Protein Synthesis and Degradation During Starvation-Induced Cardiac Atrophy in Rabbits

Allen M. Samarel, Michael S. Parmacek, Norman M. Magid, Robert S. Decker, and Michael Lesch

To determine the relative importance of protein degradation in the development of starvation-induced cardiac atrophy, in vivo fractional synthetic rates of total cardiac protein, myosin heavy chain, actin, light chain 1, and light chain 2 were measured in fed and fasted rabbits by continuous infusion of [3H]leucine. In addition, the rate of left ventricular protein accumulation and loss were assessed in weight-matched control and fasted rabbits. Rates of total cardiac protein degradation were then estimated as the difference between rates of synthesis and growth. Fasting produced left ventricular atrophy by decreasing the rate of left ventricular protein synthesis (34.8 ± 1.4, 27.3 ± 3.0, and 19.3 ± 1.2 mg/day of left ventricular protein synthesized for 0-, 3-, and 7-day fasted rabbits, respectively). Inhibition of contractile protein synthesis was evident by significant reductions in the fractional synthetic rates of all myofibrillar protein subunits. Although fractional rates of protein degradation increased significantly within 7 days of fasting, actual amounts of left ventricular protein degraded per day were unaffected. Thus, prolonged fasting profoundly inhibits the synthesis of new cardiac protein, including the major protein constituents of the myofibril. Both this inhibition in new protein synthesis as well as a smaller but significant reduction in the average half-lives of cardiac proteins are responsible for atrophy of the heart in response to fasting. (Circulation Research 1987;60:933–941)

Prolonged fasting produces atrophy and net protein catabolism of the heart and other organs by altering the balance between rates of protein synthesis and degradation. The relative importance of each pathway in the production of starvation-induced atrophy varies for individual tissues and organs. Thus, in liver, fasting rapidly leads to severe deprivation. Few studies, however, have addressed the effects of fasting on myocardial protein metabolism. Furthermore, the literature available with regard to the effect of fasting on cardiac protein synthesis and degradation is conflicting. In all likelihood, much of this uncertainty stems from the extrapolation of results obtained from in vitro preparations, where levels of circulating hormones, substrates, and hemodynamic variables do not accurately reflect in vivo conditions.

In previous studies, we and other investigators have examined the effect of prolonged fasting on the activities, subcellular distribution, transport, and proteolytic processing of cardiac lysosomal proteases and have attempted to relate these results to alterations in the rates of cardiac protein degradation as determined by in vitro perfusion or tissue incubation. While these in vitro techniques have been useful in elucidating the potential mechanisms regulating protein degradation in the atrophying heart, a clear understanding of those factors operative in vivo is not presently available.

Therefore, we undertook the present investigation to measure in vivo rates of total left ventricular protein and individual myofibrillar protein synthesis in fed and fasted rabbits by improving on established methods of continuous intravenous infusion of radiolabelled amino acids to whole animals. In addition, the rates of left ventricular growth and atrophy in both the fed and fasted states were carefully examined. Total left ventricular protein degradative rates were then derived as the difference between rates of synthesis and growth. Data are presented to indicate that fasting profoundly inhibits the synthesis of new cardiac proteins, including the major protein constituents of the myofibril. This inhibition in new protein synthesis, as well as a smaller but significant reduction in the average half-lives of cardiac proteins, contributes to the resultant atrophy of the heart in response to fasting.

Materials and Methods

Reagents

L-[4,5-3H]leucine (120 Ci/mmole) was obtained from Amersham, Arlington Heights, Ill. [Methyl "C]dansyl chloride (112 mCi/mmole) was obtained from Research Products International, Mt. Prospect, Ill. All other reagents were of the highest grade commercially available and were obtained from Sigma Chemical Co., St. Louis, Mo., and Scientific Products, McGaw Park, Ill.
Experimental Animals

Male New Zealand white rabbits (Lesser's Rabbits, Union Grove, Wis.) were used in all experiments. Animals were acclimated to our animal facility for at least 4 days prior to inclusion in either growth or infusion experiments. During that time, they were exposed to an alternating 12-hours-light and 12-hours-dark environment, weighed daily between 8:00 and 10:00 a.m., and fed ad libitum as previously described. Subsequently, some animals were deprived of food but not water for specified periods.

Determination of Growth Parameters and the Rates of Left Ventricular Protein Accumulation in Fed and Fasted Rabbits

Thirty-two rabbits with initial body weights between 1.95 and 2.05 kg were used to determine the rate of total left ventricular (LV) protein accumulation during normal cardiac growth. This weight range was chosen to reflect the rate of LV protein accumulation in fed (0-days fasted) rabbits used in protein synthesis experiments. Rabbits were randomly assigned to groups that were killed after 0, 3, 6, 9, or 12 days of ad libitum feeding.

Sixty-five rabbits were used to determine the rate of total LV protein loss in fasted rabbits. These animals all had initial body weights between 1.90 and 2.20 kg, which corresponded to fasted animals used in the infusion experiments. Rabbits were randomly assigned to groups that were killed for 0, 1, 3, 5, 7, or 10 days. At least 5 animals were assigned to each group.

Following either ad libitum feeding or fasting, animals were killed by cervical dislocation. Hearts were excised through a median sternotomy incision and homogenized for four 15-second periods in a Polytron PCU homogenizer. A portion of this homogenate was reserved for protein determination using the method of Lowry et al. 

The rates of LV protein accumulation (or loss) in fed and fasted rabbits were determined by weighted regression analysis comparing LV protein content of each group of rabbits vs. time. Data for both fed and fasted rabbits were fit to first-, second-, and third-degree polynomial equations, and respective R² and standard deviations of regression were determined. Improvement in fit was evaluated by a decrease in the standard deviation of regression with increasing polynomial order. Nonlinear curve fitting and statistical analysis was performed using the PROPHET Computer System (Division of Research Resources, National Institutes of Health).

RNA Determination

A portion of LV septum (125 mg) from fed and fasted rabbits was minced and homogenized in 20 volumes of water. RNA concentration was analyzed using the method of Munro and Fleck.

[3H]Leucine Infusion Technique

The fractional rate of protein synthesis was determined from the specific radioactivity of leucine in plasma and in total cardiac protein following 6 hours of continuous infusion of [3H]leucine in fed (0-day), 3-day, and 7-day fasted rabbits. The infusion method employed was a modification of that of Everett et al. All infusions were begun between the hours of 7:00 and 8:00 a.m. to control any diurnal variation in cardiac protein synthetic rates. Lightly restrained animals were continuously infused with a solution of L-[4,5-3H]leucine, 150 mM NaCl, 10 U/ml heparin, and 100 μM unlabelled leucine (final leucine-specific radioactivity, 1,500 μCi/μmol) at a rate of 1.33 ml/h (200 μCi/h) into a catheterized marginal ear vein. Blood samples of (0.6–1.0 ml) were withdrawn into a heparinized syringe from the catheterized central artery of the opposite ear at 0, 5, 10, 20, 30, 60, 120, 180, 240, 300, and 360 minutes during the infusion. The volume of blood withdrawn was replaced by an equal volume of sterile saline. After completion of the infusion, the rabbits were killed by cervical dislocation, and the hearts were removed and weighed as described above. LV tissue was then frozen in liquid N₂ and stored at −80°C.

Determination of Leucine-Specific Radioactivity in Plasma Samples

Blood samples obtained during [3H]leucine infusions were centrifuged at 10,000 g for 5 minutes, and the plasma (300 μl) was deproteinized by addition of an equal volume of 10% (w/v) trichloroacetic acid (TCA). Amino acids were isolated by cation exchange chromatography as described by Everett et al. Leucine-specific radioactivity was determined using the method of Airhart et al.

Isolation of Individual Myofibrillar Proteins

Radioactively labelled left ventricular tissue of fed and fasted rabbits (2 g) was homogenized in 18 ml of 100 mM KCl and 250 μM thiglycolate, pH 6.8. After removal of portions of the homogenate for protein determination (50 μl) and total cardiac protein (TCP) leucine-specific radioactivity (250 μl), the homogenate was centrifuged at 10,000 g for 10 minutes. The sediment was washed as described by Martin et al., excluding the washes with Mg-ATP relaxing solution. Myofibrillar proteins were extracted from the washed sediment by suspension in 30 ml of high salt buffer (600 mM NaCl, 20 mM MgCl₂, 100 μM dithiothreitol, 100 μM ethyleneglycol-bis(β-amino-ethyl ether) N₂,N’-tetra-acetic acid (EGTA), 10 mM ATP,
and 5 mM Na₃HPO₄, pH 7.0) as described by Clark et al. Following centrifugation at 10,000g for 10 minutes, the supernatant fraction was adjusted to 35% saturation by the addition of saturated ammonium sulfate in water. The precipitate (containing actin) was collected by centrifugation and washed twice with water prior to suspension in 1 ml of 62.5 mM Tris-HCl, pH 6.8, containing 5% (v/v) 2-mercaptoethanol and 2% (w/v) sodium dodecyl sulfate. The supernatant solution from the first addition of saturated ammonium sulfate solution was adjusted to 45% saturation, and the precipitate collected by centrifugation. This sediment (containing myosin and other myofibrillar proteins) was dissolved in 5 ml of high salt buffer and dialyzed (18 hours, 4°C) against low salt buffer (20 mM NaCl, 20 mM MgCl₂, 100 µM dithiothreitol, 100 µM EGTA, and 5 mM Na₃HPO₄, pH 7.0). The precipitated protein formed after dialysis was collected by centrifugation, washed twice with low salt buffer, and dissolved in electrophoresis sample buffer.

Preparative sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed according to Laemmli using 2.7-mm-thick, 200-mm-long, 7-17% gradient vertical slab gels in a Pharmacia GE 2/4LS electrophoresis apparatus (Pharmacia Fine Chemicals, Uppsala, Sweden). Partially purified actin (0-35% ammonium sulfate saturation) and myosin (35-45% ammonium sulfate saturation) in sample buffer were applied to 10-12 lanes of individual gels. After electrophoresis, gels were stained with Coomassie brilliant blue R-250 and destained by diffusion. Gel bands containing actin, myosin heavy chain (MHC), myosin light chain 1 (LC1), and myosin light chain 2 (LC2) were identified by coelectrophoresis with adjacent purified protein standards, were cut from the gel slabs, and were frozen at −20°C.

**Determination of Leucine-Specific Radioactivity in Total Cardiac Protein and Individual Myofibrillar Proteins**

Portions of the total homogenate of radioactively labelled tissue (250 µl) were added to an equal volume of 10% (w/v) TCA. The insoluble protein was washed 4 times by suspension and recentrifugation (10,000g, 5 minutes) in 1.5 ml of 10% TCA. Following a final wash in ethyl ether (1.5 ml), the protein sediments were transferred to vacuum hydrolysis tubes (Pierce Chemical Co., Pierce Chemical Co., Rockford, Ill.) and hydrolyzed in 2 ml of 6N HCl (110°C, 24 hours). After removal of acid by evaporation, the dried residues were dissolved in 1 ml of 100 mM sodium carbonate-bicarbonate buffer, pH 9.0.

Gel bands containing isolated myofibrillar proteins were hydrolyzed in 6N HCl (3-5 ml/10 gel bands) at 110°C for 24-48 hours using minor modifications of the method of Airhart et al. Following hydrolysis, the dissolved gels were precipitated by placing the hydrolysates in a −20°C freezer for 2 hours. Precipitated gel was removed by centrifugation at 4°C (3,000g, 15 minutes), and the supernatant HCl (containing hydrolyzed protein) was evaporated to dryness. The dried samples were then redissolved in 0.5 N KOH (2 ml) to achieve a final pH of 10 or greater, and the samples were reevaporated. This pH adjustment was necessary to remove large amounts of ammonia that were produced during hydrolysis of polyacrylamide gel and that would have interfered with the derivatization of amino acids. Subsequently, the dried residues were redissolved in 0.5 N KOH (1 ml) and freeze-dried. The freeze-dried powder was then redissolved in 0.5-1.0 ml of 100 mM sodium carbonate-bicarbonate buffer, pH 9.0.

Leucine concentration in the TCP and individual myofibrillar protein hydrolysates was determined by isotope dilution, following derivatization with [methyl-¹⁴C]dansyl chloride and separation of dansylated amino acids by thin layer chromatography. For each assay, leucine standards (125-2,000 nmol/ml) were assayed in an identical fashion as the protein hydrolysates. Leucine concentration in unknowns was obtained from a standard curve of [¹³H/¹⁴C] in the dansyl leucine vs. leucine concentration (a reciprocal plot) using an Apple IIe computer and curve-fitting software (Curve Fitter, Ver 1.2, Interactive Microware, Inc., State College, Pa.). [¹³H]leucine radioactivity (DPM/ml) of each protein hydrolysate was obtained by scintillation counting. Data were expressed as DPM/nmol. Preliminary experiments (not shown) demonstrated that the only [¹³H] radioactivity present in the protein hydrolysates was derived from [¹³H] leucine.

**Determination of Fractional Synthethic Rates of Total Cardiac Protein and Individual Myofibrillar Proteins**

The equations used to calculate in vivo fractional synthetic rates of tissue proteins following continuous intravenous infusion of radiolabelled amino acid originally were described by Garlick et al. and were summarized by Everett and Zak. These calculations are dependent on the accurate assessment of the specific radioactivity of the labelled amino acid in the precursor pool used for new protein synthesis. Recent studies have demonstrated the rapid and complete equilibration of leucine between the plasma compartment and leucyl-tRNA in rat heart, rabbit heart, and rat lung. Thus, in these experiments, the specific radioactivity of leucyl-tRNA (the intracellular leucine pool serving as the immediate precursor for new protein synthesis) can be serially estimated during the course of intravenous infusion.

The function describing the equilibration of the precursor-specific radioactivity during infusion is approximated by the following equation:

\[ F^* = F^*_{max} (1 - e^{-Kf t}) \]

where \( t \) is the length of infusion (days), \( F^*_{max} \) is the plasma leucine-specific radioactivity (DPM/nmol) at plateau (i.e., when \( t = \infty \)), and \( Kf \) is the first order rate constant (d⁻¹). In this study, plasma data were analyzed by nonweighted, nonlinear regression analysis in which the experiment results for each infusion experi-
FIGURE 2. Changes in left ventricular protein content in fed and fasted rabbits. Weighted regression analysis was used to compare left ventricular (LV) protein content vs. time in fed (control) and fasted rabbits. Data for each group were fit to first-, second-, and third-degree polynomial equations. Improvement in fit was evaluated by decrease in standard deviation of regression with increasing polynomial order. Best-fit functions for each group of control and fasted animals are depicted above.

proteins after 7 days of food deprivation. Both inhibition of new protein synthesis and an increased fractional rate of protein degradation contributed to cessation of normal cardiac growth and to eventual atrophy of the heart in response to fasting. These data directly support the results of Preedy et al. and indicate that cardiac protein synthesis indeed is much more sensitive to fasting than had previously been suggested.

Fractional Synthetic Rates of Individual Myofibrillar Proteins in Fed and Fasted States

To determine whether fasting was associated with an inhibition in the synthesis of myocyte-specific contractile proteins, the major myofibrillar proteins (actin, MHC, LC1, and LC2) were isolated from LV tissue of 10 fed rabbits, seven 3-day fasted, and eight 7-day fasted rabbits that were infused with [3H]leucine. Gel bands containing purified contractile protein subunits were subjected to acid hydrolysis, and leucine-specific radioactivity was measured by isotope dilution. As seen in Table 5, fractional protein synthetic rates in fed rabbit left ventricle differed among the four protein subunits (LC2 > MHC > LC1 > actin). Starvation was associated with an inhibition in the synthesis of all myofibrillar proteins, but their relative rates of protein synthesis were not altered to a major degree. Thus, after 7 days of fasting, fractional synthetic rates of each protein were significantly depressed, but their rates of synthesis relative to one another (LC2 > MHC > LC1 > actin) were similar to fed animals. Furthermore, the inhibition of actin and LC1 fractional synthesis was proportionally greater than the inhibition of total cardiac protein synthetic rates, indicating that the synthesis of certain myofibrillar proteins may be more sensitive than others to the effects of fasting. Interestingly, Bates et al. also demonstrated a disproportionate reduction in the fractional synthetic rate for skeletal muscle actin synthesis in response to fasting in rats. Overall, however, these data indicate that prolonged fasting leads to a generalized inhibition of myocyte-specific protein synthesis and demonstrate that fasting directly affects protein turnover within the myocytic cell population of cardiac tissue.

Table 2. Characteristics of 0-Day, 3-Day, and 7-Day Fasted Rabbits Used in [3H]Leucine Infusion Experiments

<table>
<thead>
<tr>
<th></th>
<th>0-day (n = 18)</th>
<th>3-day (n = 7)</th>
<th>7-day (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (kg)</td>
<td>2.04 ± 0.04</td>
<td>1.94 ± 0.08</td>
<td>2.09 ± 0.04</td>
</tr>
<tr>
<td>Terminal body weight (kg)</td>
<td>2.04 ± 0.04</td>
<td>1.74 ± 0.09</td>
<td>1.71 ± 0.03</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>4.21 ± 0.08</td>
<td>3.78 ± 0.12</td>
<td>3.67 ± 0.09</td>
</tr>
<tr>
<td>LV weight (g)</td>
<td>2.70 ± 0.05</td>
<td>2.52 ± 0.08</td>
<td>2.35 ± 0.06</td>
</tr>
<tr>
<td>LV protein concentration (mg/g wet wt)</td>
<td>163.0 ± 3.3</td>
<td>167.8 ± 2.5</td>
<td>163.6 ± 4.1</td>
</tr>
<tr>
<td>LV protein content (mg/LV)</td>
<td>440.0 ± 14.4</td>
<td>422.9 ± 16.3</td>
<td>384.8 ± 14.4*</td>
</tr>
</tbody>
</table>

Values are means ± SEM for each group; n, number of experiment animals in each group. p values were obtained by ANOVA followed by Student's-Newman-Keuls test, comparing data from each group of animals fasted for 0, 3, or 7 days. p > 0.10 unless otherwise indicated. *p < 0.05 vs. 0-day animals.

Discussion

Cardiac muscle is composed largely of a complex mixture of intracellular and extracellular proteins possessing relatively long half-lives. Because of these
Cardiac Protein Turnover in Starvation

Table 3. Plasma Leucine-Specific Radioactivity and Leucine Flux in Fed and Fasted Rabbits

<table>
<thead>
<tr>
<th></th>
<th>0-day (n=18)</th>
<th>3-day (n=7)</th>
<th>7-day (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kf (d⁻¹)</td>
<td>200.2 ± 32.1</td>
<td>281.2 ± 62.5</td>
<td>202.2 ± 21.4</td>
</tr>
<tr>
<td>F*max (DPM/nmol)</td>
<td>783.4 ± 47.7</td>
<td>931.4 ± 71.4</td>
<td>1218.4 ± 56.6*†</td>
</tr>
<tr>
<td>Plasma leucine flux (μmol/h/100 g)</td>
<td>30.2 ± 2.5</td>
<td>28.7 ± 2.7</td>
<td>21.6 ± 1.2*</td>
</tr>
</tbody>
</table>

Kf and F*max are computer-derived values for first order rate constant and plasma leucine-specific radioactivity at plateau for each 6-hour infusion experiment, respectively. Values are means ± SEM for each group; n, number of experiment animals in each group. p values were obtained by ANOVA followed by Student’s-Newman-Keuls test, comparing data from each group of animals fasted for 0, 3, or 7 days. p > 0.10 unless otherwise indicated. *p < 0.05 vs. 0-day animals; †p < 0.05 vs. 3-day fasted animals.

Table 4. Left Ventricular Protein Synthesis and Degradation in Fed and Fasted Rabbits

<table>
<thead>
<tr>
<th></th>
<th>0-day (n=18)</th>
<th>3-day (n=7)</th>
<th>7-day (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCP Ks (d⁻¹)</td>
<td>.079 ± .003</td>
<td>.064 ± .006*</td>
<td>.050 ± .002*†</td>
</tr>
<tr>
<td>TCP Kg (d⁻¹)</td>
<td>.019 ± .006</td>
<td>-.007 ± .003*</td>
<td>-.023 ± .008*†</td>
</tr>
<tr>
<td>TCP Kd (d⁻¹)</td>
<td>.060 ± .003</td>
<td>.071 ± .006</td>
<td>.073 ± .002*</td>
</tr>
</tbody>
</table>

TCP Ks, Kg, and Kd are fractional rates of total cardiac protein (TCP) synthesis, growth, and degradation, respectively. Unless otherwise noted, values are means ± SEM for each group; n, number of experiment animals in each group. p values were obtained by ANOVA followed by Student’s-Newman-Keuls test, comparing data from each group of animals fasted for 0, 3, or 7 days. p > 0.10 unless otherwise indicated. *p < 0.05 vs. 0-day animals; †p < 0.05 vs. 3-day fasted animals.
Table 5. Fractional Synthetic Rates of Individual Myofibrillar Proteins in Fed and Fasted Rabbits

<table>
<thead>
<tr>
<th>Protein</th>
<th>0-day (<em>n = 10</em>)</th>
<th>3-day (<em>n = 7</em>)</th>
<th>7-day (<em>n = 8</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC Ks (d⁻¹)</td>
<td>0.072 ± 0.004</td>
<td>0.055 ± 0.005*</td>
<td>0.048 ± 0.002*</td>
</tr>
<tr>
<td>Actin Ks (d⁻¹)</td>
<td>0.040 ± 0.004</td>
<td>0.016 ± 0.001*</td>
<td>0.017 ± 0.002*</td>
</tr>
<tr>
<td>LCI Ks (d⁻¹)</td>
<td>0.054 ± 0.006</td>
<td>0.042 ± 0.007</td>
<td>0.029 ± 0.001*</td>
</tr>
<tr>
<td>LC2 Ks (d⁻¹)</td>
<td>0.081 ± 0.009</td>
<td>0.098 ± 0.015</td>
<td>0.053 ± 0.004*</td>
</tr>
</tbody>
</table>

Values are means ± SEM for each group; *n*, number of experiment animals in each group. *p* values were obtained by ANOVA followed by Student’s-Newman-Keuls test, comparing data from each group of animals fasted for 0, 3, or 7 days. *p > 0.10 unless otherwise indicated. *p < 0.05 vs. 0-day animals; †p < 0.05 vs. 3-day fasted animals.

over contribute to the progressive decline in protein synthesis observed in starvation.

Furthermore, decreased levels of insulin have been shown to produce a block in peptide chain initiation in isolated perfused rat heart, leading to decreased protein synthetic efficiency. Whether low circulating levels of insulin (as seen in prolonged fasting) contribute to decreased cardiac protein synthesis in vivo is not known with certainty. It is likely, however, that starvation-induced alterations in circulating levels of this hormone, as well as growth hormone, thyroid hormones, and noncarbohydrate substrates (all of which have been demonstrated to influence protein synthetic rates in vitro), operate in a complex and interrelated manner to inhibit myocardial-specific protein synthesis in vivo.

In contrast with the results of protein synthetic measurements, our data with regard to the effect of fasting on cardiac protein degradation are more difficult to interpret. It is clear that fasting increased the fractional rate of degradation of cardiac proteins and, thus, shortened their average half-lives. When expressed as the amount of LV protein degraded per day, however, this small difference in fractional degradative rate did not appear to appreciably contribute to the resultant cessation of normal survival and initial LV atrophy since actual amounts of protein degraded per day did not differ significantly between the three experiment groups. We attribute these results to the relatively long average half-life (~12 days) of rabbit left ventricular protein compared with the longest period of observation (7 days) and to the magnitude of change in Kd.

In this regard, it is important to define the two major assumptions applied in these and other studies in which rates of protein degradation are indirectly calculated as the difference between rates of protein synthesis and protein accumulation. First, the measured rate of protein synthesis is assumed to represent an accurate index of the average synthetic rate over the time for which the change in protein content is calculated. Second, the synthetic rates and growth rates measured in different groups of animals are assumed to be valid representatives of these values in any one group. In our study, protein synthetic rates were measured over a relatively long period (6 hours) and were assumed to represent average rates of protein synthesis over the entire day.

Furthermore, infusions were performed in all three groups at the same time each day to control any diurnal variation in cardiac protein synthetic rates. In addition, we were careful in providing healthy, age- and weight-matched animals for growth and infusion experiments to minimize differences in either synthetic rates or growth rates between animals within the two experiment groups. Despite these precautions, Everett and Zak have indicated that in vivo degradative rates during non-steady-state conditions of growth or atrophy can only be estimated, since potentially large errors in estimating growth rates make these indirect measurements less reliable than directly measured protein synthetic rates. These criticisms notwithstanding, we conclude that fasting affects rates of protein synthesis more than rates of protein degradation. These results, however, are in marked contrast to the effect of even brief periods of food deprivation on in vivo hepatic protein degradation and suggest that regulation of protein degradation may be fundamentally very different in the liver and heart.

The rate-limiting steps and proteinases responsible for regulating myocyte protein degradation in response to fasting are not known. Although we and others have identified a number of fasting-induced alterations in the lysosomal-vacuolar apparatus of cardiac muscle, no clear relation exists between these alterations in lysosomal morphology and biochemistry and in vivo rates of protein degradation as described in the present study. In comparison, Mortimore and others have provided a substantial body of data implicating accelerated lysosomal proteolysis as the mechanism responsible for deprivation-induced increased rates of hepatic protein degradation and resultant liver atrophy. Based on the results of this report, we suggest that these previously described alterations in the activity, content, subcellular distribution, and processing of myocytic lysosomal proteinases may be related to the relative sparing of cardiac protein in response to fasting. Further studies are needed, however, to define this relation.

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Cardiac Protein Turnover in Starvation

Samarel et al.  

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KEY WORDS • protein turnover • myofibrillar proteins • fasting • myosin • actin
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