volume overload matched for a common parameter, stroke work, with regard to the extent of hypertrophy that developed (7). When animals with severe mitral regurgitation were matched by stroke work to those with severe aortic stenosis, the amount of left ventricular (LV) hypertrophy was nearly twice as much in the pressure-overload model as in the volume-overload model. A likely reason for this difference in hypertrophic response was found in a study in which we compared changes in the fractional rate of myosin heavy chain (MHC) synthesis (MHC Ks) in response to acute aortic stenosis vs. acute mitral regurgitation (14). There was an early increase in MHC Ks in the pressure-overload model but no increase in the volume-overload model. We hypothesized that the wall stress imposed by volume overload is not a very potent stimulus for accelerating the fractional rate of protein synthesis, particularly compared with the stimulus of pressure overload. To test this hypothesis further, we measured whether there were any changes in the mechanisms that regulate the rate of protein synthesis during the development of volume-overload hypertrophy produced in a canine model of mitral regurgitation. MHC Ks was used as a cardiocyte-specific marker for monitoring changes in protein synthesis in the myocardium. The alternative possibility was also examined, namely that the hypertrophy that does eventually
occur in this model of mitral regurgitation results from a decrease in the fractional rate of protein degradation.

**MATERIALS AND METHODS**

**Experimental Canine Preparation**

All procedures were done in accordance with institutional guidelines.

Control group. Nine adult mongrel dogs were used as controls. These dogs were matched for age and sex with the mitral regurgitation (MR) dogs. Anesthesia was induced with an infusion of 0.15 mg·kg⁻¹·min⁻¹ sufentanil supplemented by isoflurane and was maintained by a constant infusion of sufentanil and a low dose of isoflurane by inhalation. A thermolodiated Swan-Ganz catheter was inserted via the femoral vein for measuring pulmonary capillary wedge pressure (PCWP), pulmonary artery pressure, and cardiac output. A second catheter was placed in the femoral artery for measuring arterial blood pressure and for blood sampling.

MR group. MR was produced in dogs as described before (5, 6, 18). The dogs were divided into three groups according to the duration of MR as follows: 2 wk (n = 7), 4 wk (n = 5), and 3 mo (n = 6). Anesthesia was induced and maintained in a manner identical to that in the control group. An 8-Fr 30-cm sheath was placed in the right carotid artery and advanced to the LV. A pigtail catheter placed through this sheath was used for measuring LV pressure and for performing contrast left ventriculography. The femoral vein was instrumented with a Swan-Ganz catheter. After baseline measurements of hemodynamic conditions were established, the pigtail catheter was removed and replaced by a grasping forceps (5). The mitral valve chordae were grasped and severed, resulting in MR. After MR was produced, total stroke volume (SV), regurgitant fraction (RF), and LV mass were quantified. When the hemodynamic conditions were stable, continuous infusion in vivo with radiolabeled leucine was done over 6 h. A solution of [3H]leucine was prepared by mixing 20 mCi [3H]leucine (1Ci/mmol, NEN Research Products, Boston, MA) with 0.1 mM unlabeled leucine in 0.9% (wt/vol) NaCl, pH 7.4. The solution was continuously infused at a constant rate of 0.194 ml/min via the femoral vein. Blood samples were withdrawn from the ascending aorta at regular intervals during the infusion in order to measure plasma leucine specific radioactivity. On completion of the infusion period, the hearts were rapidly removed while the [3H]leucine infusion continued. The hearts were immediately rinsed in ice-cold saline, and the great vessels were perfused retrogradely with ice-cold saline. For measurements of the rate of ribosome formation and the distribution of MHC mRNA in polyosomes, portions of the LV free wall were minced and homogenized immediately. The remaining heart tissue was frozen in liquid nitrogen and stored. Samples of frozen LV free wall were used to measure the rate of MHC synthesis, MHC mRNA levels, MHC content, and total RNA content.

**Determination of Plasma Leucine Specific Radioactivity**

Blood samples were centrifuged at 1,500 × g for 15 min at 4°C. Concentrated perchloric acid (PCA) was added to the plasma to a final concentration of 6%, and the plasma proteins were pelleted by centrifugation at 1,500 × g for 15 min. The supernatant was neutralized by adding 10 N KOH and centrifuged, and a 100-µl aliquot of the supernatant was dried by vacuum centrifugation. The dried sample was resuspended in 100 µl of 0.1 mol/l NaHCO3-Na2CO3 buffer (pH 9.5), reacted with an equal volume of 5 mmol/l [methyl-14C]dansyl chloride (110–120 mCi/mmol, American Radiolabeled Chemicals, St. Louis, MO), and incubated at 37°C for 1 h (1, 2). The dansylated amino acids were purified by two-dimensional thin-layer chromatography on micropolyamide plates obtained from Schleicher & Schuell (Keene, NH). The solvent for the first dimension consisted of 2% formic acid, and the second dimension was 90% benzene-10% glacial acetic acid. The dansyl-leucine spot was excised and eluted by incubation in Solvable (NEN Research Products). The H-to-14C ratio of the dansyl-leucine spot was measured by liquid scintillation counting. For each assay, a standard curve of leucine specific radioactivity was made, and plasma leucine specific radioactivities were extrapolated from the standard curve.

**Determination of MHC Synthesis Rates**

MHC Km was determined by the continuous infusion method using the following formula

\[ K_m = \frac{P}{\int F \cdot dt} \]

where P is the specific radioactivity of leucine in MHC, and \( \int F \cdot dt \) is the area beneath the plasma leucine specific radioactivity time curve (1). To determine the specific radioactivity of leucine in MHC, we homogenized ~1-g samples of LV free wall, including the epicardium and endocardium, by means of a Polytron in 20 ml of MHC extraction buffer [1.1 mol/l K1, 0.1 mol/l K2HPO4, pH 7.4, 1 mmol/l dithiothreitol (DTT), and 1.5 mmol/l phenylmethylsulfonyl fluoride (PMSF)]. The homogenate was stirred for 1 h at 4°C and centrifuged at 10,000 × g for 15 min. The supernatant was diluted with 10 vol of H2O and allowed to settle for 2 h at 4°C. After centrifugation at
10,000 g for 15 min at 4°C, the pellet was dissolved in MHC sample buffer [0.2% (wt/vol) sodium dodecyl sulfate (SDS), 10% glycerol, 5% β-mercaptoethanol, 0.5 mol/l Tris, pH 6.8, 1.5 mmol/l \( \text{PMSE} \)]. The lysate was boiled for 5 min, pyronin Y was added to a final concentration of 0.01%, and the samples were electrophoresed for 20 h on 10–15% polyacrylamide-N, N’-diallyltartardiamide gradient gels. The gel was stained at room temperature for 1 h in 0.03% Coomassie brilliant blue R-250, 50% methanol, and 7% glacial acetic acid. The MHC band was solubilized in 2% periodic acid and 4% lactic acid. The protein was precipitated by adding PCA to a final concentration of 6% and centrifuged for 15 min at 1,500 g. The pellet was washed in 15% HCl to remove residual PCA. The pellet was dried by vacuum centrifugation and transferred to a sealable glass vial to which 1.2 ml of boiling 6 N HCl was added. Ambient air was flushed with \( \text{N}_2 \), and the vial was flame-sealed under vacuum. The vial was incubated for 24 h at 110°C to hydrolyze the protein into amino acids. The contents of the vial were removed, dried by vacuum centrifugation, and dissolved in 0.1 mol/l \( \text{NaHCO}_3\)-\( \text{Na}_2\text{CO}_3 \) buffer, pH 9.5. The amino acids were reacted with 5 mmol/l \( \text{[14C]} \) dansyl chloride and purified by two-dimensional thin-layer chromatography as described above. The amount of dansyl-leucine was determined by the isotope dilution method as modified by Samarel et al. (26). The corresponding specific radioactivity of leucine was measured by liquid scintillation counting, and the specific radioactivity of leucine in the MHC pool (dpm/nmol) was calculated, where dpm is disintegrations per minute.

**Measurement of the Rate of Ribosome Formation**

Samples of LV free wall (~100 mg) were homogenized by means of a Polytron in 2 ml of MHC extraction buffer. The homogenate was stirred for 1 h at 4°C and centrifuged at 10,000 g for 15 min. The pellet was extracted twice more, and the supernatants were combined and diluted with 10 vol of \( \text{H}_2\text{O} \). The protein extract was precipitated for 2 h at 4°C and then centrifuged at 10,000 g for 15 min. The precipitated protein was dissolved in MHC sample buffer and boiled for 5 min, and equivalent amounts of each sample were subjected to SDS-PAGE. In addition, equal amounts of the residual, nonextractable protein were also run on the gels to account for the total MHC pool. The gels were stained for 2 h in 0.03% Coomassie brilliant blue R-250, 50% methanol, and 7% glacial acetic acid and destained in 30% methanol and 10% glacial acetic acid. The optical density of the stained bands was determined by computer-assisted digital image analysis. MHC was quantified by extrapolation from a standard curve using bovine serum albumin as standard. The filters were incubated in Solvable overnight, and the radioactivity was measured by liquid scintillation counting. The portion of the gradient containing the 60S ribosomal subunits resolved as one peak, which coincided with the peak of radioactivity. Consistent with the findings of Camacho et al. (3), 40S ribosomes resolved as two peaks on the gradients, and the radioactivity peak did not coincide with the 40S protein peak. Rates of ribosome formation rates were calculated using the following formula

\[
\frac{\text{(radioactivity of 60S peak} - \text{background radioactivity)}}{\text{(protein content of 60S peak} - \text{background protein content)}} \times \left( \int F(t) \, dt \right)^{-1}
\]

where radioactivity is in dpm and protein content is in micrograms. Background radioactivity and background protein content were determined by extrapolation of baseline radioactivity and protein content immediately before and after the 60S peak.

**Measurement of Total RNA Content and Total Protein Content**

Cardiac tissue was homogenized in 6% PCA and centrifuged for 10 min at 10,000 g. The pellet was washed three times with 6% PCA and hydrolyzed in 0.3 N \( \text{NaOH} \) at 37°C for 2 h. Protein and DNA were precipitated by adding PCA to a final concentration of 1 N, incubating on ice for 15 min, and centrifuging at 10,000 g for 10 min. The supernatant was removed, and total RNA content was determined spectrophotometrically at a wavelength of 260 nm as before (28). For measuring total cardiac protein (TCP) content, the residual pellet was washed three times with 0.2 N PCA, resuspended with 0.3 N \( \text{NaOH} \), and incubated at 37°C overnight. The concentration of TCP was measured by the bicinchoninic acid method (Pierce, Rockford, IL).

**Distribution of MHC mRNA in Polysomes**

Cardiac polysomes were prepared as described before with modifications (16). Fresh samples of LV and RV free wall (0.5–0.8 g) were minced and homogenized with a Polytron in 9 ml of resuspension buffer [10 mmol/l Tris, pH 7.5, 250 \( \text{MgCl}_2 \), and 1 EGTA. After centrifugation at 50,000 rpm for 2 h, the supernatant was aspirated and the walls of the tube were washed with

\[
\text{H}_2\text{O}. \text{The pellet was resuspended in 10 mmol/l Tris, pH 7.4, to which 0.5% Brij-58 and 0.5% deoxycholate were added. The ribosomes were aggregated by adding \( \text{MgCl}_2 \) to a final concentration of 70 mmol/l. The ribosomes were pelleted by centrifugation at 10,000 g for 15 min and resuspended in a buffer containing (in mmol/l) 10 Tris, pH 7.2, 200 KCl, and 3 \( \text{MgCl}_2 \). DTF and puromycin were added to yield concentrations of 2 and 0.3 mmol/l, respectively, and the material was centrifuged at 10,000 g for 15 min. The supernatant was layered over a 15–68% exponential sucrose gradient containing 10 mmol/l Tris, pH 7.4, 0.5 mol/l KCl, and 3 mmol/l \( \text{MgCl}_2 \). Gradients were centrifuged at 32,000 rpm for 16 h in an SW41 rotor and fractionated by upward displacement. Each individual 0.6-ml fraction was diluted with \( \text{H}_2\text{O} \), and the optical density was determined at 260 nm. Protein content was measured in each fraction by the method of Schaffner and Weissmann (27). The gradient fractions were precipitated with 50% trichloroacetic acid in the presence of SDS, filtered through a 0.8-µm Millipore membrane (Bedford, MA), and stained with napthol blue black. The dye was eluted in a solution containing 50% ethanol, 25 mmol/l \( \text{NaOH} \), and 0.05 mmol/l EDTA, and its absorbance was determined at 630 nm. The protein concentration was extrapolated from a standard curve using bovine serum albumin as standard. The filters were incubated in Solvable overnight, and the radioactivity was measured by liquid scintillation counting. The portion of the gradient containing the 60S ribosomal subunits resolved as one peak, which coincided with the peak of radioactivity. Consistent with the findings of Camacho et al. (3), 40S ribosomes resolved as two peaks on the gradients, and the radioactivity peak did not coincide with the 40S protein peak. Rates of ribosome formation rates were calculated using the following formula

\[
\frac{\text{(radioactivity of 60S peak} - \text{background radioactivity)}}{\text{(protein content of 60S peak} - \text{background protein content)}} \times \left( \int F(t) \, dt \right)^{-1}
\]
mmol/l KCl, 2 mmol/l MgCl₂, 0.5% (vol/vol) Triton X-100, 2 mmol/l DTT, 100 µg/ml cycloheximide, and 2 U/ml RNasin (Promega, Madison, WI)]. The material was homogenized further with 12 strokes in a chilled Dounce homogenizer. The homogenate was vortexed and incubated on ice for 15 min. Nine-hundred microliters of a solution containing 10% (vol/ vol) Tween 80–5% (wt/vol) deoxycholate were added, and the homogenates were vortexed and then centrifuged for 10 min at 10,000 g. Aliquots of the postmitochondrial supernatant were layered on 15–50% linear sucrose gradients containing 10 mmol/l Tris, pH 7.5, 250 mmol/l KCl, 10 mmol/l MgCl₂, and 2 U/ml RNasin and were centrifuged for 95 min at 35,000 rpm in an SW41 rotor. The gradients were fractionated by upward passes a highly conserved region of mammalian rRNA signal was processed for autoradiography and measured by digital image analysis.

**RESULTS**

H68 MECHANISMS OF CARDIAC HYPERTROPHY IN CANINE VOLUME OVERLOAD

**Table 1.** Hemodynamics of experimental MR

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 9)</th>
<th>2-wk MR (n = 7)</th>
<th>4-wk MR (n = 5)</th>
<th>3-mo MR (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate,</td>
<td>87.4 ± 0.4</td>
<td>91.0 ± 0.4</td>
<td>92.9 ± 10.3</td>
<td>93.3 ± 5.5</td>
</tr>
<tr>
<td>beats/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP,</td>
<td>121.6 ± 4.1</td>
<td>98.4 ± 4.4*</td>
<td>101.7 ± 5.5*</td>
<td>110.1 ± 4.6*</td>
</tr>
<tr>
<td>mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic BP,</td>
<td>86.7 ± 2.6</td>
<td>67.1 ± 5.4*</td>
<td>70.6 ± 2.4*</td>
<td>79.2 ± 5.8</td>
</tr>
<tr>
<td>mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic Ppa,</td>
<td>18.1 ± 0.7</td>
<td>24.7 ± 12.2</td>
<td>25.6 ± 5.3</td>
<td>20.1 ± 1.5</td>
</tr>
<tr>
<td>mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pcw, mmHg</td>
<td>6.8 ± 0.4</td>
<td>14.7 ± 1.1*</td>
<td>13.7 ± 2.4*</td>
<td>12.0 ± 1.1*</td>
</tr>
<tr>
<td>Total SV, ml/min</td>
<td>43.2 ± 1.7</td>
<td>74.2 ± 5.4*</td>
<td>70.0 ± 7.5*</td>
<td>68.1 ± 6.5*</td>
</tr>
<tr>
<td>Stroke work, g·m</td>
<td>55.8 ± 2.6</td>
<td>68.3 ± 9.6</td>
<td>77.1 ± 9.8</td>
<td>75.6 ± 9.4</td>
</tr>
<tr>
<td>RF, %</td>
<td>63.5 ± 4.0*</td>
<td>57.2 ± 5.1*</td>
<td>56.7 ± 5.2*</td>
<td></td>
</tr>
<tr>
<td>LVEDV, ml</td>
<td>67.4 ± 3.8</td>
<td>92.9 ± 7.9†</td>
<td>89.9 ± 6.2†</td>
<td>101.6 ± 6.1†</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>56.4 ± 2.7</td>
<td>63.2 ± 4.4</td>
<td>61.0 ± 4.3</td>
<td>63.7 ± 3.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of dogs. MR, mitral regurgitation; BP, blood pressure; Ppa, pulmonary artery pressure; Pcw, pulmonary capillary wedge pressure; SV, stroke volume; RF, regurgitant fraction; LVEDV, left ventricular (LV) end-diastolic volume; LVEF, LV ejection fraction. Controls for LVEDV and LVEF are means ± SE of baseline values for all of the MR dogs. *P < 0.05 vs. control group; †P < 0.05 vs. baseline value of all MR dogs.

Changes in LV mass were determined in the MR dogs by ventriculography (Fig. 1). The baseline value for LV mass was measured in each dog before the imposition of MR. There was a progressive increase in LV mass compared with the corresponding baseline values that reached 27% after 3 mo of chronic MR (Fig. 1A). These data indicated that cardiac hypertrophy occurred in response to MR. The data in Fig. 1B show parallel increases in the LV mass-to-body weight ratio. These results confirm that the increase in LV mass was attributable to compensatory cardiac hypertrophy.

In Fig. 2, echocardiography was used to measure LV growth as a function of time in a different set of MR dogs. Changes in LV mass were measured in nine dogs at weekly intervals over 3 mo of chronic MR, and the...
best-fit nonlinear function for growth is shown in Fig. 2. This function was then used to fit the data points for LV mass at baseline (time 0) and at 2 wk, 4 wk, and 3 mo of MR that are shown in Fig. 1A. The baseline was derived from the mean of all of the baseline measurements for LV mass in the MR dogs. These data points for LV mass (y) were fit along the curve as described by the function

\[ y = 24.53(1 - e^{-0.063x}) + 90.58 \]

where \( x \) is the time of experimental MR (\( R^2 = 0.92 \)).

Effect of MR on MHC Accumulation

For calculation of the fractional rate of protein accumulation (\( K_g \)), the first derivative of the function above describing changes in LV mass was determined at each experimental time point of MR and divided by the corresponding value for LV mass. These \( K_g \) values derived from LV mass were substituted for MHC \( K_g \) values (Table 2). This substitution was validated by the fact that there were no significant differences in MHC content in the controls or any of the MR dogs, thereby confirming that MHC as a fraction of total LV protein remained constant (Table 2). However, MHC did accumulate during hypertrophy when the increase in total LV protein was taken into account. In Table 2, the rate of MHC accumulation (mg g^{-1} LV^{-1} \cdot day^{-1}) was calculated by multiplying the \( K_g \) values (%/day) at each time point of MR by the corresponding values for MHC content (mg MHC/g LV). These calculations show that MHC protein accumulated as a function of LV mass during MR-induced hypertrophy.

**Fig. 1.** Cardiac hypertrophy in dogs in response to experimental mitral regurgitation (MR). A: for each group of MR dogs, left ventricular (LV) mass was determined by ventriculography before the imposition of MR (baseline) and after 2 wk (2W-MR), 4 wk (4W-MR), or 3 mo of MR (3M-MR). B: LV mass normalized to body weight in the same dogs. Values are means ± SE. *P < 0.05 vs. corresponding baseline value as determined by paired t-test.

**Fig. 2.** Cardiac hypertrophy in dogs in response to experimental MR. LV mass was determined in a separate group of 9 dogs by serial echocardiography at weekly intervals. Values are means ± SE. Curve represents best-fit nonlinear function for growth as indicated by equation.

**Table 2.** MHC accumulation during cardiac growth in MR dogs

<table>
<thead>
<tr>
<th>Time, wk</th>
<th>MHC ( K_g ), %/day</th>
<th>MHC Content, mg/g LV</th>
<th>MHC Accumulation, mg/g LV \cdot day^{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>23.4 ± 1.0 (9)</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.592</td>
<td>25.0 ± 1.5 (7)</td>
<td>0.148 ± 0.009 (7)</td>
</tr>
<tr>
<td>4</td>
<td>0.247</td>
<td>23.8 ± 1.1 (5)</td>
<td>0.059 ± 0.003 (5)</td>
</tr>
<tr>
<td>12</td>
<td>0.007</td>
<td>23.5 ± 1.8 (6)</td>
<td>0.002 ± 0.0001 (6)</td>
</tr>
</tbody>
</table>

Values are means ± SE; nos. in parentheses are no. of dogs. Fractional rate of myosin heavy chain (MHC) accumulation (\( K_g \)) values were derived by calculating the first derivative of the best-fit nonlinear equation for growth at each time point of MR and dividing by the corresponding value for LV mass. MHC content was measured as described in MATERIALS AND METHODS. There were no significant differences in MHC content as determined by ANOVA. MHC accumulation was calculated by multiplying \( K_g \) by the corresponding values for MHC content.
Specific Radioactivity of Plasma Leucine During Continuous Infusion

In these studies, the rate of MHC synthesis was measured after continuous infusion with [$^3$H]leucine. We have shown previously that the plasma leucine specific radioactivity equilibrates with leucyl-tRNA specific radioactivity during continuous infusion in both control and MR dogs (14). Therefore, in these studies, the plasma leucine specific radioactivity was used as the precursor pool for calculating the rate of MHC synthesis and the rate of ribosome formation. Figure 3 shows the plasma leucine specific radioactivity time curve during 6 h of continuous infusion with [$^3$H]leucine. The specific radioactivity rose rapidly during the first 30 min and plateaued to a constant value over the remainder of the infusion period. There were no significant differences in plasma leucine specific radioactivity or $\int F^*$ in the control dogs compared with any of the MR groups.

Effect of MR on MHC $K_s$ Values

The effect of MR on cardiac protein synthesis was examined by measuring MHC $K_s$. There are three advantages of measuring the rate of MHC synthesis in cardiac tissue rather than the rate of total protein synthesis. First, MHC is a cardiocyte-specific protein expressed at constitutively high levels. Second, because MHC is a singular protein pool, it obviates the problem of interpreting changes in synthesis rates derived from a mixed pool of total cardiac protein. During a 6-h period of continuous infusion, short-lived proteins in myocardial tissue are preferentially labeled over long-lived proteins. Third, measuring the rate of MHC synthesis avoids any potential problems associated with labeling a total protein pool whose tissue composition may be changing during hypertrophic growth. In Fig. 4A, $K_s$ values for MHC synthesis are shown in control dogs and in MR dogs measured at 2-wk, 4-wk, and 3-mo time points. MHC $K_s$ increased by 1.5 and 5.8% after 2 and 4 wk of MR, respectively, but these increases did not achieve statistical significance. These data indicate that MHC accumulated during the development of volume-overload hypertrophy without a substantial increase in MHC $K_s$. As a positive control, MHC $K_s$ was measured in dogs subjected to acute pressure overload (14). Similar to our previous findings, MHC $K_s$ was accelerated by 45% in response to 6 h of acute pressure overload ($P < 0.05$, $n = 4$ dogs). Thus the lack of significant increases in MHC $K_s$ in response...
to MR was not due to an inability to detect changes in MHC synthesis rates by the continuous infusion method.

Effect of MR on Fractional Rates of MHC Degradation

The fractional rate of MHC degradation \( (k_d) \) was calculated indirectly using the formula \( k_g = k_s - k_d \). In the control dogs, MHC \( k_s \) and MHC \( k_d \) were considered to be equivalent because they were in a steady state with respect to cardiac growth. Figure 4A shows that the calculated MHC \( k_d \) value was decreased significantly after 2 wk of MR compared with control. Because LV growth was most rapid during the first 2 wk of MR, changes in MHC accumulation can be accounted for by a decrease in MHC \( k_d \) rather than an acceleration of MHC \( k_s \). These calculations also show that fractional rates of MHC synthesis and degradation were essentially the same at 12 wk, a time point at which a steady state with respect to growth had been reestablished.

In Fig. 4B, rates of total MHC synthesis and total MHC degradation (mg MHC·LV\(^{-1}·\text{day}^{-1}\)) were calculated to assess their relative contributions to MHC accumulation during volume-overload hypertrophy. These calculations take into account the size of the LV in the MR dogs. Total MHC synthesis rates were higher at each time point of MR measured compared with control and were attributable to increased LV mass. However, these increases did not reach statistical significance. These data indicate that the rate of total MHC synthesis increased as hypertrophy developed in MR dogs by maintaining MHC \( k_s \) constant. Figure 4 also shows that the rate of total MHC degradation did not initially increase as hypertrophy developed in the MR dogs because MHC \( k_d \) values were suppressed, particularly after 2 wk of MR. Consequently, the accumulation of MHC protein during hypertrophic growth resulted from the net difference between MHC synthesis and degradation.

Effect of MR on the Capacity for Protein Synthesis

Cardiac protein synthesis rates are regulated during sustained hypertrophic growth by increasing the capacity for protein synthesis, defined as an increase in translational machinery including the ribosome pool. Total RNA content is a well-described marker for changes in translational capacity of cardiac muscle because ribosomal RNA is \( \sim 85\% \) of the total RNA pool. Figure 5A shows that RNA content did not change at any time point during MR-induced hypertrophy. To confirm that translational capacity was unchanged during volume-overload hypertrophy, we measured rates of 60S ribosome formation in both the control and MR dogs (Fig. 5B). Although the mean values for the rate of 60S ribosome formation were lower at 4 wk and 3 mo of MR, these rates were not significantly different compared with either the control or the 2-wk MR group.

Effect of MR on MHC mRNA Expression

MHC mRNA levels were measured to determine whether MR had any effect on MHC mRNA expression. In Fig. 6, MHC mRNA levels were compared in the MR dogs by slot blotting using a \( \beta \)-MHC cDNA probe. To correct for differences in the amount of total RNA added to the slots, we normalized MHC mRNA levels to 28S rRNA by stripping the blots and hybridizing them with a 28S rDNA probe. The MHC mRNA-to-28S rRNA ratio did not change significantly in the LV of the MR dogs compared with the controls. Thus MHC mRNA abundance was unaltered, suggesting that the stimulus of volume overload does not trigger any transcriptional or posttranscriptional increases in MHC gene expression.
MHC mRNA Levels in Polysome Fractions

Because MHC mRNA levels were unchanged in the MR dogs, we examined whether MR had any effect on the ability of MHC mRNA to be translated into protein. In Fig. 7, the functional distribution of MHC mRNA in free, nonpolysome fractions and bound, actively translating polysome fractions was determined in both the LV and RV of the MR dogs. Cardiac polysomes were resolved on 15–50% linear sucrose gradients, and MHC mRNA levels in the gradient fractions were measured by slot blotting using the β-MHC cDNA probe. The amount of recovered rRNA in each fraction was measured by stripping the blot and hybridizing it to the 28S rRNA probe. The percentage of MHC mRNA in each gradient fraction was calculated from the sum total of MHC mRNA in all of the gradient fractions. The values were divided by the percentage of 28S rRNA in each corresponding fraction, thereby correcting for any differences in recovery of ribosomes and polysomes. Figure 7 shows that there were no differences in the distribution of MHC mRNA in the LV of the MR dogs at any time point measured during MR, and the same distribution was observed in the companion, more normally loaded, RV from the same hearts. MHC mRNA did not mobilize from the unbound region of the gradient into the polysome fractions, nor was there any significant shift of MHC into heavier polysomes. These data demonstrate that volume overload did not significantly alter the efficiency of protein synthesis as the amount and distribution of MHC mRNA in nonpolysome and polysome fractions were unchanged.

DISCUSSION

Hypertrophy in MR

In both experimental and clinical MR, the amount of hypertrophy that occurs is relatively modest compared with that found in other types of valvular heart disease, even when the MR is extremely severe (4, 7, 18). Modest hypertrophy was confirmed in the current study when after 3 mo of severe MR, LV mass normalized to body weight had increased by only 23% compared with the baseline values. The current study, in tandem with our previous work (14), helps explain the processes whereby only modest hypertrophy develops in severe MR. For hypertrophy to occur, the rate of myocardial protein synthesis must exceed the rate of protein degradation either because K_s increases or K_d decreases. In acute MR, there is no significant increase in K_s for MHC (14). This contrasts to acute pressure overload, in which K_s for MHC increases by 45% within the first 6 h after pressure overload is created. The present study extends these findings by demonstrating that MHC K_s did not significantly increase even during more chronic MR. At 80% power, it would require a minimal sample size of 20 dogs per group to detect a type II error for the small differences in MHC K_s measured in this study. Thus, although a larger sample size might have found the increases in MHC K_s to be statistically significant, the decrease in MHC K_d accounted for most of the MHC accumulation during LV growth. Taken together, these studies indicate that a decrease in the fractional rate of protein degradation has a major role in regulating hypertrophic growth during chronic MR.

The key question of this study is why there is not a significant acceleration of the fractional rate of protein synthesis in response to MR. The most plausible explanation is that the translational mechanisms that regulate the rate of cardiac protein synthesis respond differently to volume overload compared with pressure overload. For example, MR does not produce a significant increase in translational efficiency, defined as the rate of protein synthesis divided by ribosome content, whereas acute pressure overload does produce a significant increase in translational efficiency (14). Consistent with these findings, we have shown that acute differences in anabolic responsiveness to pressure overload vs. volume overload may be due to the extent of phosphorylation of eukaryotic initiation factor (eIF) 4E.
(eIF-4E), a rate-limiting factor for protein synthesis (29). Phosphorylation of eIF-4E is increased in response to acute pressure overload but not in response to acute volume overload. The functional significance of eIF-4E phosphorylation is that it increases the rate of translational initiation (22). An alternative explanation for the lack of anabolic responsiveness to volume overload would be an actual decrease in the rate of translational initiation. Studies using a rat model of head-down tilt to produce an increase in preload have shown that translational efficiency is decreased via a mechanism involving phosphorylation of eIF-2α (21). It is well known that phosphorylation of translational eIF-2α causes a block in peptide chain initiation (12). However, this possibility is unlikely in the MR model because we did not observe a decrease in translational efficiency as reflected either by the distribution of MHC mRNA in polysome gradients or by simply dividing the rate of MHC synthesis by RNA content.

To further support our conclusion that MR does not trigger an increase in translational activity, we found that MR-induced hypertrophy occurred without an increase in the capacity for protein synthesis as measured by either the rate of 60S ribosome formation or ribosome content. An increase in the capacity for protein synthesis is a well-documented mechanism for accelerating the rate of protein synthesis during sustained pressure-overload hypertrophy (23). These studies indicate that Ks for MHC does not increase during volume-overload hypertrophy because the stimulus of MR does not elicit a significant increase in translational efficiency or translational capacity.

### Role of Protein Degradation

The amount of protein that accumulates during cardiac hypertrophy is a function of the net difference between the fractional rate of protein synthesis and the fractional rate of protein degradation. Because MHC protein accumulates during MR-induced hypertrophy with so small an increase in the MHC Ks, we conclude that a decrease in the fractional rate of protein degradation is the primary mechanism regulating MHC content. Previous studies have shown that MHC degradation rates may actually decrease during hypertrophic growth in response to the combined pressure and volume overload of aortic regurgitation, causing a net accumulation of MHC in the cardiocyte (19). Our study extends these findings to the hemodynamically different condition of pure volume overload in MR. The mechanism by which the MHC Kd is regulated is not known, but in vitro studies suggest that MHC degradation may be linked to assembly of myosin into myofibrils. For example, it has been shown in isolated adult cardiac myocytes that there is a relatively large, stable pool of sarcomeric MHC characterized by a long half-life, and a smaller pool of nascent MHC that has been shown kinetically to have a much faster rate of degradation (9). Thus MHC could accumulate during MR-induced hypertrophy either by increasing assembly of MHC into sarcomeres or by maintaining MHC in the sarcomeric pool.

### Ventricular Geometry and the Hemodynamic Overload of MR

We find it extraordinary that such severe volume overload, which causes a regurgitant fraction of >60% and increases total stroke volume by 70%, produces so little increase in myocardial mass. This only modest increase in mass occurs because despite a large increase in LV radius, LV wall thickness actually declines. These changes in LV geometry are in keeping with Grossman’s hypothesis (11) that LV systolic pressure and systolic wall stress primarily regulate wall thickness, whereas diastolic stress primarily regulates cavity size. In MR, LV systolic pressure is lower than normal, potentially accounting for a decline in LV thickness (24), whereas end-diastolic pressure and stress are higher than normal, contributing to the eccentric hypertrophy and/or remodeling of the ventricle that occurs. It should be emphasized that the type...
of volume overload that occurs in MR is different from other volume overloads that occur in clinical disease (30). In MR, the extra volume that the LV expels is ejected into the low-pressure left atrium, whereas in nearly all other types of volume overload, the extra volume is expelled into the relatively high pressure of the aorta. Thus the volume overload of MR has been termed “pure” volume overload, whereas most other volume overloads really constitute combined pressure and volume overload. These differences are paralleled by the type of hypertrophy that occurs. For instance, in aortic regurgitation, in which LV pressure is higher than normal, there is an increase in wall thickness as well as an increase in radius (10), and LV mass greatly increases. We conclude that 1) the mechanisms for increasing myocardial protein content differ according to the type of overload; and 2) although there is a small, but not statistically significant, increase in the fractional rate of protein synthesis, the primary mechanism for increasing LV mass in response to the overload of MR is a decrease in the fractional rate of protein degradation.

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Address for reprint requests: P. J. McDermott, Gazes Cardiac Research Institute, Rm. 303, Strom Thurmond Biomedical Research Bldg., 114 Doughty St., Charleston, SC 29403.

REFERENCES


