Acute Changes in Myosin Heavy Chain Synthesis Rate in Pressure Versus Volume Overload

Takuro Imamura, Paul J. McDermott, Robert L. Kent, Masayoshi Nagatsu, George Cooper IV, Blase A. Carabello

Abstract The left ventricular hypertrophy that develops with the volume overload of mitral regurgitation is relatively less than that which develops with the pressure overload of aortic stenosis even when both lesions are severe. The hypertrophy that develops must be the sum of changes in the rate of myocardial protein synthesis and degradation. In the present canine study, we explored early changes in the synthesis rate of myosin heavy chain in response to severe acute pressure overload versus that of the severe acute volume overload of mitral regurgitation. We tested the hypothesis that in acute overload, the rate of protein synthesis would increase less in the volume-overload model than in the pressure-overload model, a potential partial mechanism for the discrepancy in the eventual total amount of hypertrophy that develops in these two lesions. Acute pressure overload was produced by inflating a balloon in the descending aorta, and acute volume overload was produced by using our closed-chest mitral chordal rupture technique. In both models, the hemodynamic lesion that was created was severe. In eight dogs with pressure overload, the average gradient across the balloon was 119.8±6.1 mm Hg. In six dogs with volume overload, the average regurgitant fraction was 0.67±0.06. Six other dogs served as controls. The average rate of myosin heavy chain synthesis in control dogs was 2.7±0.2% per day, virtually identical to the rate we found in the severe volume-overload model. In contrast, the rate was increased in the pressure-overload model by 30% to 3.5±0.3% per day (P<.05). We conclude that the rate of myocardial protein synthesis increases measurably after 6 hours of severe pressure overload but does not after 6 hours of severe volume overload. If these early qualitative differences persisted, they would help explain the relative lack of hypertrophy in the volume overload of mitral regurgitation. (Circ Res. 1994;75:418-425.)

Key Words • hypertrophy • myosin synthesis rate • pressure overload • volume overload

The imposition of long-standing cardiac hemodynamic overload results in the development of ventricular hypertrophy. In pressure overload, concentric hypertrophy develops, whereas in volume overload, eccentric hypertrophy develops. These adaptations are at least initially beneficial, since concentric hypertrophy in pressure overload normalizes systolic load, thereby maintaining pump performance, whereas eccentric hypertrophy in volume overload allows the ventricle to pump the required extra stroke volume.1,2 However, the volume overload of mitral regurgitation, in which the excess volume is ejected into the left atrium under low pressure, typically results in less hypertrophy (a smaller increase in left ventricular mass) than does either pressure overload or high-pressure volume overload.1-23 It should be noted that these observations were derived from studies in which the severity and duration of the overload varied substantially.1,3-23 However, under more controlled conditions, we recently found that the severe volume overload of mitral regurgitation produced substantially less hypertrophy than the severe pressure overload of aortic stenosis when animals with the two types of overload were matched by stroke work (SW, a commonly shared hemodynamic parameter).24 Further, when both aortic stenosis and mitral regurgitation were so severe that they led to heart failure, the hypertrophy of volume overload was less than half that found in pressure overload. Thus, the hypertrophic compensation in mitral regurgitation appears limited compared with that of other overloads.

The amount of hypertrophy that develops during chronic overload is the summation of changes in the corresponding rates of myocardial protein synthesis and degradation. In the present study, we examined the acute change in contractile protein synthesis rate after the imposition of a severe acute pressure overload versus that after the imposition of a severe acute volume overload. The purpose of the present study was to test the hypothesis that severe acute low-pressure volume overload causes a smaller increase in myocardial protein synthesis rate than does acute pressure overload. If so, these initial events might be a first step in explaining the relative less hypertrophy that occurs in low-pressure volume overload versus pressure overload.

Materials and Methods

Study Design

The rate of myosin heavy chain (MHC) synthesis was studied by examining the rate of incorporation of radiolabeled Leu ([3H]Leu) into myosin25 over a 6-hour infusion period.
Three groups of animals were studied: (1) control dogs, (2) dogs with acute pressure overload (POL group), and (3) dogs with acute volume overload (VOL group). Obviously, for the study to have meaning, the severity of the overload had to be similar; it would be pointless to compare the hypertrophic response of mild acute volume overload with that of severe acute pressure overload. Unfortunately, comparing these two entities is difficult because they are hemodynamically so dissimilar. In the present study, our definition of “severe” for pressure and volume overload was identical: the most overload that could be created and tolerated by the animal without causing deterioration for the 6 hours of [3H]Leu infusion. In the POL group, the overload was created by an intra-aortic balloon placed in the descending aorta and inflated to the maximum degree, which allowed a mean distal perfusion pressure of at least 50 mm Hg. With this degree of stenosis, no dog developed acidosis, as determined by arterial blood gas determinations; other parameters, including forward stroke volume (SV) and pulmonary capillary wedge pressure (PCW), did not deteriorate, further indicating experimental stability. However, in experiments reported in the present study in which distal perfusion pressure was <50 mm Hg, deterioration inevitably occurred. Thus, distal perfusion pressure was a good guideline for assessing the maximum severity of aortic obstruction that could be tolerated. Volume overload was created by producing acute mitral regurgitation by use of our previously reported closed-chest chordal rupture technique.34,35 Enough chords were severed to reduce SV by 50%, to increase PCW to 20 mm Hg, and as in pressure overload to allow for a mean arterial pressure of at least 50 mm Hg. When these criteria were used, no VOL animal developed pulmonary edema or acidosis, but the amount of regurgitation was +4/+4 on the standard angiographic scale, and all subjects had a regurgitation fraction (RF) of >50%. Since RFs of >70% inevitably cause pulmonary edema in this model, this guideline also seemed appropriate in defining the maximum tolerated lesion.

Experimental Preparation

Control Dogs
Six parasite-free adult mongrel dogs of both sexes served as controls. Anesthesia was induced with an infusion of 0.15 mg·kg⁻¹·min⁻¹ sufentanil supplemented by isoflurane and maintained by a constant infusion of sufentanil and a low dose of isoflurane by inhalation. A thermodilution Swan-Ganz catheter was inserted from the femoral vein for measuring PCW and cardiac output. A second catheter was placed in the femoral artery for measuring arterial blood pressure and for blood sampling. After baseline hemodynamic conditions were measured, the infusion of [3H]Leu was begun from the femoral vein and maintained for 6 hours. Hemodynamic conditions were monitored constantly to ensure that they remained stable.

Pressure Overload
In eight dogs, anesthesia was induced in a fashion identical to that in control dogs. Cutdowns were performed over both groins. A Swan-Ganz catheter was advanced to the pulmonary artery from the femoral vein. A pigtail catheter was advanced to the ascending aorta through a sheath for the purpose of recording aortic pressure proximal to the balloon. The side arm of the sheath was used to measure femoral arterial pressure distal to the balloon. A 23-mm balloon catheter was placed into the descending aorta from the opposite femoral artery. The balloon catheter was then inflated to maintain a proximal pressure of >170 mm Hg and a mean femoral artery pressure of 50 mm Hg. Heart rate was maintained by the continuous infusion of atropine if bradycardia occurred. When the hemodynamic conditions were stable, the [3H]Leu infusion was begun and maintained for 6 hours. Hemodynamics were monitored constantly throughout the infusion.

Volume Overload
Mitral regurgitation was produced in six dogs via the transarterial approach. Anesthesia was induced and maintained in a manner identical to that in the other groups. An 8F 30-cm sheath was placed in the right carotid artery and advanced to the left ventricle. A pigtail catheter placed through this sheath was used for measuring left ventricular pressure and for performing ventriculography. The femoral vein was instrumented with a Swan-Ganz catheter. After baseline measurements of hemodynamics, the pigtail catheter was removed and then replaced by a grasping forceps.22 The mitral valve chordae were grasped and severed, resulting in mitral regurgitation. When PCW increased to 20 mm Hg and SVR was reduced by 50%, previous experience demonstrated that RF would be 60% to 70%. This was confirmed by contrast left ventriculography, which allowed for visual assessment of the mitral regurgitation and for calculation of RF and total stroke volume (SVt). After a 15-minute stabilization period, [3H]Leu was infused for 6 hours while the hemodynamics were closely monitored.

[3H]Leu Infusion Protocol
The same protocol was followed in all dogs. For in vivo labeling, a [3H]Leu solution was prepared by mixing 10 mCi [3H]Leu (1,4,5-3H]Leu, Amersham Corp) with 0.1 mmol/L unlabeled Leu in a 0.09% (wt/vol) NaCl solution, pH 7.4. The solution was continuously infused at a constant rate of 0.915 mL/min. Blood samples (3 mL) were withdrawn from the ascending aorta at 0, 15, 30, 60, 90, 120, 180, 240, 300, and 360 minutes during the infusion. After 6 hours of infusion, the hearts were rapidly removed while the [3H]Leu infusion continued. The hearts were frozen in liquid nitrogen and stored at −70°C.

Calculation of MHC Synthesis Rate
The fractional rate of MHC synthesis (Kd) was calculated by measuring the incorporation of [3H]Leu into electrophoretically purified MHC. Mathematically, the change in specific radioactivity of MHC (dP/dt) is described as follows:

\[
\frac{dP}{dt} = K_d E^* - K_s P^*
\]

where P* is the specific activity of the radiolabeled amino acid in the product pool, F* is the specific activity of radiolabeled amino acid in the precursor pool, and Kd and Ks are the rate constants for MHC synthesis and degradation, respectively. Because MHC has a relatively long half-life, only a small fraction of the MHC pool is degraded over the 6-hour labeling period. Thus, the degradation constant (Kd) can be eliminated from Equation 1, resulting in the following formula:

\[
\frac{dP^*}{dt} = K_d F^*
\]

or

\[
K_d = \frac{\int F^* dt}{P^*}
\]

where the \(\int F^* dt\) term in Equation 3 is the area beneath the precursor pool specific radioactivity–time curve. Thus, the specific radioactivity of the immediate precursor pool of the radiolabeled amino acid incorporated into protein must also be measured in order to calculate protein synthesis rates. The immediate precursor pool for protein synthesis is aminoacyl-tRNA, which can only be measured at one time point in the study, at death. However, Everett et al24 have demonstrated that the specific radioactivity of the plasma Leu pool equilibrates rapidly with the leucyl-tRNA pool during constant infusion with [3H]Leu and thus can be substituted for leucyl-tRNA. In the present study, MHC synthesis rates were calculated by using the
specific radioactivity of plasma Leu as the precursor pool. To further validate the use of the plasma Leu pool for calculations of MHC $K_t$, the extent of equilibration between plasma Leu-specific radioactivity and leucyl-tRNA–specific radioactivity was determined at the time of death.

**Determination of Leu-Specific Radioactivity in Plasma**

Blood samples were centrifuged at 1500 g for 15 minutes at 4°C. Concentrated perchloric acid (PCA) was added to the plasma to a final concentration 6%, and the samples were centrifuged at 15 000g for 15 minutes. The supernatant was neutralized by adding 10N KOH and centrifuged, and a 100-$\mu$L aliquot of the supernatant was dried by vacuum centrifugation. The dried sample was resuspended in 100 $\mu$L of 0.1 mol/L NaHCO$_3$, Na$_2$CO$_3$ buffer (pH 9.5), reacted with an equal volume of $^{[3]C}$dansyl chloride (5 mmol/L, 5 dpm/pmol, Amershams Corp), and incubated at 37°C for 1 hour. The dansylated amino acids were purified by two-dimensional thin-layer chromatography on micropolyamide plates (Michromolyamide foil, F 1700, Schleicher & Schuell, Inc). The solvent for the first dimension consisted of 2% formic acid, and the solvent used for the second dimension was 90% benzene:10% glacial acetic acid. The first and second dimensions were repeated as necessary to reduce tailing of the spots. The dansyl-Leu spot was excised and eluted by an overnight incubation in protonsol (New England Nuclear). The ratio of $^1$H to $^{13}$C of the dansyl-Leu spot was measured by liquid scintillation counting by use of a dual-label program (Beckman Instruments). For each assay, a standard curve of Leu-specific radioactivity was developed, and plasma Leu–specific radioactivities were extrapolated from the standard curve.

**Determination of Leu-Specific Radioactivity in MHC**

Left and right ventricular tissue (1 g) was homogenized in a solution consisting of 1.1 mol/L KI, 0.1 mol/L KH$_2$PO$_4$, pH 7.4, 1.0 mmol/L dithiothreitol, and 1.5 mmol/L phenylmethylsulfonyl fluoride (PMSF). The homogenate was stirred for 1 hour at 4°C and centrifuged at 10 000g for 15 minutes. The supernatant was aspirated, diluted with 10 vol cold water, and allowed to settle for 2 hours at 4°C. After centrifugation at 10 000g for 15 minutes at 4°C, the pellet was dissolved in sample buffer containing 0.2% (wt/vol) sodium dodecyl sulfate, 10% glycerol, 5% $\beta$-mercaptoethanol, 0.5 mol/L Tris-HCl, pH 6.8, and 1.5 mmol/L PMSF. The lysate was boiled for 5 minutes, pyronin Y was added to a final concentration of 0.01%, and the samples were electrophoresed for 20 hours on 10% to 13% polyacrylamide-$N,N'$-diallyltartardiamide gradient gels. The gel was stained at room temperature overnight in 0.05% Coomassie brilliant blue R-250 (BioRad), 50% methanol, and 7% glacial acetic acid and destained in 30% methanol and 10% glacial acetic acid. A typical gel is shown in Fig 1. The MHC band was cut from the gel and solubilized in 2% periodate acid (Fishers) and 4% lactic acid (Fisher). The protein was precipitated by adding PCA to a final concentration of 6% PCA and centrifuged for 15 minutes at 1500g. The pellet was washed twice in 10% HCl to remove any residual PCA. The pellet was dried by vacuum centrifugation and transferred to a sealable glass vial to which 1 mL of boiling 6N HCl (Pierce) was added. Ambient air was flushed with N$_2$, and the vial was flame-sealed under vacuum. The sealed vial was incubated for 24 hours at 110°C to hydrolyze the protein into amino acids. The contents of the vial were removed, the supernatant was dried by vacuum centrifugation, and the remaining dried residues were dissolved in 0.1 mol/L NaHCO$_3$, Na$_2$CO$_3$ buffer, pH 9.5. The amino acids were reacted with 5 mmol/L dansyl chloride (5 dpm/pmol) and purified by two-dimensional thin-layer chromatography as described above. The amount of dansyl-Leu was determined by the isotope dilution method as modified by Samarel et al. The corresponding $[3$H]Leu radioactivity was measured by liquid scintillation counting, and the specific radioactivity of Leu in the MHC pool (in disintegrations per minute per nanomole) was calculated.

**Determination of Leu-Specific Radioactivity**

Approximately 2 g frozen left ventricular tissue was homogenized with 20 mL cold RNA zol (RNA isolation solvent, Cinna/Biotech). An equal volume of chloroform (chloroform:isoamyl alcohol [24:1]) was added, and the mixed solution was homogenized again. The homogenate was vortexed for 10 minutes at 4°C and centrifuged at 10 000g for 15 minutes at 4°C. The aqueous phase was transferred to another tube, mixed with an equal volume of isopropanol, and precipitated overnight at -20°C. RNA was pelleted by centrifugation at 10 000g for 15 minutes at 4°C. The pellets were air-dried for 50 minutes and resuspended in 2 mL of 20 mmol/L Tris, pH 7.5, and 50 mmol/L EDTA, pH 8.0. The sample was extracted with an equal volume of phenol:chloroform (1:1) followed by precipitation overnight in 70% ethanol at -20°C. The RNA was pelleted by centrifugation and washed four times with 75% ethanol. The deacylation of tRNA was performed by the method of Everett et al. The specific radioactivity of Leu was determined by the same method used for plasma Leu.

**Measurement of Total RNA Content**

Approximately 100 mg tissue was resected from each right and left ventricle and homogenized in 6% PCA. The homogenate was centrifuged for 10 minutes at 10 000g. After washing three times with 6% PCA, the pellet was hydrolyzed in 0.3N NaOH at 37°C for 2 hours. After addition of 1 mL of 4N PCA, the mixture was put on ice for 15 minutes and spun at 10 000g for 10 minutes. The supernatant was removed, and RNA content was determined by spectrophotometry at a wavelength of 260 nm. The residual pellet was washed three times with 4 mL of 0.2N PCA and resuspended with 4 mL of 0.3N NaOH and incubated at 37°C overnight. The concentration of protein was measured by the bicinchoninic acid method. RNA content was expressed as milligrams RNA per gram protein.

**Other Calculations**

RF was calculated as follows:

$$\text{RF} = \frac{\text{SV}_t - \text{SV}_f}{\text{SV}_t}$$

where SV$_t$ is angiographic end-diastolic volume minus end-systolic volume and SV$_f$ is thermodilution cardiac output divided by heart rate.

Total external SW was calculated as follows:
TABLE 1. Hemodynamic Data

<table>
<thead>
<tr>
<th></th>
<th>Control (n=6)</th>
<th>POL (n=8)</th>
<th>VOL (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>80.0±4.1</td>
<td>120.7±5.0†</td>
<td>110.1±8.6†</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg*</td>
<td>124.4±8.5</td>
<td>177.9±5.1†</td>
<td>96.6±4.3‡</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg*</td>
<td>84.4±3.0</td>
<td>121.8±5.6‡</td>
<td>73.6±5.2‡</td>
</tr>
<tr>
<td>Pulmonary wedge pressure, mm Hg</td>
<td>7.6±0.6</td>
<td>10.0±0.4†</td>
<td>17.5±0.7‡</td>
</tr>
<tr>
<td>Total stroke volume, mL/min</td>
<td>46.3±2.3</td>
<td>36.6±1.7</td>
<td>72.5±6.1‡</td>
</tr>
<tr>
<td>Stroke work, g-m</td>
<td>59.0±3.4</td>
<td>69.5±3.8‡</td>
<td>78.0±8.1†</td>
</tr>
</tbody>
</table>

POL indicates dogs subjected to pressure overload; VOL, dogs subjected to volume overload; and bpm, beats per minute. Values are mean±SEM.

*In the POL group, these values are proximal to the balloon.
†P<.05 vs the control group.
‡P<.05 vs the POL group.

(5) \[ SW = [\text{MAP} - \text{PCW}] \times \text{SV}_i \times 0.0136 \]

where MAP is mean arterial pressure (millimeters of mercury) and 0.0136 is a factor for converting millimeters of mercury×milliliters to gram-meters. \text{SV}_i was total angiographic stroke volume in the VOL model and was assumed to be equal to thermodilution cardiac output divided by heart rate in control dogs and POL dogs.

Statistical Analysis

Data are presented as mean±SEM. Significant differences between means were tested by ANOVA followed by a Newman-Keuls test. Differences were considered statistically significant at a level of \( P<.05 \).

Results

Hemodynamic Data

Table 1 shows hemodynamic data averaged over the 6-hour infusions from data taken at hourly intervals. Heart rate was significantly increased in POL and VOL models compared with control dogs; however, there was no difference between the POL and VOL model. The average gradient across the balloon in the POL model was 119.8±6.1 mm Hg. Appropriately, systolic pressure was increased in the POL model by 44% compared with control dogs and by 84% compared with the VOL model. The average RF produced by the VOL model was 0.67±0.06. The VOL model produced a substantial increase in \text{SV}_i, which was 100% greater than that of the POL model. External SW was increased in both overload models and tended to be higher in the VOL model than in the POL model.

Because of concerns that coronary flow driving pressure was reduced in the VOL model, potentially causing ischemia, left ventricular lactate extraction was measured in three additional dogs undergoing the creation of mitral regurgitation in a fashion identical to that in dogs in which \( K_v \) was measured. Lactate extraction was identical before and after the creation of mitral regurgitation, giving no indication that ischemia had developed.

Equilibration of Plasma and Leucyl-tRNA Pools During Continuous Infusion

Fig 2 shows the plasma Leu–specific radioactivity time curve for all dogs during 6 hours of constant infusion of \(^{[3]H}\)Leu. Characteristically, the specific radioactivity rose rapidly and plateaued during the first 60 minutes of infusion. The plasma Leu–specific radioactivity then remained constant over the remaining 5 hours of infusion.

Leucyl-tRNA–Specific Activity

Previous studies have demonstrated that the specific radioactivity of the plasma Leu and leucyl-tRNA pools equilibrates rapidly during the continuous infusion method.26 However, to be certain of this equilibration in our hands, the specific radioactivities of the plasma and leucyl-tRNA pools were measured at the completion of the 6-hour infusion to establish the extent to which equilibration between these pools was achieved. The ratio of leucyl-tRNA–specific radioactivity to plasma Leu–specific radioactivity was similar in control, POL, and VOL dogs (Fig 3). Thus, in all groups, the plasma Leu–specific radioactivity equilibrated to a similar extent with leucyl-tRNA, the immediate precursor pool for protein synthesis. These findings validated the substitution of plasma Leu–specific radioactivity for that of leucyl-tRNA in the calculations of MHC \( K_v \).

MHC Synthesis Rate

In Fig 4, \( K_v \) values for MHC in control, VOL, and POL dogs are shown. MHC \( K_v \) in the left ventricle was significantly increased in response to pressure overload. In contrast, MHC \( K_v \) was not accelerated in the left ventricle of VOL dogs. As an internal control, values for MHC \( K_v \),
were also measured in the right ventricle of the same hearts; there were no significant differences (control, 2.5±0.15%/d; POL, 2.7±0.25%/d; VOL, 2.5±0.2%/d).

In Table 2, the specific radioactivity of plasma Leu and MHC Leu pools that were used to calculate the corresponding MHC $K_i$ values are shown. These data document that the acceleration of $K_i$ in response to pressure overload in the left ventricle was due to an increase in the rate of incorporation of Leu into MHC rather than a change in the specific radioactivity of the precursor pool.

**RNA Content in Control, POL, and VOL Dogs**

RNA content, which is an index of protein synthetic capacity because it reflects primarily ribosomal content, was measured in control, POL, and VOL groups (Fig 5). There were no significant differences in RNA content in the left ventricle or right ventricle among the three groups.

**Discussion**

An important finding of the present study was that although the MHC synthesis rate increased in severe acute pressure overload, there was no detectable change in MHC synthesis rate during severe acute “pure” (low-pressure) volume overload. To our knowledge, this is the first report of the response of MHC rate in mitral regurgitation. Mitral regurgitation is distinct from other volume overloads because the extra volume pumped is pumped into the low pressure of the left atrium instead of the high pressure of the aorta.1,2 Thus, we have considered mitral regurgitation “pure” volume overload because it lacks the elements of pressure overload. As shown in Table 3, of the common clinical valvular lesions, this type of volume overload produces the least hypertrophy. This relative lack of hypertrophy in mitral regurgitation could obviously be due to a relatively smaller increase in myocardial protein synthesis rates or an increase in degradation rate. Our data indicate that at least initially there is a quantitative difference in the response of MHC synthesis rate to pressure versus low-pressure volume overload.

**Previous Studies of Myocardial Protein Synthesis Rate**

In discussing the present study in relation to previous studies of protein synthesis, several differences among the studies must be taken into consideration. These include the type and severity of the overload, whether synthesis constants were studied during the acute or the chronic phase of the hypertrophy, whether the studies were performed in vivo or in vitro, whether total protein versus contractile protein synthesis was studied, and the species and type of labeling procedure used. Table 4 summarizes some of these studies. In general, in pressure overload there is a modest acute increase in protein synthesis rate, which increases progressively more over the next several days.3,4 In one reported study of high-pressure volume overload (aortic regurgitation),4 the increase in synthesis was delayed for 10 days and then increased progressively over the next 2 weeks. These data would be consistent with our finding of an early lack of response to acute volume overload. Other studies found that in papillary muscle, passive stretch such as might occur in volume overload increased protein synthesis less than did active tension development, which is required to increase in pressure overload.36,37 These in vitro data are also consistent with our in vivo data. Thus, MHC synthesis rate in acute pressure overload increases, whereas it does not in the volume overload of mitral regurgitation. Since hypertrophy does occur in volume overload, the protein synthesis rate must eventually increase unless degradation rate decreased. However, if this relatively lesser response in low-pressure volume overload persisted throughout the course of the overload, it would result in relatively less hypertrophy in volume overload unless degradation rate was decreased.

**Analysis of the Stimulus to Hypertrophy**

The obvious question is why would the protein synthesis rate be so different in the different types of severe overload? Presumably, the hemodynamic demands of the overload are transduced into genetic signals that increase transcription or translation or both, resulting in the accelerated synthesis of the myocardial proteins. It has been postulated that wall stress is a major mechanical stimulus to the two types of hypertrophy, with
increased systolic stress leading to the concentric hypertrophy of pressure overload and increased diastolic stress leading to the eccentric hypertrophic response in volume overload. Indeed, there is substantial evidence that at the cellular, tissue, and intact organ level, load is a predominant factor in regulating hypertrophy. Thus, Mann et al. found an 89% increase in protein synthesis in isolated cardiocytes subjected to the mechanical load of simple stretch. These studies were performed in a plasma-free medium, a medium void of putative hormonal growth factors, suggesting that load alone was the major cause of increased protein synthesis. At the tissue level, Kent et al. found that papillary muscles subjected to stretch increased protein synthesis, whereas active force development produced yet a greater increase in protein synthesis. In an elegant study in the intact animal, Cooper et al. pressure-overloaded the right ventricle in the cat by pulmonary artery banding. At the same time, they locally unloaded a right ventricular papillary muscle by transsecting its chorda tendinea. Thus, in the milieu of general ventricular pressure overload, a portion of the muscle was unloaded. While the ventricle hypertrophied, the transsected papillary muscle atrophied, demonstrating that it was local load rather than milieu that regulated cardiac growth. β-Adrenergic and α-adrenergic blockade did not alter these results, diminishing the likelihood that the adrenergic nervous system played a role.

Although mechanical load (wall stress) was not measured in the present study, systolic stress was almost surely elevated in response to pressure overload, whereas diastolic stress must have been elevated in response to volume overload. Stress is most simply described by the following Laplace relation: 
\[(P \times r)/2h\]
where P is pressure, r is radius, and h is wall thickness. We know that systolic pressure was elevated in the POL model by 84% compared with the VOL model and 44% compared with the control group. Since PCW was slightly elevated in the POL model (Table 1), it is likely that the ventricular radius increased slightly in the POL model (and thickness decreased slightly). Thus, both terms in the denominator increased so that systolic stress must have been increased in the POL model compared with the control group and the VOL model. On the other hand, diastolic stress must have been greatly increased in the VOL model, since PCW (diastolic pressure) was increased by 70% compared with the POL model and 100% compared with the control group. At the same time, we know that in the acute response, the left ventricular radius increases in this model by ≈20%. Thus, diastolic stress must have been increased compared with the POL model, since diastolic pressure and radius were increased. The effects of the VOL model on systolic stress are more difficult to speculate about. The increase in radius would tend to increase systolic stress, and the decrease in pressure would reduce stress. However, we can conclude from these data that the putative mechanical signals for protein synthesis to increase (increased systolic stress in pressure overload and increased dia-

### Table 2. Leu-Specific Activity

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>POL</th>
<th>VOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Leu–specific radioactivity, dpm/nmol</td>
<td>1318 ± 125</td>
<td>1486 ± 120</td>
<td>1540 ± 245</td>
</tr>
<tr>
<td>Leu-specific radioactivity in LV MHC, dpm/nmol</td>
<td>8.2 ± 1.0</td>
<td>13.3 ± 1.2†</td>
<td>9.8 ± 0.7</td>
</tr>
<tr>
<td>Leu-specific radioactivity in RV MHC, dpm/nmol</td>
<td>7.6 ± 0.8</td>
<td>9.7 ± 0.6</td>
<td>8.5 ± 0.6</td>
</tr>
</tbody>
</table>

POL indicates dogs subjected to pressure overload; VOL, dogs subjected to volume overload; LV, left ventricular; MHC, myosin heavy chain; and RV, right ventricular. Values are mean ± SEM.

Plasma Leu–specific radioactivity was obtained from the integral of the activity–time curve.

*P<.05 vs the control group.
†P<.05 vs the VOL group.

### Table 3. Hypertrophy in Human Valvular Disease

<table>
<thead>
<tr>
<th>Mass Index, g/m²</th>
<th>r/h</th>
<th>m/v</th>
</tr>
</thead>
<tbody>
<tr>
<td>NL</td>
<td>86 (259)</td>
<td>3.05 (68)</td>
</tr>
<tr>
<td>MR</td>
<td>158 (146)</td>
<td>4.03 (64)</td>
</tr>
<tr>
<td>AR</td>
<td>230 (148)</td>
<td>3.52 (31)</td>
</tr>
<tr>
<td>AS</td>
<td>178 (302)</td>
<td>2.35 (93)</td>
</tr>
</tbody>
</table>

r/h indicates the ratio of left ventricular radius to thickness; m/v, the ratio of left ventricular mass to volume; NL, normal subjects; MR, subjects with mitral regurgitation; AR, subjects with aortic regurgitation; and AS, subjects with aortic stenosis. Numbers within parentheses indicate the number of subjects analyzed. Data are compiled from References 1 and 3 through 21.

![Bar graph shows total RNA content normalized to total protein for both ventricles for control dogs, dogs subjected to pressure overload (POL), and dogs subjected to volume overload (VOL). LV indicates left ventricle; RV, right ventricle. No differences existed. Although the protein synthesis rate was increased (Fig 4), protein content would not have been expected to change significantly during the 6-hour infusion period.](http://circres.ahajournals.org/Downloaded from http://circres.ahajournals.org/).
TABLE 4. Summary of Some Previous Studies of the Rate of Cardiac Protein Synthesis

<table>
<thead>
<tr>
<th>Reference</th>
<th>Species</th>
<th>Protein Studied</th>
<th>Preparation</th>
<th>Type of Overload</th>
<th>Time From Onset of Overload</th>
<th>Increase in Protein Synthesis Rate, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>Guinea pig</td>
<td>Myosin</td>
<td>Isolated heart</td>
<td>Aortic constriction</td>
<td>2 h</td>
<td>No increase</td>
</tr>
<tr>
<td>31</td>
<td>Dog</td>
<td>Myosin</td>
<td>In vivo</td>
<td>Pulmonary artery constriction</td>
<td>24 h</td>
<td>32%</td>
</tr>
<tr>
<td>32</td>
<td>Rat</td>
<td>Myosin</td>
<td>In vivo</td>
<td>Aortic constriction</td>
<td>48-96 h</td>
<td>140%</td>
</tr>
<tr>
<td>33</td>
<td>Rat</td>
<td>Total protein</td>
<td>In vivo</td>
<td>Aortic constriction</td>
<td>0-30 d</td>
<td>Progressive increase to 91% at day 6</td>
</tr>
<tr>
<td>34</td>
<td>Rabbit</td>
<td>Myosin</td>
<td>In vivo</td>
<td>Aortic constriction</td>
<td>0-15 d</td>
<td>Progressive increase from day 2 to day 7; peak at 117%</td>
</tr>
<tr>
<td>35</td>
<td>Rabbit</td>
<td>Total protein</td>
<td>Papillary muscle</td>
<td>Aortic constriction</td>
<td>...</td>
<td>Active tension increased synthesis by 53% compared with only 36% with passive stretch</td>
</tr>
<tr>
<td>36</td>
<td>Ferret</td>
<td>Myosin</td>
<td>Papillary muscle</td>
<td>...</td>
<td>Progressive increase in synthesis as active tension increased</td>
<td></td>
</tr>
</tbody>
</table>

stroke stress in volume overload) were present and produced a substantially different acute response in MHC synthesis rate.

We wish to again emphasize that lesions were qualitatively similar in severity. We induced as much pressure overload and volume overload as would allow for a stable preparation over 6 hours. One could argue that the hemodynamic lesions were so severe that the induced ischemia possibly prevented protein synthesis from occurring, especially in the VOL model. Since lactate was not produced in three comparable VOL dogs, we believe that ischemia is an unlikely reason that K, failed to increase. In another comparison of severity, we also examined total external left ventricular SW, a hemodynamic parameter held in common between pressure and volume overload. External SW does not take internal work (potential energy) into account. However, the fact that external work actually tended to be greater in the VOL model than in the POL model further suggests that it was not less overload in the VOL model that led to the lack of an acute increase in the MHC synthesis rate.

In the acute POL model, in which the MHC rate increased, there was no increase in total RNA content. Since most of the RNA analyzed is rRNA, this finding implies that increased protein synthesis occurred without a detectable increase in ribosomes. In turn, these data suggest that this early protein synthesis rate increase was likely due to enhanced efficiency of existing ribosomes in producing proteins.

We conclude that MHC protein synthesis rate increases in acute severe pressure overload but not in severe acute low-pressure volume overload. These findings may help to explain quantitative differences in the magnitude of hypertrophy seen in pressure versus volume overload. These data also indicate a qualitative difference in the way the left ventricular myocardium responds to different types of hemodynamic stress.

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