Suppression of Myocardial Protein Degradation in the Rat during Fasting
Effects of Insulin, Glucose, and Leucine

GREGORY D. CURFMAN, DONALD S. O’HARA, BARRY E. HOPKINS, AND THOMAS W. SMITH

SUMMARY To study the effects of leucine, glucose, and insulin on myocardial protein degradation in fed and fasted nutritional states, we developed and validated a sensitive method for measuring rates of total protein degradation in rat isolated left atrial preparations. Fasting resulted in a progressive decrease in myocardial protein breakdown to 71% of control over a 24-hour period, with no further reduction in degradation rate between 24 and 72 hours of fasting. Insulin (100 mU/ml) suppressed atrial protein degradation by 38% in fed animals (P < 0.001) and by 51% in fasted animals (P < 0.001). Glucose alone had no effect on protein degradation in either nutritional state. At 5 times normal plasma levels, leucine suppressed protein breakdown by 21% in fed and by 15% in fasted animals. The decrease in degradation induced by fasting and the absence of an effect of glucose are in contrast to the behavior reported for skeletal muscle. Circ Res 46: 000-000, 1980

PROTEINS within mammalian cells are degraded continuously to their constituent amino acids during the life of the cell. Several studies have indicated that alterations in the rate of intracellular protein degradation may be an important mechanism regulating the quantities of proteins within cells (Goldberg and Dice, 1974; Goldberg and St. John, 1976; Li and Goldberg, 1976; Scornik and Botbol, 1976). However, the physiological significance of this regulatory process has not been defined completely, in part because of technical problems involved in quantifying rates of protein breakdown. Protein degradation has been estimated by determining the rate of release of amino acids from a tissue or cell system under conditions in which amino acid reutilization is prevented (Goldberg and Dice, 1974). Inhibitors of protein synthesis have been employed to block amino acid reutilization, but use of such agents for this purpose has been accompanied by problems in interpretation because of suppression of protein breakdown in a number of experimental systems (Ceccarini and Eagle, 1976; Epstein et al., 1976; Fulks et al., 1975; Gunn et al., 1976; Hershko and Tomkics, 1971; Hopgood et al., 1977; Jefferson et al., 1977; Khairallah and Mortimore, 1976; Rannels et al., 1975; Wildenthal and Griffin, 1976; Woodside, 1976). In this communication, we describe a sensitive method for measuring the rate of total protein degradation in rat isolated left atrial preparations. The approach is based on the observation that the protein synthesis inhibitors, cycloheximide and puromycin, appear to have little effect on atrial protein degradation in vitro for 2 hours. During this time, protein synthesis is blocked, and valid observations about rates of protein breakdown can be made by quantifying the rate of release of tyrosine, an amino acid which is neither synthesized nor degraded by this tissue. This technique has been used to study the effects of leucine, insulin, and glucose on myocardial protein degradation in left atrial explants from animals in fed and fasted nutritional states.

Methods

Chemicals

Cycloheximide, puromycin hydrochloride, and unlabeled L-amino acids were obtained from Sigma Chemical Co. Insulin was purchased from Eli Lilly and Co. L-tyrosine[carboxyl-14C], L-tyrosine[14C(U)], and L-phenylalanine[14C(U)] were obtained from New England Nuclear. All other reagents were of the best grade commercially available.

Thin Layer Chromatography and CO₂ Release Studies

To determine whether L-tyrosine is metabolized to other compounds by atrial myocardium under the conditions employed in these experiments, atrial preparations were incubated (as outlined below) in oxygenated Krebs-Ringer bicarbonate
(KRB) buffered medium (Umbreit et al., 1964) containing 10 mM glucose and L-tyrosine[^14C(U)] (10^6 dpm/μmol) for 2 hours at 37°C. The tissues were homogenized, treated with trichloroacetic acid (TCA) (final concentration 10%), and centrifuged at 2500 g for 20 minutes. Aliquots of the supernatant phase were chromatographed on silica gel (Eastman) plates with standard amino acids using several solvents: (1) propan-1-ol:30% NH₄OH (70:30); (2) 100% ethanol:30% NH₄OH (65:35); (3) butan-1-ol:acetic acid:water (80:20:20). After development of the plates and identification of individual amino acids, the plates were cut into 0.5-cm squares, each of which was counted in liquid scintillation medium (Instagel, Packard Instrument Co.). To determine whether tyrosine is synthesized by atrial tissue, similar studies were performed in which tissues were incubated in the presence of L-phenylalanine[^14C(U)].

In additional experiments, left atria were incubated in the presence of L-tyrosine[carboxyl-¹⁴C], and radioactivity in CO₂, released into the atmosphere and medium, was quantified as described by Odessy and Goldberg (1972).

**Tissue High Energy Phosphate Content and O₂ Consumption**

Atria used for measurement of ATP and creatine phosphate levels were frozen rapidly by clamping in metal tongs maintained at liquid nitrogen temperatures. Aliquots of a perchloric acid tissue extract were analyzed by fluorometric measurement of NADPH produced in the coupled enzyme assay described by Lowry and Passonneau (1972).

Oxygen consumption was measured with a Gilson oxygraph using a water-jacketed Clark electrode maintained at 37°C. Atria were studied in an incubation medium initially equilibrated with 95% O₂-5% CO₂. Oxygen consumption was further assessed by measuring the rate of release of radioactivity from atrial muscle prelabeled in vitro with L-tyrosine[^3H(U)]. Labeling was carried out by

---

[^14C(U)]: Udenfriend and Goldberg (1972).
[^3H(U)]: Odessey and Goldberg (1972).
incubating tissues for 4 hours in 3.0 ml oxygenated KRB containing normal rat plasma levels (Morgan, et al., 1971) of all amino acids (except tyrosine), 1 U/ml insulin, 25 mM glucose, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.1 mM L-tyrosine, and 10 μCi/ml L-tyrosine[3H(U)]. To remove unincorporated label, the tissues were then incubated for 1 hour in 3.0 ml of the same medium, except that 2.5 mM unlabeled tyrosine was present instead of L-tyrosine[3H(U)]. The rate of release of radioactivity was determined during a subsequent chase period in identical medium containing 2.5 mM unlabeled tyrosine. Designated media also contained 0.5 mM cycloheximide. At the end of the chase period, aliquots of each medium were treated with TCA (final concentration 10%) followed by centrifugation to precipitate protein. The supernatants were decanted and added to 10 ml of scintillation fluid. The tissues were blotted, dissolved in 1.0 ml Soluene-350, (Packard Instrument Co.), and counted in 15 ml of scintillation fluid. Differences in quenching were corrected by the use of internal standards.

The rate of protein degradation was expressed as the fraction of total counts in protein at the beginning of the chase period that was released as a function of incubation time.

Results

Studies of Synthesis and Catabolism of Tyrosine by Atrial Myocardium

Thin layer chromatograms of homogenates of tissues incubated in the presence of labeled tyrosine or phenylalanine confirmed that the only radioactivity detectable was in the appropriate amino acid and a small amount (less than 2%) at the origin. The latter probably represents amino acids incorporated into small TCA-soluble peptides, and there was no evidence of interconversion of tyrosine to phenylalanine or other metabolic transformation of the tyrosine molecule that would invalidate its use as an indicator of protein degradation. The results of studies using labeled phenylalanine are in agreement with previous findings that rat heart does not contain phenylalanine hydroxylase (McGee et al., 1972) and, therefore, is unable to synthesize tyrosine from phenylalanine precursor.

CO2 release studies indicated that no 14CO2 was recoverable from either the atmosphere or medium of tissues incubated in the presence of L-tyrosine[carboxyl-14C]. These results confirm that rat atrial myocardium is unable to carry out the obligatory decarboxylation step (Lehninger, 1975) of tyrosine catabolism.

Tissue High Energy Phosphate Content and O2 Consumption

Tissue ATP and creatine phosphate (CP) concentrations as a function of incubation time in media with designated additions are shown in Table 1. These data indicate that atrial ATP levels are stable during incubations for up to 3 hours in all media tested, and CP levels tend to rise slightly under the same conditions.

Data on tissue oxygen consumption are shown in Table 2. In most experiments, tissue O2 consumption fell by 25-30% during a 2-hour incubation in KRB. This reduction in O2 consumption was not seen when the medium was supplemented with glucose, amino acids, and insulin, suggesting improved tissue stability in the richer medium. The decrease in O2 consumption seen in tissues incubated in unsupplemented medium is quantitatively similar to the 22% reduction over a 3-hour period found in the perfused rat heart by Neely et al. (1967).

The Effect of Inhibitors of Protein Synthesis on Atrial Total Protein Degradation

Figure 1 illustrates typical tyrosine release profiles from left atrial preparations incubated in KRB. In the absence of cycloheximide, tyrosine was released linearly (A). After 40 minutes of incubation (arrow), cycloheximide was added to the medium at a final concentration of 0.5 mM. In initial experiments, cycloheximide inhibited protein synthesis.

Table 1

<table>
<thead>
<tr>
<th>Incubation medium</th>
<th>Nutritional state</th>
<th>ATP content (nmol/mg protein)</th>
<th>CP content (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(in vivo)</td>
<td>0 hr</td>
<td>1 hr</td>
</tr>
<tr>
<td>KRB</td>
<td>Fed</td>
<td>33.69 ± 0.41</td>
<td>30.95 ± 1.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.03 ± 1.30</td>
<td>25.81 ± 4.40</td>
</tr>
<tr>
<td>KRB</td>
<td>Fasted</td>
<td>35.00 ± 2.20</td>
<td>32.10 ± 0.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36.81 ± 7.30</td>
<td>65.40 ± 6.30</td>
</tr>
<tr>
<td>KRB-G</td>
<td>Fed</td>
<td>27.61 ± 1.50</td>
<td>31.30 ± 1.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26.58 ± 4.30</td>
<td>24.20 ± 1.90</td>
</tr>
<tr>
<td>KRB-G</td>
<td>Fasted</td>
<td>33.85 ± 1.01</td>
<td>29.88 ± 1.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36.08 ± 3.31</td>
<td>30.29 ± 3.94</td>
</tr>
</tbody>
</table>

Mean ATP and CP contents of five atria (± SE) are shown for freshly excised tissue (0 hour) and for tissues incubated for 1 or 2 hours at 37°C. KRB-G is KRB + 25 mM glucose.
TABLE 2

<table>
<thead>
<tr>
<th>Incubation medium</th>
<th>Nutritional state (in vivo)</th>
<th>Oxygen consumption rate (nmol/min per mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 hr</td>
</tr>
<tr>
<td>KRB</td>
<td>Fed</td>
<td>13.9 ± 1.1</td>
</tr>
<tr>
<td>KRB</td>
<td>Fasted</td>
<td>13.9 ± 2.0</td>
</tr>
<tr>
<td>KRB-G</td>
<td>Fed</td>
<td>16.2 ± 1.6</td>
</tr>
<tr>
<td>KRB-G</td>
<td>Fasted</td>
<td>16.1 ± 1.0</td>
</tr>
<tr>
<td>Complete</td>
<td>Fed</td>
<td>15.7 ± 1.0</td>
</tr>
</tbody>
</table>

Mean O2 consumption of five atria (± se) are shown for freshly excised tissue (0 hour) and for tissues incubated for 1 or 2 hours at 37°C. Complete medium contains insulin, 0.10 U/ml, and plasma levels of all amino acids in KRB-G.

The same time period (Fig. 1), the reduction in amino acid release rate at 120 minutes appears to reflect an inhibitory effect of cycloheximide on proteolysis. A slight decline in tyrosine release rate of some control tissues at 120 minutes was not statistically significant. Studies using puromycin (0.5 mM) to block protein synthesis produced identical results.

To investigate further the effect of cycloheximide on myocardial proteolysis, protein degradation was assessed by determining the rate of release of L-tyrosine [1H(U)] from left atrial protein prelabeled in vitro. Initial experiments demonstrated that atrial preparations maintained in the enriched medium employed in this experiment released total tyrosine at the same rate after 1 and 24 hours of incubation when measured during a 1-hour exposure to cycloheximide. To prevent reincorporation of labeled tyrosine into protein, 2.5 mM unlabeled tyrosine was present in all incubation media during the 24-hour release period. Media for half of the atria also contained 0.5 mM cycloheximide. Results of these studies are summarized in Figure 2. During the first 4 hours of incubation, the fraction of total counts released from prelabeled protein was the same in the presence and absence of cycloheximide (Fig. 2A). Since protein synthesis is reduced by more than 95% by cycloheximide at the concentration used, these data confirm that 2.5 mM unlabeled tyrosine is sufficient to prevent reincorporation of L-tyrosine[1H(U)] into protein. After 5 hours of incubation, the rate of release of radioactivity from tissues incubated in the presence of cycloheximide was significantly lower than that of controls (Fig. 2B). At 24 hours, the cumulative fraction of counts released from tissues incubated in the presence of the drug was 66% of the control value. Thus, using an independent method of assessing protein degradation, we found that cycloheximide suppressed protein breakdown after a lag period. The fractional protein degradation rate of 40% per 24 hours indicated in Figure 2B is higher than other values reported in this paper because, during the 4-hour labeling period employed in this experiment, the radioactivity is taken up primarily by proteins with shorter half lives. This preferential labeling of proteins with higher turnover rates is also the reason that release of labeled tyrosine shows a progressive...
Effect of Food Deprivation on Myocardial Protein Degradation

The effect of fasting on atrial protein degradation is shown in Figure 3. Protein degradation decreased to 82% of control at 12 hours of fasting and to 71% at 24 hours. Degradation rate then remained stable through 72 hours of food deprivation. These results are in contrast to previous studies of skeletal muscle, in which an increase in protein degradation rate during fasting has been reported (Fulks et al., 1975; Li and Goldberg, 1976; Wassner et al., 1977).

Effect of Insulin and Glucose on Atrial Protein Degradation

To investigate the effect of insulin on protein degradation quantified by the release of tyrosine from atrial myocardium, we incubated atrial preparations in the presence and absence of the hormone at a concentration of 100 mU/ml. The results of these experiments are summarized in Table 3. Insulin suppressed protein degradation by 38% (P < 0.001) in preparations from animals fasted for 48 hours. Glucose alone (25 mM) had no significant effect on atrial total protein degradation in either nutritional state (Table 3), and the combination of glucose plus insulin had no greater suppressive effect on protein degradation than insulin alone. It should be noted that there were slight day-to-day variations in total protein degradation rate. For this reason, separate controls were performed for each individual experiment, as indicated in Table 3.

Effect of Leucine on Atrial Protein Degradation

Previous studies on incubated rat skeletal muscles have demonstrated that amino acids, and spec...
specifically the branched chain amino acids, leucine, isoleucine, and valine, suppress protein breakdown in this tissue (Buse and Reid, 1975; Buse and Weigand, 1977; Fulks et al., 1975). The effect of leucine on protein degradation in the heart is shown in Table 3. At 5 times normal plasma concentration, leucine suppressed protein degradation in both fed (21%) and fasted (15%) animals.

**Discussion**

Quantification of protein degradation by measurement of the rate of release from tissue of an amino acid requires that the marker amino acid be neither synthesized nor degraded by the tissue under study. Previous studies have shown that tyrosine meets these requirements in rat skeletal muscle (Fulks et al., 1975), as does phenylalanine in the rat heart (Morgan et al., 1971). The data presented here indicate that tyrosine is not metabolized by explanted rat atrial myocardium and is, therefore, a suitable marker amino acid for studies of protein breakdown in this tissue. The use of tyrosine affords substantial advantages in the assessment of protein degradation in small tissue samples, since the fluorometric assay for its quantification (Waalkes and Underfriend, 1957) is sensitive, specific, and convenient.

An additional requirement for obtaining valid estimates of protein degradation rate is that reutilization of the marker amino acid must be prevented. This frequently has been achieved by inclusion in media of high concentrations of the appropriate amino acid while release of radioactivity from prelabeled tissues or cells is measured (Goldberg and St. John, 1976). An alternative approach is to block amino acid reincorporation with an inhibitor of protein synthesis. The usefulness of the latter approach has been limited by the fact that protein synthesis inhibitors suppress protein breakdown in a number of tissues (Ceccarini and Eagle, 1976; Epstein et al., 1975; Fulks et al., 1975; Gunn et al., 1976; Hershko and Tomkins, 1971; Hopgood et al., 1977; Jefferson et al., 1977; Khairallah and Mortimore, 1976; Rannels et al., 1975; Wildenthal and Griffin, 1976; Woodside, 1976). However, studies of the perfused rat liver (Khairallah and Mortimore, 1976; Woodside, 1976) and of cell culture systems (Epstein et al., 1975; Gunn et al., 1976) have indicated that the suppression of protein breakdown by inhibitors of protein synthesis occurs only after a delay that varies with the particular tissue or cell type. In the isolated left atrial preparation, the duration of this lag period is approximately 2 hours. During this interval, protein synthesis is blocked, and the rate of release of free tyrosine from the tissue is essentially constant. The time course of this behavior is similar for both cycloheximide and puromycin and appears to be independent of the mechanism by which protein synthesis is inhibited.

To determine how the rate of tyrosine release during the 2-hour linear phase relates to protein degradation in the absence of cycloheximide, we examined the release of $^3$H-tyrosine from labeled atria when reincorporation was minimized by an excess of unlabeled tyrosine. In this experiment, cycloheximide did not significantly alter $^3$H-tyrosine efflux for the first 5 hours. The curvilinearity of the tyrosine release in this case is an indication of the fact that the atrial proteins are not all labeled to the same specific activity. By virtue of a higher specific radioactivity, the more rapidly turning-over proteins contribute a disproportionately larger fraction of the $^3$H-tyrosine efflux during the initial phase of release. The apparent lack of effect of cycloheximide in the first 5 hours does not necessarily indicate that it has no influence on protein degradation during this interval. Epstein et al. (1975) reported that cycloheximide preferentially suppresses the breakdown of proteins with longer turnover times. Consequently, we cannot eliminate the possibility that cycloheximide immediately suppresses the degradation of slowly turning-over atrial proteins and that tyrosine efflux measured in the presence of this inhibitor somewhat underestimates the degradation rate for total protein.

The isolated atrial preparation employed in the present studies has several distinct advantages and some disadvantages compared with other systems used for studies of myocardial protein turnover. The advantages include its simplicity and stability. Tissue ATP content remains constant during a 2-hour incubation, and $O_2$ consumption is stable for...
2 hours when tissues are maintained in a medium supplemented with glucose, amino acids, and insulin. Tyrosine release in the presence of cycloheximide has been documented to be linear for 2 hours, a period adequate for measurements of protein degradation rate. When maintained in an enriched medium containing insulin, glucose, and amino acids, these atrial preparations demonstrate a constant total protein degradation rate for at least 24 hours. In separate studies we also have shown that total protein synthesis proceeds linearly for at least 24 hours in the presence of insulin. Under these conditions, we have shown that rates of protein synthesis and degradation are equal. Prolonged stability of the preparation in the presence of insulin permits studies of protein degradation to be performed by labeling cell protein in vitro and determining the subsequent rate of release of radioactivity. When atria are incubated in KRB without additions, the highest fractional rate of total protein degradation is 28% per day. In the presence of insulin, this value is reduced to 8.4% per day. These degradation rates are similar quantitatively to those reported for other in vitro systems, including fetal mouse hearts in organ culture (Wildenthal et al., 1976), perfused rat hearts (Rannels et al., 1975), and incubated skeletal muscles (Fulks et al., 1975). Disadvantages of the preparation include exchange of oxygen and substrates by diffusion from the medium rather than through the capillary bed, and the fact that it is resting rather than contracting tissue. The latter disadvantage can be overcome in part by use of the isolated right atrium, which will contract spontaneously in vitro for several hours (Cohen et al., 1969).

The results presented in this report indicate that, in 150-g rats, food deprivation is associated with a 30% reduction in total myocardial protein degradation rate. This reduction occurs during the first 24 hours of fasting, and degradation rate then remains stable for the next 48 hours. In skeletal muscle, most investigations have indicated that protein breakdown increases during periods of starvation (Fulks et al., 1975; Li and Goldberg, 1976; Wassner et al., 1977). However, a recent study by Li et al. (1979), using the perfused rat hemicorpus, concluded that the influence of fasting on skeletal muscle proteinolysis is related to the weight of the animal. For 100-g rats, starvation for 48 hours produced a 30-50% increase in protein degradation, but 220-g rats faster for 72 hours showed no change in degradation rate. Measurements of protein breakdown in vivo by Millward et al. (1976) demonstrated that protein breakdown is decreased in 400-g rats after starvation for 2 days and then exceeds that of controls by 4 days. It appears, therefore, that heart and skeletal muscle respond differently to the initial stages of starvation and that the decrease in protein degradation in the heart acts to preserve myocardial protein at a time when skeletal muscle is being broken down to supply precursors for hepatic gluconeogenesis. A possible mechanism for this effect could be the difference in sensitivity of heart and skeletal muscle to by-products of fatty acid metabolism. This concept is supported by reports that fatty acids and ketone bodies diminish protein degradation in the perfused rat heart (Rannels et al., 1974) but have no effect on proteolysis in isolated diaphragm preparations (Fulks et al., 1975) or the perfused hemicorpus (Jefferson et al., 1977).

Since fasting causes a significant reduction in plasma insulin levels (Cahill, 1970), it was of interest to compare the effect of insulin on protein degradation in atria from fed and fasted animals. We found that insulin caused a 38% reduction in tyrosine release rate in fed animals and a 51% reduction in fasted ones. The magnitudes of the changes in degradation rate for fed (1.40 ± 0.23 nmol/mg per hr) and fasted (1.71 ± 0.12 nmol/mg per hr) rats were not significantly different. These results agree with the 50% reduction in protein breakdown noted in the perfused rat heart (Rannels et al., 1975) and the 22% reduction found in fetal mouse heart (Wildenthal et al., 1976). Thus, these three in vitro myocardial systems, using different methods to assess protein breakdown, respond similarly to insulin.

In rat diaphragm, glucose significantly reduces protein degradation, and glucose in combination with insulin has a greater suppressive effect than either agent alone (Fulks et al., 1975). Consequently, the drop in circulating glucose levels attending starvation is a possible factor in the observed increases in protein degradation for skeletal muscle. This mechanism does not appear to be important in the heart, however, since glucose did not significantly alter protein degradation rates in atria incubated either in the presence or absence of insulin.

Previous investigations of both skeletal and cardiac muscle have indicated that amino acids may play a part in regulating protein metabolism in these tissues (Buse and Reid, 1975; Buse and Weigand, 1977; Fulks et al., 1975). Fulks et al. (1975) reported that the branched chain amino acids, leucine, isoleucine, and valine, enhance protein synthesis and diminish protein breakdown in rat diaphragm. Similar results were obtained by Buse and co-workers (1975, 1977) using the same tissue preparation. These investigations further suggested that, of the three branched chain amino acids, leucine may be the most important in regulating protein turnover in skeletal muscle. Studies using the perfused rat heart (Rannels et al., 1974) have indicated that the three branched chain amino acids in combination promote protein synthesis. In liver, tryptophan appears to influence protein synthesis, whereas proline, methionine, tryptophan, and phenylalanine retard protein degradation (Woodside and Mortimore, 1972). The effect of leucine on myocardial...
dial protein breakdown has not been reported previously. In the isolated atrial preparation, leucine at 5 times normal plasma concentration significantly decreased the rate of total protein degradation in both fed and fasted rats.

Since leucine levels are elevated in the initial stages of starvation (Bray, 1974), the suppressive effect of this amino acid suggests a possible regulatory role in the reduction of myocardial protein degradation during fasting. Part of the effect of leucine may be attributable to the production of ketone bodies during intracellular catabolism. The physiological significance of this process, however, will require further investigation.

The results presented here indicate that, in the isolated atrium from fasted rats, there is a decreased rate of protein degradation that is evident in the absence of added hormones or substrates. Since insulin suppresses intracellular proteolysis, it might be expected in the live animal that the low circulating insulin levels characteristic of fasting could partially offset the effect seen in vitro. In studies of atrophied hearts from rabbits and mice subjected to 3 days of food deprivation, Wildenthal et al. (1975) found that there was an elevation in both lysosomal and free cathepsin D activity. Neither the rate of total protein synthesis nor degradation was reported for these experiments, so it is uncertain whether the enhanced cathepsin activity in this instance was correlated with an increase in intracellular proteolysis. However, in experiments with quail using a double isotope procedure to measure the in vivo rate of protein degradation after a 3-day fast, myocardial protein breakdown was reported to be reduced for soluble proteins for all molecular weights (Bush and Marquardt, 1978). This behavior was organ-specific since skeletal muscle from the same animals had an increased degradation rate. Therefore, the double isotope experiments are consistent with the response observed with isolated atria and suggest that fasting produces a significant readjustment in the rate of myocardial protein turnover.

Acknowledgments

The expert technical assistance of Carolyn Trumbull is gratefully acknowledged.

Note Added in Proof

A recent study using perfused rat hearts found that 1–5 days of fasting reduced cardiac proteolysis to the same degree as reported here for atria (Crie JS, Sanford CF, Wildenthal K (1980) Influence of starvation and refeeding on cardiac protein degradation in rats. J Nutr 110: 22–27)

References


Gunn JM, Ballard FJ, Hanson RW (1976) Influences of hormones and medium composition on the degradation of phosphoanhydride carboxykinase (GTP) and total protein in Reuber H35 cells. J Biol Chem 251: 3586–3593


Lehninger AL (1975) Biochemistry, ed 2. New York, Worth, p 569


Rannels DE, Hjalmarson AC, Morgan HE (1974) Effects of...
noncarbohydrate substrates on protein synthesis in muscle.

Am J Physiol 226: 528-539


Wildenthal K, Poole AR, Dingle JT (1975) Influence of starvation on the activities and localization of cathepsin D and other lysosomal enzymes in hearts of rabbits and mice. J Mol Cell Cardiol 1: 76-81

Woodside KH (1976) Effects of cycloheximide on protein degradation and gluconeogenesis in the perfused rat liver. Biochim Biophys Acta 421: 70-79

Suppression of myocardial protein degradation in the rat during fasting. Effects of insulin, glucose, and leucine.
G D Curfman, D S O'Hara, B E Hopkins and T W Smith

Circ Res. 1980;46:581-589
doi: 10.1161/01.RES.46.4.581

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/46/4/581