Suppression of Protein Degradation in Progressive Cardiac Hypertrophy of Chronic Aortic Regurgitation

Norman M. Magid, MD; Jeffrey S. Borer, MD; Mason S. Young, MD; Donald C. Wallerson, MD; and Chinnavuth DeMonteiro, MD

Background. The heart adapts to the volume overload of aortic regurgitation with dilation and hypertrophy. The development of left ventricular hypertrophy at the protein level is a dynamic process resulting from an imbalance between cardiac protein synthesis and degradation. The objective of the present study was to determine in vivo the relative contributions of cardiac protein synthesis and degradation to the progressive hypertrophy that occurs in response to chronic aortic regurgitation and to compare these with responses earlier in the course of this stress.

Methods and Results. Continuous intravenous infusions of [3H]-leucine were administered 3 days and 1 month after surgical induction of aortic regurgitation and sham operation in rabbits. Total cardiac protein and myosin heavy chain fractional synthesis rates were obtained by analysis of plasma and protein hydrolysate data using [3H]-dansyl chloride assays. Left ventricular growth rates were determined from serial echocardiographic and postmortem left ventricular weight and protein concentration measurements; protein degradation rates were determined by subtraction of growth rates from synthesis rates.

Conclusions. In comparison with sham-operated control rabbits, protein fractional synthesis rates were increased at 3 days but not at 1 month after induction of aortic regurgitation. Progressive cardiac hypertrophy occurring at 1 month was caused by a decrease in protein fractional degradation rates. An increase in protein synthesis contributes only to the early phase of hypertrophy caused by acute aortic regurgitation, whereas progressive eccentric hypertrophy in chronic volume overload is due to suppression of protein degradation. (Circulation 1993;87:1249-1257)

Key Words • proteolysis • protein synthesis • heart disease, valvular • volume overload

In experimental animals and in humans, the heart adapts to the volume overload of aortic regurgitation with left ventricular dilation and hypertrophy.1-3 Left ventricular mass at the protein level can be viewed as a dynamic process resulting from the balance between cardiac protein synthesis and degradation. In the normal heart, left ventricular mass is stable and rates of protein synthesis and degradation are equal. However, in the enlarging heart, the development of cardiac hypertrophy results from an imbalance between protein synthesis and degradation.4-7 Thus, cardiac growth can be caused either by an increase in protein synthesis or a decrease in protein degradation. Many studies of pathological hypertrophy have shown increases in protein synthesis early after stimulus introduction.6-8-17 However, few have assessed protein metabolism beyond this early time, fewer still have assessed the impact of alterations in protein degradation rate, and none has provided both these assessments in eccentric hypertrophy caused by chronic volume overload. The present study was undertaken to determine the relative contributions of cardiac protein synthesis and degradation to the progressive cardiac hypertrophy that occurs in response to chronic volume overload caused by aortic regurgitation.

Recently, we developed an animal model of chronic aortic regurgitation that simulates the human disease in terms of left ventricular hypertrophy, dilation, and performance characteristics, making characterization of cellular processes of particular interest.2,3 In this model, left ventricular growth manifests a curvilinear relation, which can be approximated to consist of two phases, after which a steady state again develops.2,3 Our data indicate that left ventricular mass increases most rapidly during the first week after induction of aortic regurgitation. The second phase is characterized by a continued increase in left ventricular mass at a slower rate during the following 2-3 months, after which a steady state (plateau) is reestablished.2,3 In the present study, we undertook assessments of total cardiac protein synthesis and degradation during the middle portions of both the acute and progressive phases of growth, at 3

From the Division of Cardiology, Department of Medicine, Cornell University Medical College, The New York Hospital–Cornell Medical Center, New York, N.Y.

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Address for correspondence: Norman M. Magid, MD, Starr 463, The New York Hospital–Cornell Medical Center, 525 E. 68th St., New York, NY 10021.

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days, and at 1 month after induction of aortic regurgitation to determine their relative contributions to the development of left ventricular hypertrophy. In addition, since total cardiac protein comprises many structurally and functionally different intracellular and extracellular molecules obtained from several types of cells, we also undertook to define synthesis and degradation rates of myosin heavy chain, reasoning that this contractile protein found primarily within cardiac myocytes is of particular interest because of its functional importance in our experimental model.

**Methods**

**Reagents**

L-[4,5-3H]-leucine (120 Ci/mmol) was obtained from Amersham, Arlington Heights, Ill. Methyl [14C]-dansyl chloride (112 mCi/mmol) was obtained from Research Products International Corp., Mount Prospect, Ill. All other reagents were obtained from Sigma Chemical Co., St. Louis, Mo., or J.T. Baker Chemical Co., Phillipsburg, N.J.

**Animal Population**

Our study population consisted of a total of 55 New Zealand White rabbits (Hazleton Research Products, Inc., Denver, Pa.). Aortic regurgitation was surgically induced in 24 rabbits; 12 of these animals were killed 3 days after and 12 animals were killed 1 month after induction of aortic regurgitation for biochemical studies. Sham operation (see below) was performed in 12 rabbits; six of these animals were killed 3 days after and six animals were killed 1 month after sham operation for biochemical studies. Three-day and 1-month time points were selected for biochemical assessments because these corresponded to the middle portions of the acute and progressive growth phases that occurred in our aortic regurgitation model.2 Seven normal rabbits that did not undergo surgery were killed for biochemical studies. Twelve additional normal rabbits were studied with serial echocardiography for 6 months to determine the normal growth of left ventricular mass over time. Normal rabbits were selected so that initial body and left ventricular weights were matched to those of the aortic regurgitant and sham-operated animals. Before operation, average body weight was 2.5±0.3 kg among rabbits in which aortic regurgitation was induced and 2.5±0.2 kg among sham-operated control rabbits; initial weight was 2.4±0.1 kg among normal control rabbits.

**Surgery**

To induce aortic regurgitation and quantify its severity, a sharp 5F catheter was advanced retrogradely through an aortic valve cusp, and an electromagnetic flow probe was placed around the ascending aorta via thoracotomy, as we have described previously.2,3 After the aortic valve was perforated, the catheter was withdrawn, the regurgitant fraction (the ratio of retrograde to antegrade flow) was measured, and the catheter was advanced through the aortic valve a second or third time as necessary to attain a mild to moderate degree of aortic regurgitation (acute regurgitant fraction approximately 30%; range, 20–50%). Sham-operated rabbits were prepared in identical fashion except that the aortic valve was not perforated. Dietary supplementation (12–15 mL daily, Nutri-Cal, Evsco Pharmaceuticals, IGI, Inc., Buena, N.J.), systemic antibiotics, and analgesics were administered.

**Echocardiography**

Two-dimensional, M-mode, and Doppler echocardiograms were performed before the operation and at 3 days and 1, 2, 3, and 4 weeks after the operation in aortic regurgitant and sham-operated animals and at monthly intervals in the normal rabbits in the manner we have described previously.2,3,18 Left ventricular internal dimension (LVID), posterior wall thickness (PW), and interventricular septal thickness (IVS) were measured at end diastole from M-mode echocardiograms according to the American Society of Echocardiography recommendations.19 Left ventricular mass was calculated from the cube function formula \( \text{LVM (g)} = [\text{LVID} + \text{IVS} + \text{PW}]^3 - \text{LVID}^3 \times 1.05 \), where 1.05 = specific gravity of myocardium), a measurement that we previously demonstrated closely with anatomic left ventricular weights in both normal and aortic regurgitant rabbits.18

**[3H]-Leucine Infusion**

A continuous intravenous infusion of 1.2 mCi of L-[4,5-3H]-leucine was administered over 6 hours, serial arterial blood samples were drawn, and plasma leucine-specific radioactivity was determined using N-methyl [14C]-dansyl chloride as previously described.6,7,20,21 Plasma leucine-specific radioactivity measurements were used to assess leucyl-tRNA–specific radioactivity permitted by rapid equilibration between compartments.6,8,22–24 Animals then were killed by intravenous barbiturate administration, and the hearts were rapidly removed.

**Protein Samples**

Left ventricular free wall tissue was homogenized in low-salt buffer (LSB; 100 mM KCl, 5 mM MgCl₂, 5 mM EGTA, 1 mM dithiothreitol, 10 mM sodium phosphate, pH 6.8) and protease enzyme inhibitors (leupeptin, 5 µg/mL; antipain, 5 µg/mL; E64, 1 µg/mL; PMSF, 0.1 mM; aprotonin, 1 µg/mL). Total cardiac protein was obtained from the homogenate by precipitation with 10% (wt/vol) trichloroacetic acid, and myofibrillar protein was obtained by washing the homogenate in LSB and LSB containing 0.5% Triton X-100. Myosin heavy chain was obtained from total myofibrillar protein by preparative sodium dodecyl sulfate polyacrylamide gel electrophoresis, as previously described.7,25 Total cardiac protein and myosin heavy chain leucine-specific radioactivities were determined from protein hydrolysates using an isotope dilution dansylation procedure, as previously described.6,7,20,21 Total cardiac protein and total myofibrillar protein concentrations were determined by the Lowry method.26 Myosin heavy chain concentration was determined by densitometry27 using a laser densitometer and computer-assisted analyses of polyacrylamide gels containing myofibrillar protein and myosin heavy chain standards (UltroScan XL laser densitometer, Gelscan XL software package, Pharmacia LKB Biotechnology, Piscataway, N.J.). A portion of the left ventricle was dried to constant weight at 106°C for determination of dry weight to wet weight ratios.
Protein Synthesis, Growth, and Degradation Rates

The cardiac protein fractional synthesis rates (%/d, percentage per day) were measured from determinations of the specific radioactivity of protein precursor and the specific radioactivity subsequently found in the resulting cardiac proteins, as previously described.4-7,9 This determination is based on the observation that plasma leucine–specific radioactivity reflects that of cardiac leucyl-tRNA, the amino acyl-tRNA precursor of cardiac proteins.6-8,22-24 Specific radioactivities of plasma leucine (F*) and protein (P*) were determined as described above, and first-order rate constants (kf) and fractional synthesis rates (KF) were determined from the equations F*=F*max (1-e-kt) and KF=P*/[F*max {T/(1-e-kt)-(1/kf)}], where F*max=plateau plasma leucine–specific radioactivity and T=time.4-7,9

The amounts of total cardiac protein and myosin heavy chain synthesized daily by 1 g of myocardium (mg/g/d) were calculated by multiplying the protein fractional synthesis rates (%/d) by the protein concentrations (mg/g), and the amount of protein synthesized daily by the entire left ventricle (mg/LV/d) was calculated by multiplying by the left ventricular weight (g).

The total cardiac protein and myosin heavy chain fractional degradation rates were determined by subtraction of protein fractional growth rates from protein fractional synthesis rates, KF=Kt-Kr (see References 4-7).

Left ventricular growth curves were assessed from preoperative and serial postoperative left ventricular weights determined from echocardiographic left ventricular mass measurements made before the animal was killed and anatomic left ventricular weights measured immediately after. Premortem left ventricular weight was determined from echocardiographic left ventricular mass measurements using regression equations that we previously validated in both normal and aortic regurgitant rabbits;15 in these animals, left ventricular mass measurements had standard errors of the estimate of 0.59 g and 0.71 g, respectively.18

In the present study, the relation between left ventricular weight and time after surgery in aortic regurgitant animals was assessed by regression analyses of both mean and individual left ventricular weight data. To compare linear and exponential mathematical models, the equations y=m*x+b and y=m*ln(1+x)+b, where y=LV wt (g), m=slope (g/day), x=time, and b=intercept, were applied to preoperatively obtained left ventricular weights (day 0) and at 3, 7, 14, 21, and 28 days. Left ventricular growth rates in aortic regurgitant animals were estimated from the first derivative of the exponential equation above, y=m/(1+x), at the midpoints of the rapid and progressive growth phases, 3 and 28 days, respectively.

Left ventricular growth rate was estimated in sham-operated animals from linear regression analysis of mean left ventricular weight determined from echocardiograms performed before the operation (day 0) and at 3, 7, 14, and 21 days after operation and from mean anatomic left ventricular weight measured directly after the animal was killed at 1 month. Left ventricular growth rate was estimated in normal animals from the slope of the line between the mean echocardiographic left ventricular weight determined at the initial matched body weight and that obtained 1 month later. Total cardiac and myosin heavy chain fractional growth rates (%/d) were determined by multiplying protein concentration (mg/g) by the left ventricular growth rate (g/d) and dividing by protein content (mg).

The amounts of total cardiac protein and myosin heavy chain degraded daily by the entire left ventricle (mg/LV/d) was determined by subtraction of the amount of protein accumulated daily from the amount of protein synthesized daily by the entire left ventricle.6,7 Total cardiac and myosin heavy chain accumulated daily by the entire left ventricle (mg/LV/d) was determined by multiplying protein concentration (mg/g) by the left ventricular growth rate (g/d).

Plasma Leucine Flux

Plasma leucine flux, a measurement used to estimate whole-body amino acid metabolism and protein turnover, can be calculated from the infusion rate of l-[4,5-3H]-leucine and plateau plasma leucine–specific activity, F*max (Reference 7). During continuous infusion of a radiolabeled amino acid, the specific radioactivity of that amino acid in the plasma reaches a plateau value when the amount entering the plasma compartment equals the amount leaving the plasma compartment. Plasma leucine flux represents the rate of metabolism of free amino acid from the free amino acid pool to whole-body protein synthesis, oxidation, other chemical reactions, and excretion. Plasma leucine flux was calculated from this formula: leucine flux (μmol/hr)=infusion rate (μCi/hr)/[F*max (μCi/μmol)×100 g body wt] (Reference 7).

Statistical Analyses

Results were expressed as mean±SD unless otherwise indicated. Linear regression of left ventricular weight and time data was used to determine left ventricular growth rates, and nonlinear regression was used to determine first-order rate constants (kf) from plasma-specific radioactivity data using the Marquardt algorithm and a statistical software program (CRUNCH Software Corporation, Oakland, Calif.). A two-way randomized block analysis of variance followed by Dunnett’s test was performed to compare multiple samples and baseline measurements; Newman-Keuls test was performed for statistical comparisons of sequential multiple samples.28 Statistical comparisons between two samples were made with the Student’s paired and unpaired t tests.28 A value of p<0.05 was considered significant.

The experimental protocol was reviewed and approved by the Cornell Institutional Animal Care and Use Committee in accordance with U.S. Public Health Service policy.

Results

Left Ventricular Growth and Protein Content

Aortic regurgitation (acute regurgitant fraction, 31±6%) was surgically induced in 24 rabbits that exhibited marked hypertrophy compared with control rabbits (Figure 1). Left ventricular weight increased approximately 30% in aortic regurgitant rabbits during the first month after surgery, from 3.3±0.6 g before operation to 3.8±0.7 g at 3 days and 4.3±0.6 g at 1 month (both p<0.01) after operation (3 days versus 1 month, p<0.07;
Figure 1. Curves of left ventricular (LV) growth during the development of LV hypertrophy caused by aortic regurgitation (AR). Mean LV weights (g) ±SEM are represented by diamonds (*), squares (□), and triangles (△) in AR, sham-operated, and normal animals, respectively. The results of linear regression analyses of LV weight vs. time (r=0.7–0.9), LV growth, are indicated by an exponential curve (y=m*ln[1+x]+b) for aortic regurgitant rabbits and by straight lines (y=mx+b) for sham-operated and normal animals. Identical growth curves were obtained for myosin heavy chain, myofibrillar protein, and total cardiac protein content except that protein content (mg) rather than LV weight (g) defined the ordinate (see text).

Figure 1). Left ventricular weight decreased in sham-operated animals after carotid cutdown and thoracotomy, from 3.6±0.5 g before operation to 3.4±0.3 g at 1 month, with the variation from 3 days to 1 month attaining statistical significance (p<0.02), even though supplemental nutrition was administered routinely after surgical intervention (Figure 1). Left ventricular weight increased minimally in normal animals that underwent no surgical intervention (Figure 1).

The results of regression analyses of left ventricular weight and time data for aortic regurgitant, normal, and sham-operated rabbits are shown in Figure 1. Regression analysis of mean left ventricular weights obtained before operation (day 0) and at 7, 14, 21, and 28 days after operation in aortic regurgitant animals using the exponential equation y=m*ln(1+x)+b yielded an r value of 0.93, whereas regression analysis using the linear equation y=mx+b yielded an r value of 0.78. The rates of increase in mean left ventricular weight (g/d) were therefore obtained from the first derivative of the exponential equation y'=m/(1+x) at 3 and 28 days. Regression analyses of left ventricular weight data for mean data yielded rates of increase in left ventricular weight and fractional growth rates that were similar to those determined from individual animals. Fractional growth rates for mean data were 2.4 and 0.7 (%±SEM) %/d compared with 2.7±1.6 and 0.7±0.6 %/d for individual animals at 3 and 28 days, respectively.

The relation of protein content versus time was identical with the relation of left ventricular weight versus time (the left ventricular growth curves, Figure 1) among aortic regurgitant, sham-operated, and normal rabbits as shown in Figure 1, except that myosin heavy chain, total myofibrillar protein, and total cardiac protein content (mg) rather than left ventricular weight (g), was plotted on the ordinate. Myosin heavy chain content (mg) was increased 13% (p=NS) at 3 days and 72% (p<0.05) at 1 month after induction of aortic regurgitation compared with sham-operated control rabbits, documenting the development of myocyte hypertrophy in parallel with increases in left ventricular weight (Table 1 and Figure 1). Myofibrillar protein and total cardiac protein content (mg) after 1 month of aortic regurgitation were increased 49% and 17%, respectively, although these variations did not attain statistical significance in comparison with sham-operated control rabbits (Table 1). Protein content (mg), the product of protein concentration (mg protein/g myocardial tissue) and left ventricular weight (g), was increased in aortic regurgitant animals because left ventricular weight was increased (Table 1 and Figure 1). Myosin

| Table 1. Cardiac Protein Content, Concentration, and Left Ventricular Anatomic and Body Weight in Aortic Regurgitant and Sham-Operated Control Rabbits 3 Days and 1 Month After Surgical Intervention and in Normal Animals |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                 | Normal (n=7)    | 3-Day sham (n=6) | 3-Day AR (n=12) | 1-Month sham (n=6) | 1-Month AR (n=12) |
| MHC (mg)                       | 81.9±24.8       | 85.3±20.3       | 96.3±40.7       | 79.3±25.2       | 136.4±77.3*     |
| Myofibrillar protein (mg)      | 138.4±16.9      | 156.7±41.3      | 156.4±59.8      | 194.0±86.0      | 289.6±139.7     |
| TCP (mg)                       | 461.8±44.8      | 516.0±66.8      | 565.3±179.9     | 569.6±90.1*     | 665.1±88.6†     |
| MHC (mg/g)                     | 26.5±7.0        | 24.9±4.6        | 27.5±11.1       | 23.5±6.1        | 32.7±19.3       |
| MHC/myofibrillar protein (%)   | 48.7±6.2        | 46.4±5.1        | 52.0±11.6       | 44.5±4.3        | 49.8±7.0        |
| Myofibrillar protein (mg/g)    | 45.0±5.4        | 44.5±11.2       | 44.4±14.5       | 57.0±19.8       | 67.4±27.6       |
| TCP (mg/g)                     | 149.7±8.5       | 150.5±12.4      | 161.0±48.3      | 169.5±17.8      | 156.5±6.4       |
| LV wt (g)                      | 3.1±0.2         | 3.4±0.2         | 3.5±0.4         | 3.4±0.2         | 4.3±0.6†        |
| LV dry/wet wt (ratio)          | 0.2±0.0         | 0.2±0.0         | 0.2±0.0         | 0.2±0.0         | 0.2±0.0         |
| Body wt (kg)                   | 2.4±0.1         | 2.4±0.1         | 2.4±0.2         | 2.7±0.3         | 2.5±0.2         |
| AR (%)                         | 0               | 0               | 33±7            | 0               | 29±5            |

AR, aortic regurgitant rabbits; MHC, myosin heavy chain; TCP, total cardiac protein; LV, left ventricular.

*p<0.05; †p<0.06; ‡p<0.01, AR vs. sham-operative value at same time interval or sham-operative vs. normal (no surgical intervention) value.
TABLE 2. Comparison of Cardiac Protein Fractional Synthesis, Degradation, and Growth Rates in Aortic Regurgitant and Sham-Operated Control Rabbits 3 Days and 1 Month After Surgical Intervention and in Normal Animals

<table>
<thead>
<tr>
<th></th>
<th>Normal (n=7)</th>
<th>3-Day sham (n=6)</th>
<th>3-Day AR (n=12)</th>
<th>1-Month sham (n=6)</th>
<th>1-Month AR (n=12)</th>
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<tbody>
<tr>
<td>TCP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_s$ (%/d)</td>
<td>4.8±0.8</td>
<td>4.8±0.8</td>
<td>6.5±1.3*</td>
<td>5.7±0.4</td>
<td>4.9±0.8*</td>
</tr>
<tr>
<td>$K_d$ (%/d)</td>
<td>4.7±0.8</td>
<td>5.2±0.8</td>
<td>4.1±1.3†</td>
<td>6.2±0.4</td>
<td>4.2±0.8*</td>
</tr>
<tr>
<td>$K_r$ (%/d)</td>
<td>+0.05</td>
<td>-0.4</td>
<td>+2.4</td>
<td>-0.5</td>
<td>+0.7</td>
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<tr>
<td>MHC</td>
<td></td>
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</tr>
<tr>
<td>$K_s$ (%/d)</td>
<td>8.6±6.1</td>
<td>5.0±2.6</td>
<td>6.3±5.7</td>
<td>9.9±2.7</td>
<td>7.3±4.1</td>
</tr>
<tr>
<td>$K_d$ (%/d)</td>
<td>8.6±6.1</td>
<td>5.4±2.6</td>
<td>3.9±5.7</td>
<td>10.2±2.6</td>
<td>6.6±4.1†</td>
</tr>
<tr>
<td>$K_r$ (%/d)</td>
<td>+0.05</td>
<td>-0.4</td>
<td>+2.4</td>
<td>-0.5</td>
<td>+0.7</td>
</tr>
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</table>

AR, aortic regurgitant rabbits; TCP, total cardiac protein; $K_s$, fractional synthesis rate; $K_d$, fractional degradation rate; $K_r$, fractional growth rate; MHC, myosin heavy chain.

*p<0.01, †p<0.05, AR vs. sham-operative value at same time interval or sham-operative vs. normal (no surgical intervention) value.

Myosin Heavy Chain Fractional Synthesis and Degradation Rates

Similar directional relations between protein fractional synthesis and degradation rates were found for myosin heavy chain and total cardiac protein (Table 2), although statistically significant variations were not found for myosin at all relevant observations. At 3 days after surgical induction of aortic regurgitation, myosin heavy chain fractional synthesis rate was increased 26% and fractional degradation rate was decreased 28%, although these variations did not attain statistical significance in comparison with sham-operated control rabbits (Table 2). At 1 month after induction of aortic regurgitation, myosin fractional synthesis rate decreased 26% ($p=NS$) and myosin fractional degradation rate decreased 35% ($p<0.05$) compared with sham-operated control rabbits (Table 2). Thus, the continued increase in myosin heavy chain content during the slower, progressive growth phase was due to a reduction in myosin fractional degradation rate, paralleling the findings for total cardiac protein.

Plasma- and Protein-Specific Radioactivity

Total cardiac protein fractional synthesis rates were importantly influenced by variations in the plateau plasma-specific radioactivity data from which they were calculated (Table 3). At 3 days after surgical induction of aortic regurgitation, plateau plasma leucine–specific radioactivity was 20% lower than in sham-operated animals ($p<0.05$). At 1 month after induction of aortic

TABLE 3. Comparison of Plasma and Cardiac Protein–Specific Radioactivity and Plasma Leucine Flux in Aortic Regurgitant and Sham-Operated Control Rabbits 3 Days and 1 Month After Surgical Intervention and in Normal Animals

<table>
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<tr>
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<th>3-Day AR (n=12)</th>
<th>1-Month sham (n=6)</th>
<th>1-Month AR (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{plateau}$ (dpm/nmol)</td>
<td>625±123</td>
<td>610±79</td>
<td>490±125†</td>
<td>513±54</td>
<td>657±121*</td>
</tr>
<tr>
<td>Leu flux (µmol/hr/100 g)</td>
<td>30.3±6.5</td>
<td>30.8±3.3</td>
<td>41.3±17.3‡</td>
<td>33.2±2.6</td>
<td>27.7±4.5*</td>
</tr>
<tr>
<td>TCP $P*$ (dpm/nmol)</td>
<td>7.0±1.5</td>
<td>7.0±1.9</td>
<td>7.7±2.3</td>
<td>7.0±0.8</td>
<td>7.4±0.6</td>
</tr>
<tr>
<td>MHC $P*$ (dpm/nmol)</td>
<td>12.0±6.4</td>
<td>7.3±4.2</td>
<td>7.2±5.2</td>
<td>12.7±3.5</td>
<td>10.8±5.1</td>
</tr>
</tbody>
</table>

AR, aortic regurgitant rabbits; $F_{plateau}$, plateau plasma leucine–specific radioactivity; Leu flux, plasma leucine flux; TCP, total cardiac protein; $P*$, protein-specific radioactivity; MHC, myosin heavy chain.

*p<0.01, †p<0.05, ‡p<0.06, AR vs. sham-operative value at same time interval or sham-operative vs. normal (no surgical intervention) value.

heavy chain, myofibrillar protein, and total cardiac protein concentrations (mg protein/g myocardial tissue) and myosin heavy chain concentration expressed as percentage of total myofibrillar protein were not significantly different among aortic regurgitant, sham-operated, and normal rabbits (Table 1).

Total Cardiac Protein Fractional Synthesis and Degradation Rates

Total cardiac protein fractional synthesis and degradation rates at 3 days and 1 month after surgical induction of aortic regurgitation are compared with those of sham-operated values in Table 2. At 3 days after induction of aortic regurgitation, total cardiac protein fractional synthesis rate was increased 35% ($p<0.01$) and total cardiac protein fractional degradation rate was decreased 22% ($p<0.05$) compared with sham-operated control rabbits; the rapid increase in total cardiac protein content during the rapid growth phase was due both to an increase in total cardiac protein fractional synthesis rate and to a decrease in degradation rate. At 1 month after induction of aortic regurgitation, total cardiac protein fractional synthesis rate decreased 14% ($p<0.01$) compared with sham-operated control rabbits, and total cardiac protein fractional degradation rate decreased 32% ($p<0.0001$). Thus, the continued increase in total cardiac protein content during the slower, progressive growth phase was due solely to a reduction in total cardiac protein fractional degradation rate.
regurgitation, plateau plasma leucine-specific radioactivity was 28% higher than in sham-operated animals \((p<0.01)\). Total cardiac protein fractional protein synthesis rates \(K_c\) are inversely proportional to plateau plasma-specific radioactivity \(F^*_{\text{max}}\) and directly proportional to protein-specific radioactivity \(P^*\) measurements (see equation above). Therefore, even though there were no significant variations in \(P^*\) for total cardiac protein (Table 3), \(K_c\) for total cardiac protein was significantly increased at 3 days and decreased at 1 month after induction of aortic regurgitation (Table 2) because of the significant variations in \(F^*_{\text{max}}\) occurring at these time intervals (Table 3).

Myosin heavy chain fractional synthesis rates were similarly influenced by variations in the \(F^*_{\text{max}}\) data from which they were calculated (Table 3). Thus, even though there were no marked variations in \(P^*\) for myosin heavy chain (Table 3), \(K_c\) for myosin was directionally increased at 3 days and decreased at 1 month after induction of aortic regurgitation (Table 2) because of the significant variations in \(F^*_{\text{max}}\) occurring at these time intervals (Table 3).

\(F^*_{\text{max}}\) variations appeared to be due to time-dependent alterations in whole-body free leucine metabolism in aortic regurgitant animals. Leucine flux was 34% higher at 3 days \((p<0.06)\) and 17% lower at 1 month \((p<0.01)\) in aortic regurgitant than in sham-operated animals (Table 3).

Alterations in total cardiac protein and myosin heavy chain fractional synthesis and degradation rates in aortic regurgitant animals cannot be explained by alterations in nutritional status or weight loss. Total body weight 3 days after surgical intervention decreased identically in aortic regurgitant and sham-operated animals, from 2.6±0.2 kg to 2.4±0.2 kg \((p<0.05)\) and from 2.6±0.1 kg to 2.4±0.1 kg \((p<0.05)\), respectively. Total body weight in aortic regurgitant and sham-operated animals was unaltered 1 month after surgical intervention. Preoperative and 1-month postoperative total body weights were 2.6±0.2 kg and 2.5±0.2 kg \((p=\text{NS})\) and 2.6±0.2 kg and 2.6±0.3 kg \((p=\text{NS})\) in aortic regurgitant and sham-operated animals, respectively.

### Table 4. Comparison of Amounts of Cardiac Protein Synthesized, Degraded, and Accumulated Daily by the Entire Left Ventricle in Aortic Regurgitant and Sham-Operated Control Rabbits 3 Days and 1 Month After Surgical Intervention and in Normal Animals

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<th>3-Day AR ((n=12))</th>
<th>1-Month sham ((n=6))</th>
<th>1-Month AR ((n=12))</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCP S (mg/LV/d)</td>
<td>22.0±4.5</td>
<td>24.7±5.4</td>
<td>36.6±13.5†</td>
<td>32.4±7.0*</td>
<td>32.7±7.9</td>
</tr>
<tr>
<td>TCP D (mg/LV/d)</td>
<td>21.8±4.5</td>
<td>26.8±5.6</td>
<td>23.0±10.0</td>
<td>35.2±7.5*</td>
<td>28.0±7.4</td>
</tr>
<tr>
<td>TCP G (mg/LV/d)</td>
<td>+0.22</td>
<td>-2.1</td>
<td>+13.5*</td>
<td>-2.8</td>
<td>+4.7*</td>
</tr>
<tr>
<td>MHC S (mg/LV/d)</td>
<td>8.1±7.5</td>
<td>4.0±1.7</td>
<td>6.6±0.7</td>
<td>7.6±1.5</td>
<td>11.1±13.8</td>
</tr>
<tr>
<td>MHC D (mg/LV/d)</td>
<td>8.0±7.5</td>
<td>4.3±1.7</td>
<td>4.3±6.2</td>
<td>8.0±1.6</td>
<td>10.2±13.4</td>
</tr>
<tr>
<td>MHC G (mg/LV/d)</td>
<td>+0.04</td>
<td>-0.3</td>
<td>+2.3*</td>
<td>-0.4</td>
<td>+1.0*</td>
</tr>
</tbody>
</table>

AR, aortic regurgitant rabbits; TCP, total cardiac protein; S, amount synthesized; D, amount degraded; G, amount accumulated daily by the entire left ventricle (LV); MHC, myosin heavy chain.

\(*p<0.01, \uparrow p<0.05\), AR vs. sham-operate value at same time interval or sham-operative vs. normal (no surgical intervention) value.

#### Total Cardiac Protein Amount Synthesized and Degraded Daily

The amounts of total cardiac protein synthesized and degraded daily by the entire left ventricle (mg/LV/d) at 3 days and 1 month after surgical induction of aortic regurgitation are compared with those of sham-operated animals in Table 4. At 3 days after induction of aortic regurgitation, the amount of total cardiac protein synthesized daily by the entire left ventricle was increased 48% \((p<0.05)\) compared with sham-operated control rabbits, and the amount of total cardiac protein degraded daily was directionally decreased 14% \((p=\text{NS})\). At 1 month after induction of aortic regurgitation, the amount of total cardiac protein synthesized daily by the entire left ventricle was similar in the aortic regurgitant and sham-operated animals (32.7 versus 32.4 mg/d, respectively, \(p=\text{NS}\)), and the amount of total cardiac protein degraded daily was directionally decreased 20% \((p=\text{NS})\), although the aortic regurgitant ventricle was 26% larger than the sham-operated ventricle (Figure 1 and Table 4).

**Myosin Heavy Chain Amount Synthesized and Degraded Daily**

At 3 days and at 1 month after surgical procedures, the amount of myosin heavy chain synthesized daily by the entire left ventricle was directionally similar to that of total cardiac protein (Table 4). However, whereas sham-operated and aortic regurgitant animals did not differ in the amount of myosin heavy chain degraded daily at 3 days, at 1 month, unlike total cardiac protein, the amount of myosin degraded daily by the entire left ventricle was directionally increased when aortic regurgitant were compared with sham-operated values (Table 4). The amounts of myosin heavy chain synthesized and degraded daily by the entire left ventricle (mg/LV/d) had more variability (larger standard deviations) than those of total cardiac protein (Table 4) partly because these values were calculated from protein concentration (mg/g) measurements (Table 1) derived from densitometric analyses of polyacrylamide gels; total cardiac protein concentration (mg/g) measurements were determined directly.

Thus, whereas both fractional synthesis rates and synthesis rates per gram of myocardium increased at 3 days and decreased at 1 month for both total cardiac protein and myosin heavy chain, the absolute amounts of protein synthesized daily by the larger left ventricle of the aortic regurgitant rabbits were greater than control.
at 3 days and also, to a lesser extent, at 1 month. Similarly, whereas both fractional degradation rates and degradation rates per gram of myocardium were decreased in aortic regurgitant compared with sham-operated control rabbits both at 3 days and at 1 month, the absolute amount of myosin heavy chain (but not of total cardiac protein) degraded daily by the larger ventricle of the aortic regurgitant rabbits was slightly greater than control at 3 days and at 1 month.

**Effect of Sham Operation Versus Normal Values**

Total cardiac protein fractional synthesis and degradation rates were not altered by surgical intervention alone, as seen from comparison of 3-day sham-operated rabbits with normal animals (Table 2). However, myosin heavy chain fractional synthesis rate decreased 42% \( (p=\text{NS}) \) and myosin fractional degradation rate decreased 37% \( (p=\text{NS}) \) when 3-day sham-operated rabbits were compared with normal animals (Table 2). At 1 month after surgical intervention, myosin heavy chain fractional synthesis rate increased 98% \( (p<0.05) \) and myosin fractional degradation rate increased 88% \( (p<0.05) \) in sham-operated rabbits compared with the values observed 3 days after sham operation and returned to values similar to those of normal rabbits.

The decreases in left ventricular weight and myosin heavy chain fractional synthesis rate in sham-operated rabbits compared with normal animals may be partially attributable to poorer nutritional status after surgical intervention. Although nutritional supplements were administered, total body weight decreased 8% at 3 days and was not increased at 1 month after surgical intervention in both sham-operated and aortic regurgitant animals. Normal animals increased total body weight about 8% over 1 month. These findings were in accordance with our previous report of the effects of starvation-induced atrophy in rabbits.7

**Discussion**

Cardiac hypertrophy can be induced by a variety of hemodynamic, hormonal, and nutritional stimuli that cause an alteration in the balance between protein synthesis and degradation. In the present study, we found that induction of mild to moderate aortic regurgitation produced rapid, progressive, and marked left ventricular hypertrophy, with a similar time course to what we previously reported in rabbits with comparatively less severe aortic regurgitation.2 The initial, most rapid phase of growth, consisting of a marked increase in total cardiac protein content at 3 days after induction of aortic regurgitation, was due to both an increase in total cardiac protein fractional synthesis rate and a decrease in total cardiac protein fractional degradation rate. However, the continued augmentation of total cardiac protein content during the slower, progressive growth phase evaluated at 1 month was due solely to a reduction in total cardiac protein fractional degradation rate. Results of assessment of myosin heavy chain metabolic kinetics similarly demonstrated that enhanced myosin fractional synthesis rate contributes only to the early, most rapid phase of hypertrophy, whereas later, progressive myocyte hypertrophy was due to the continued suppression of myosin fractional degradation rate. These data represent the first evidence that alteration in protein fractional degradation rate is importantly involved in regulating the development of both early and progressive hypertrophy in an intact animal model of volume overload.

Few earlier investigators have assessed cardiac growth specifically in the setting of aortic regurgitation or volume overload. Nonetheless, in experimental hypertrophy in a variety of settings, an increase in total cardiac protein synthesis has been observed early after various hemodynamic and hormonal stimuli.6,8-17 This finding is paralleled by the results early after induction of aortic regurgitation in the present study. However, the importance of alterations in total cardiac protein degradation to the early development of hypertrophy has not been clearly demonstrated, and assessments using several animal models of disease have provided conflicting results.6,9-13,29

The relative contributions of total cardiac protein synthesis and degradation later during the evolution of cardiac hypertrophy have not been defined previously. Three weeks after surgical intervention, protein synthesis rates in rats were increased in aortic regurgitation but were not increased in aortic banding, whereas at later points up to 8 weeks after surgery, protein synthesis rates no longer were increased in either aortic regurgitation or aortic banding.12 Neither growth nor degradation rates were determined in this study, nor was aortic regurgitation quantified, precluding direct comparison with the present data. However, the lack of an increase in synthesis rates after 1–2 months of aortic regurgitation is consistent with the results of the present study. No prior assessments of both protein synthesis and degradation rates during the course of progressive left ventricular hypertrophy caused by aortic regurgitation or other volume-loading lesions were found on review of the literature.

The results of the present study demonstrate the need to determine fractional synthesis rates and to carefully measure the precursor pool in assessments of protein synthesis in vivo. Although all animals received identical infusions of \( L-[4,5^3H]\)-leucine, time-dependent variations in plateau plasma leucine-specific radioactivity occurred in aortic regurgitant animals that importantly influenced total cardiac protein and myosin heavy chain fractional synthesis rates. Total cardiac protein and myosin heavy chain–specific radioactivity determinations were not notably different, suggesting that measurements of protein incorporation alone would not have revealed important variations in protein synthesis occurring in aortic regurgitant and sham-operated animals.

The reason for the time-dependent variations in plateau plasma leucine–specific radioactivity and leucine flux in aortic regurgitant animals is unclear. We previously reported time-dependent variations in leucine–specific radioactivity and plasma leucine flux influencing protein fractional synthesis measurements during thyrotoxicosis6 and starvation.7 Thyrotoxicosis decreased plasma leucine–specific radioactivity and increased leucine flux,6 whereas starvation increased plasma leucine–specific radioactivity and decreased plasma leucine flux.7 In the aortic regurgitant rabbits, whole-body free leucine metabolic rates appeared to be somewhat higher during rapid hypertrophy at 3 days and lower during progressive hypertrophy at 1 month than in the sham-operated control rabbits, although surgical interventions in the two groups were identical.
other than valve perforation. These variations could not be explained by differences in nutritional status or weight loss. Thus, the observed variations in metabolism between these two groups would appear to be attributable in some way to the hemodynamic effects of aortic regurgitation.

The method used to assess degradation in the present study is similar to that used by several previous investigators in that it is dependent upon determinations of protein fractional synthesis rates, requiring accurate measurements of protein precursors and products, and growth rates, requiring accurate measurements of left ventricular mass and protein concentrations. Like some of the earlier investigators, we used [\(^{1}\)H]-leucine to quantitatively identify protein precursors and products. However, to optimize estimates of left ventricular growth, unlike the earlier investigators, we assessed left ventricular mass serially in the same group of animals that underwent biochemical studies. To this end, we used the noninvasive approach that we previously developed and validated for this purpose. Nonetheless, although our method for degradation rate determination is accurate in theory, our results must be understood within the context of several potential limitations. The sample size was small, the leucyl-tRNA precursor pool was not measured directly, the quantitative method used to assess degradation was indirect and dependent upon an accurate assessment of left ventricular growth rates, and the gel electrophoresis technique did not eliminate other proteins that could have comigrated with myosin heavy chain bands. To minimize the effects of methodological variations in individual animals, we used mean left ventricular weight in calculation of growth rates. Moreover, growth rates calculated in the present study were similar to those previously derived from a greater number of left ventricular weight determinations. Nonetheless, suboptimal precision of growth rate assessment in individual animals may have limited the accuracy of degradation rate determinations.

Importantly, however, we found that total protein and myosin heavy chain fractional synthesis rates were not increased during the progressive cardiac growth occurring 1 month after induction of aortic regurgitation. Although accurate quantitative assessments of fractional growth and degradation rates in individual animals are difficult, as long as some degree of cardiac growth occurred, protein degradation must have decreased proportionately. Cardiac protein growth must have occurred because myosin heavy chain, myofibrillar protein, and total cardiac protein content measurements, derived directly from protein concentrations and anatomic left ventricular weights, were increased in aortic regurgitant animals at 1 month in comparison with sham-operated control rabbits. Therefore, despite the potential difficulties associated with quantitative assessment of protein degradation, a reduction in protein degradation of some degree must have taken place in order to account for the progressive hypertrophy that occurred in our experimental model.

Calculations of total cardiac protein synthesized and degraded daily by the entire left ventricle (mg/LV/d) further supported the importance of the suppression of protein degradation in contributing to the progressive hypertrophy phase of chronic aortic regurgitation. The amount of total cardiac protein synthesized daily by the entire left ventricle was similar in the aortic regurgitant and sham-operated animals, and the amount of total cardiac protein degraded daily was directionally decreased, although the aortic regurgitant ventricle was substantially larger than that of the sham-operated animal.

Myocardial tissue is composed of a variety of cells, including myocytes, fibroblasts, endothelial cells, and interstitium containing many proteins present in varying quantities with varying turnover rates. We found that approximately 50% of the myofibrillar protein content of the ventricle was myosin heavy chain, in accordance with previous reports. Thus, to facilitate understanding of the cellular concomitants of pathophysiological cardiac mechanical changes, information regarding total cardiac protein synthesis and degradation rates was supplemented by assessment of myosin heavy chain metabolism. Information regarding individual proteins in experimental aortic regurgitation and other volume loading lesions has not been presented previously. However, our data obtained early (3 days) after induction of aortic regurgitation generally are consistent with assessments of myosin synthesis obtained early in a pressure-loaded model. No previous studies have examined myosin heavy chain metabolic kinetics at the later stage of growth evaluated in the present study.

The mechanisms responsible for the decreased rates of total cardiac protein and myosin heavy chain degradation resulting in progressive hypertrophy as yet have not been defined. Both lysosomal and nonlysosomal processes may be involved in control of cellular degradation; however, alterations in lysosomal morphology and lysosomal protease activities are not clearly related to protein degradation. Protein degradation may be regulated by a cytosolic ATP-dependent pathway involving the peptide ubiquitin, which has evidenced enhanced localization in specific regions of the myofibril. Multiple potential control sites are suggested by the demonstration that skeletal muscle contains enzymes that both conjugate ubiquitin to protein and degrade ubiquitin–protein conjugates. Enzymes present in rabbit cardiac muscle have the capability to conjugate ubiquitin to many proteins, including calmodulin. Additional pathways potentially responsible for regulation of protein degradation include insulin and insulin-like growth factors that have been shown to inhibit proteolysis.

Our results indicate that alterations in cardiac protein fractional degradation rate are of primary importance in the regulation of the progressive eccentric hypertrophy that occurs in experimental aortic regurgitation. Such alterations may be importantly involved in the cardiac response to aortic regurgitation in humans, although no data as yet are available to assess this possibility. Further study to evaluate the phenomenon that we have observed is appropriate. Such studies should include, among other goals, the determination of 1) protein metabolic kinetics in the setting of severe aortic regurgitation more closely modeling clinically important disease than does the model used in this study, 2) the patterns of protein synthesis and degradation in later stages of eccentric hypertrophy caused by aortic regurgitation close to the time of development of heart failure, and 3) the mechanisms responsible for
regulation of cardiac protein fractional degradation rates.

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