Physiological Hyperinsulinemia Inhibits Myocardial Protein Degradation In Vivo in the Canine Heart

Lawrence H. Young, Douglas M. Dahl, Deborah Rauner, and Eugene J. Barrett

Myocardial protein turnover in vivo was examined in anesthetized dogs following a 16- or 36-hour fast and again during a hyperinsulinemic (2 mU/kg per minute) euglycemic clamp with or without amino acid replacement or during saline infusion. We measured myocardial phenylalanine balance and rates of protein synthesis and degradation, using the extraction of intravenously infused L-[ring-2,6-3H]phenylalanine and the dilution of its specific activity across the heart at isotopic steady state. After both a 16-hour (n=19) and 36-hour fast (n=10), there was net myocardial release of phenylalanine indicated by the negative balances for phenylalanine of −52±9 (p<0.001) and −38±9 (p<0.005) nmol/min, respectively. Overall, the basal rate of myocardial protein degradation was lower in the 36-hour-fasted animals (81±13 versus 121±12 nmol/min, p<0.05). Myocardial phenylalanine balance and rates of protein synthesis and degradation did not change during insulin and glucose infusion in the 36-hour-fasted animals (n=10). In these animals, there was a 30–40% decline in plasma amino acid concentrations, including branched chain (p<0.001) and essential amino acids (p<0.001). In the 16-hour-fasted animals receiving an infusion of amino acids during the euglycemic clamp (n=11), myocardial phenylalanine balance shifted from negative to neutral (from −48±3 to 0±15 nmol/min, p<0.05) because of a 50% inhibition of heart protein degradation (from 118±12 to 60±7 nmol/min, p<0.001); heart protein synthesis was unchanged. In the additional 16-hour-fasted animals receiving saline (n=8), the net negative myocardial phenylalanine balance persisted throughout the infusion. These results demonstrate that physiological increments in plasma insulin have a relatively anabolic effect on myocardial protein turnover in vivo caused by inhibition of protein degradation. Furthermore, hypoaminoacidemia appears to blunt insulin’s anabolic action on heart muscle. (Circulation Research 1992;71:393–400)

KEY WORDS • protein degradation • hyperinsulinemia

Limitations in the available methods for measuring heart protein turnover have slowed attempts to achieve an integrated understanding of the regulation of this process in vivo. Heart protein synthesis, estimated from the incorporation of radiolabeled amino acids into heart protein, has been studied extensively and the effects of acute metabolic and hemodynamic interventions have been examined (for review see References 1 and 2). Heart protein balance has been assessed from changes in heart mass and protein content over time.3–5 Protein degradation has been assessed only indirectly by comparing measured rates of protein synthesis with changes in net heart protein mass over time in groups of comparably treated animals.5–7 A method recently developed in our laboratory allows for repeated estimates of heart8,9 and skeletal muscle10–11 protein synthesis, degradation, and overall protein balance in vivo. This approach, based on measurements of labeled phenylalanine kinetics across the muscle bed, permits the assessment of short-term changes in protein turnover in individual subjects and has proved useful in characterizing the response to experimental interventions.9,10,12–16

In a variety of in vitro studies of both heart17–19 and skeletal muscle,20,21 insulin stimulates protein synthesis and inhibits protein degradation. In humans, recent studies have shown that the primary effect of physiological hyperinsulinemia on both whole-body22,23 and skeletal muscle10 is to inhibit protein degradation. Much less is known about insulin’s role in the regulation of heart protein metabolism in vivo. In insulin-deficient diabetic rats, higher rates of heart protein synthesis are observed after chronic (2–3 days) insulin treatment.24,25 Although in one study glucose infusion appeared to stimulate heart protein synthesis in normal young rats,26 in other studies hyperinsulinemia did not stimulate synthesis of myocardial protein.24,27 Insulin’s effect on heart protein degradation in vivo in normal animals is unknown, although it may slow myocardial proteolysis in insulin-deficient diabetic rats.25

In the present study, we examine the effect of physiological increases in plasma insulin on net heart protein
balance, synthesis, and degradation measured using the phenylalanine isotope dilution technique.\textsuperscript{8} Anesthetized dogs were studied with and without a replacement infusion of amino acids to prevent the hypoaminoacidemia that normally accompanies insulin infusion in vivo.\textsuperscript{28,29}

**Materials and Methods**

**Animal Preparation**

Adult mongrel dogs, weighing 20–25 kg, were fasted either 16 hours or 36 hours before the study, which began at 8–10 AM. Animals were anesthetized with sodium pentobarbital (20 mg/kg), intubated, and ventilated on room air. Anesthesia was maintained with either intravenous \(\alpha\)-chloralose urethane or sodium pentobarbital. The right external jugular vein and femoral artery were isolated through a right lateral neck and femoral incision, respectively. A 7F arterial catheter was introduced for blood sampling and to monitor blood pressure. Under fluoroscopic guidance, a 6F multipurpose catheter, for blood sampling, and a 7F Bain thermodilution flow catheter (Electrocardioth Co., Rahway, N.J.) were placed in the mid coronary sinus. Catheter patency was maintained with intermittent saline flush (500 units heparin per liter of 0.9% saline). The electrocardiogram and arterial blood gases were monitored throughout.

**Infusion Protocols**

A total of 29 dogs were studied. A continuous infusion of L-[ring-2,6-\(\text{\textsuperscript{3}}\text{H}\)]phenylalanine (0.75 \(\mu\)Ci/min) (Amersham Corp., Arlington Heights, Ill.) was administered to each animal via a hind limb vein. After 60–90 minutes of tracer equilibration, initial baseline samples of arterial and coronary sinus blood were drawn in quadruplicate over a 20-minute period, and coronary sinus blood flow was measured in triplicate using the thermodilution method.\textsuperscript{30} Repeat quadruplicate blood samples and triplicate measurements of coronary blood flow were again made during the last 20 minutes of each of the following three infusion protocols. In the first group, 10 animals (fasted 36 hours) received a primed (4 mM/kg×10 minutes) continuous (2 mM/kg per minute×110 minutes) infusion of porcine regular insulin (Eli Lilly Corp., Indianapolis, Ind.); blood glucose was maintained at a constant level using the euglycemic clamp technique.\textsuperscript{31} In the second group, 11 animals (fasted 16 hours) received amino acids in addition to insulin to prevent hypoaminoacidemia during the euglycemic clamp, which was extended to 150 minutes to ensure steady-state levels of the plasma amino acids at the time of final measurements. Amino acids were given as a continuous infusion (1.2 mg/kg per minute, 10% Travesol; Clintec Nutrition Co., Deerfield, Ill.) to prevent the hypoaminoacidemia associated with insulin infusion.\textsuperscript{28,29} Each 100 ml of this solution contained leucine, 730 mg; isoleucine, 600 mg; lysine, 580 mg; valine, 580 mg; phenylalanine, 560 mg; histidine, 480 mg; threonine, 420 mg; methionine, 400 mg; tryptophan, 180 mg; alanine, 2.07 g; arginine, 1.15 g; glycine, 1.03 g; proline, 680 mg; serine, 500 mg; and tyrosine, 40 mg. In the third group, eight dogs (fasted 16 hours) received only a 2-hour saline infusion after the initial measurements were obtained.

The tracer infusion times used in the present study are typical of those used to study steady-state skeletal muscle and whole-body protein turnover.\textsuperscript{12,15,22,23} Equilibration between plasma and cellular amino acid pools appears to be more rapid for heart than skeletal muscle.\textsuperscript{1,8,32} During both the baseline and experimental sampling periods, both plasma phenylalanine concentrations and specific activities were observed to be at a steady state (coefficient of variation of 2–3% in the quadruplicate samples).

**Analytic Measurements**

Whole-blood glucose concentration was measured using the glucose oxidase method (Yellow Springs Instruments Co., Yellow Springs, Ohio). Concentrations of acidic and neutral amino acids were measured in duplicate sulfosalicylic acid extracts of arterial plasma with an automated ion-exchange chromatographic technique (Dionex D-500, Sunnyvale, Calif.).\textsuperscript{8,28} Plasma insulin was assayed using a double-antibody radioimmunoassay technique. Plasma phenylalanine concentration was measured using a reverse-phase high pressure liquid chromatographic technique as previously described,\textsuperscript{8,9} and the eluant under the phenylalanine peak was collected and measured for \([\text{H}]\)radioactivity. \([\text{H}]\)Phenylalanine specific activity was calculated by dividing the phenylalanine radioactivity (disintegrations per minute per milliliter) by concentration (nmol/ml). The intra-assay coefficients of variation for the measurement of phenylalanine concentration and specific activity were 1.8% and 2.2%, respectively. All phenylalanine measurements were made in quadruplicate.

**Calculations**

The net myocardial balance of glucose was calculated from the product of the arterial–coronary sinus concentration difference and coronary sinus blood flow. For amino acids the coronary sinus plasma flow and plasma concentrations were used. Net balance = (\([A]–[\text{CS}]\) × flow, where \([A]\) and \([\text{CS}]\) are the arterial and coronary sinus concentrations, and flow is the thermodilution coronary blood flow. The whole-body glucose utilization rate\textsuperscript{31} was calculated from 30 minutes after starting the insulin infusion until the conclusion of the study, and results are expressed as milligrams per kilogram per minute of glucose.

Rates of myocardial protein synthesis and degradation were calculated from the extraction of labeled phenylalanine and the dilution of phenylalanine specific activity across the heart, respectively.\textsuperscript{1,8} Because phenylalanine is neither synthesized nor metabolized in muscle,\textsuperscript{33} at steady state the measured rate of disappearance of tracer across the heart reflects the rate of incorporation of phenylalanine into protein, i.e., PHE synthetic uptake = \([\text{DPMA}–\text{DPMCS}]\)/SA\textsubscript{CS}×flow, where PHE is phenylalanine and DPMA and DPMCS are disintegrations per minute per milliliter in the artery and coronary sinus, respectively. In addition, the dilution of phenylalanine specific activity across the heart at steady state reflects the release of unlabeled phenylalanine from myocardial protein into plasma.\textsuperscript{1,8} The rate of phenylalanine release from myocardial protein degradation is calculated according to the equation PHE degradative release = \([\text{SA}\textsubscript{A}–\text{SA}\textsubscript{CS}–1]\)×\([A]×\text{flow}\), where \(\text{SA}\textsubscript{A}\) and \(\text{SA}\textsubscript{CS}\) denote the specific activity (dpm/
nmol) of phenylalanine in the artery and CS, respectively. The second and third equations assume that phenylalanine released from heart protein degradation is reutilized for new protein synthesis in a fashion similar to phenylalanine entering the heart from arterial plasma, and that the specific activity of heart phenylalanyl-tRNA equilibrates rapidly with that in plasma.8,34

**Statistical Analysis**

All data are presented as mean±SEM. Comparison of data between the fasting period and euglycemic clamp was performed on the mean values from quadruplicate samples drawn during those periods using a paired Student’s *t* test. Comparison between the infusion protocols used analysis of variance (ANOVA) and unpaired Student’s *t* test analysis.

**Results**

**Metabolic Effects of the Euglycemic Clamps**

In the animals fasted 36 hours, plasma insulin increased from 9±1 to 106±9 μU/ml (*p<0.001*), whereas blood glucose was maintained constant (65±1 mg/dl) during insulin infusion without amino acid replacement. During hyperinsulinemia, whole-body glucose utilization averaged 8.0±0.6 mg/kg per minute, and myocardial glucose uptake increased 2.2-fold (Table 1). In the animals fasted 16 hours that received combined insulin and amino acid infusions, blood glucose concentrations (70±2 mg/dl) were slightly higher (*p<0.01*) than in 36-hour-fasted animals. Plasma insulin levels, however, both before and during the clamp, were similar in both groups (Table 1). The whole-body glucose utilization rate (8.7±0.8 mg/kg per minute) and the increase in myocardial glucose utilization (2.2-fold) during the clamp were similar to those measured in animals undergoing the euglycemic clamp without amino acid infusion (Table 1). Blood glucose, plasma insulin, and myocardial glucose utilization were similar in both groups of animals fasted 16 hours and remained constant during the saline infusion (Table 1).

**Coronary Blood Flow and Hemodynamics**

Coronary sinus blood flow was similar in each group and appeared unchanged during both euglycemic clamps and the saline infusions (Table 2). Animals fasted 36 hours had somewhat higher baseline heart rates and significantly greater mean arterial blood pressures (*p<0.02*) than animals fasted 16 hours (Table 2). The only significant hemodynamic change seen during the course of the infusions was a 10–15% rise in arterial pressure accompanying combined insulin and amino acid infusion (Table 2).

**Amino Acid Concentrations**

Plasma arterial amino acid concentrations were measured before and then during the last 20 minutes of each infusion. Mean values of individual amino acids are shown in Table 3; summed values of total amino acids, branched chain amino acids, and essential amino acids are displayed in Figure 1. Insulin infusion without amino acid replacement led to a substantial fall in the concentrations of most amino acids measured, including the essential amino acids threonine, phenylalanine, leucine, isoleucine, valine, and methionine (Table 3). Total amino acids decreased 34%, and there was a 37% decrease in both the branched chain amino acids and essential amino acids analyzed (Figure 1). Amino acid infusion during the euglycemic clamp maintained the overall concentrations of the branched chain amino acids, essential amino acids, and total amino acids close to basal values (Figure 1). Because of the formulation of the amino acid mixture, there were variable effects on the concentrations of individual amino acids: among the essential amino acids, methionine, isoleucine, and phenylalanine increased, whereas leucine decreased and valine and threonine were unchanged (Table 3). Total amino acid concentrations remained relatively stable during the course of saline infusion (Figure 1), although

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**Table 1. Arterial Glucose and Insulin Concentrations, Whole-Body and Heart Glucose Uptake**

<table>
<thead>
<tr>
<th></th>
<th>Blood glucose (mg/dl)</th>
<th>Plasma insulin (μU/ml)</th>
<th>M (mg/kg/min)</th>
<th>Myocardial glucose uptake (mg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Euglycemic clamp</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>n=10, 36-hour fasted</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>63±2</td>
<td>9±1</td>
<td>...</td>
<td>3.1±0.6</td>
</tr>
<tr>
<td>Insulin</td>
<td>65±1</td>
<td>106±9*</td>
<td>8.0±0.6</td>
<td>6.8±0.9†</td>
</tr>
<tr>
<td><strong>Euglycemic clamp + AA</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>n=11, 16-hour fasted</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>70±2‡</td>
<td>9±1</td>
<td>...</td>
<td>2.6±0.8</td>
</tr>
<tr>
<td>Insulin</td>
<td>71±2‡</td>
<td>106±14*</td>
<td>8.7±0.8</td>
<td>5.7±0.9*</td>
</tr>
<tr>
<td><strong>Saline infusion</strong></td>
<td></td>
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</tr>
<tr>
<td><em>n=8, 16-hour fasted</em></td>
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</tr>
<tr>
<td>Fasting</td>
<td>66±2</td>
<td>10±1</td>
<td>...</td>
<td>2.6±0.6</td>
</tr>
<tr>
<td>Saline</td>
<td>65±3</td>
<td>7±1</td>
<td>...</td>
<td>2.6±0.6</td>
</tr>
</tbody>
</table>

*M* is the mean whole-body glucose utilization rate during the euglycemic clamp (see “Methods”). Data are mean±SEM for the arterial concentrations of glucose and insulin and for myocardial glucose uptake initially in the fasting state and during the last 20 minutes of the infusion protocols (insulin or saline).

*†p<0.001, ‡p<0.005 compared with initial values by paired *t* test. §p<0.01, §p<0.05 compared with euglycemic clamp by unpaired *t* test.
there was a 26% increase in essential amino acids, including significant increases in the branched chain amino acids and phenylalanine (Figure 1 and Table 3).

**Phenylalanine Kinetics**

In the animals fasted for 36 hours, the phenylalanine concentration was higher in coronary sinus than in arterial plasma in nine of 10 animals (mean difference, 1.02±0.22 nmol/ml; p<0.001; range, 0.40–2.08 nmol/ml). Thus, there was a net negative phenylalanine balance initially (−38±9 nmol/min, p<0.005 different from 0), indicating that the rate of protein degradation by the myocardium exceeded the rate of protein synthesis. There was also significant extraction of [3H]phenylalanine (3±1%, p<0.05) and dilution of [3H]phenylalanine specific activity (6±1%, p<0.001) across the heart. The initial rates of protein synthesis and degradation calculated from the phenylalanine kinetics using the second and third equations (see “Calculations”) were 38±17 and 81±13 nmol/min, respectively (Figure 2). Neither the phenylalanine balance nor the rates of heart protein synthesis or degradation during the last 20 minutes of the euglycemic clamp differed from initial rates in these animals (Figure 2).

In the 16-hour-fasted animals before insulin and amino acid infusion, we also observed higher coronary sinus than arterial phenylalanine concentrations (mean difference, 1.12±0.15 nmol/ml; p<0.001; range, 0.45–

**Table 2. Hemodynamics and Coronary Blood Flow**

<table>
<thead>
<tr>
<th></th>
<th>Heart rate (min⁻¹)</th>
<th>Systolic BP (mm Hg)</th>
<th>Mean BP (mm Hg)</th>
<th>Double product (×10⁻²)</th>
<th>Coronary blood flow (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Euglycemic clamp</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>n=10, 36-hour fasted</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>159±9</td>
<td>165±4</td>
<td>146±6</td>
<td>25.7±2.2</td>
<td>57±4</td>
</tr>
<tr>
<td>Insulin</td>
<td>154±11</td>
<td>167±3</td>
<td>149±5</td>
<td>25.3±2.2</td>
<td>58±6</td>
</tr>
<tr>
<td><strong>Euglycemic clamp+AA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=11, 16-hour fasted</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>142±9</td>
<td>139±9</td>
<td>113±6</td>
<td>20.0±2.2</td>
<td>60±9</td>
</tr>
<tr>
<td>Insulin</td>
<td>139±7</td>
<td>157±5†</td>
<td>128±4‡</td>
<td>22.1±1.7</td>
<td>63±9</td>
</tr>
<tr>
<td><strong>Saline infusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=8, 16-hour fasted</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>138±16</td>
<td>165±9</td>
<td>137±7</td>
<td>23.0±3.2</td>
<td>62±8</td>
</tr>
<tr>
<td>Saline</td>
<td>159±11</td>
<td>173±5</td>
<td>146±2</td>
<td>27.7±2.3</td>
<td>64±9</td>
</tr>
</tbody>
</table>

BP, Blood pressure; double product, systolic blood pressure×heart rate; AA, amino acids. Data are mean±SEM of quadruplicate values measured before fasting and during the last 20 minutes of the infusion protocols (insulin or saline).

*p<0.05 compared with 16-hour-fasted dogs by unpaired t test.  tp<0.02,  tp<0.05 compared with fasting values by paired t test.

**Table 3. Arterial Amino Acid Concentrations (μM)**

<table>
<thead>
<tr>
<th></th>
<th>Euglycemic clamp</th>
<th>Euglycemic clamp+AA</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasting</td>
<td>Insulin</td>
<td>Fasting</td>
</tr>
<tr>
<td>Threonine</td>
<td>193±45</td>
<td>124±30*</td>
<td>139±14</td>
</tr>
<tr>
<td>Serine</td>
<td>94±8</td>
<td>66±8†</td>
<td>84±10</td>
</tr>
<tr>
<td>Asparagine</td>
<td>39±4‡</td>
<td>21±2§</td>
<td>29±2</td>
</tr>
<tr>
<td>Glutamate</td>
<td>15±2</td>
<td>13±1</td>
<td>14±1</td>
</tr>
<tr>
<td>Glutamine</td>
<td>386±39</td>
<td>265±26§</td>
<td>382±28</td>
</tr>
<tr>
<td>Glycine</td>
<td>129±16</td>
<td>91±10‡</td>
<td>91±6</td>
</tr>
<tr>
<td>Alanine</td>
<td>375±72†</td>
<td>280±36</td>
<td>209±16</td>
</tr>
<tr>
<td>Valine</td>
<td>160±14</td>
<td>123±16*</td>
<td>159±10</td>
</tr>
<tr>
<td>Cysteine</td>
<td>24±2</td>
<td>24±2</td>
<td>22±2</td>
</tr>
<tr>
<td>Methionine</td>
<td>30±2#</td>
<td>15±2§</td>
<td>26±3#</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>34±4#</td>
<td>10±1§</td>
<td>39±4</td>
</tr>
<tr>
<td>Leucine</td>
<td>90±11</td>
<td>41±8§</td>
<td>101±9</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>30±4</td>
<td>17±2§</td>
<td>25±2</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>44±3</td>
<td>32±2§</td>
<td>49±4</td>
</tr>
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</table>

AA, amino acids. Data represent mean±SEM for arterial plasma amino acid concentrations in the fasting state (36-hr fast for euglycemic clamp [n=9]; 16-hr fast for euglycemic clamp+AA [n=10] and saline groups [n=7] and the last 20 minutes of the infusions [insulin or saline]); n=4 for threonine values available in each group.

*p<0.02,  tp<0.01 compared with initial values by paired t test.  tp<0.05 compared with 16-hour fasted dogs.  †p<0.001,  †p<0.05 compared with initial values by paired t test.  ‡p<0.05 compared with saline-infused 16-hour-fasted dogs by analysis of variance.
FIGURE 1. Bar graphs showing arterial concentrations of total (nonbasic) amino acids (panel A), total branched chain amino acids (BCAA) (panel B), and measured essential amino acids (panel C). Data are mean±SEM before experimental infusion (open bars) and during the last 20 minutes (hatched bars) of the euglycemic clamp (INSULIN), euglycemic clamp with amino acid infusion (INSULIN+AA), or saline infusion (SALINE). *p<0.005, **p<0.05 compared with initial values.

1.79 nmol/ml), and again there was a net negative phenylalanine balance during the initial period (−40±6 nmol/min, p<0.001 different from 0). There was also significant extraction of [3H]phenylalanine (5±1%, p<0.001) and dilution of [3H]phenylalanine specific activity (7±1%, p<0.001) across the heart. The rates of protein synthesis and degradation were 79±8 nmol/min and 118±12 nmol/min, respectively (Figure 2). When insulin was infused with amino acid replacement, there was a significant change in the myocardial phenylalanine balance (p<0.05) from negative (−40±6 nmol/min) to slightly positive (6±15 nmol/min, NS), indicating that proteolysis no longer exceeded protein synthesis (Figure 2). This net anabolic effect was caused by an inhibition of myocardial protein degradation, which fell almost 50% from a basal rate of 118±12 to 60±7 nmol/min (p<0.001) (Figure 2).

In the 16-hour-fasted animals given only saline, there was also a negative arterial–coronary sinus phenylalanine concentration difference (−1.72±0.36 nmol/ml, p<0.005) and phenylalanine balance (−68±19 nmol/min, p<0.01 different from 0). During the saline infusion, phenylalanine balance remained negative (−60±17 nmol/min) and differed significantly from the neutral balance seen in insulin- and amino acid–infused dogs (p<0.01). The rate of heart protein degradation at the end of the saline infusion (101±18 nmol/min) exceeded (p<0.05) that in insulin- and amino acid–infused dogs (60±7 nmol/min) (Figure 2). Heart protein synthesis did not change significantly during saline infusion and was similar to that in insulin- and amino acid–infused animals (Figure 2).

Discussion

Three significant findings emerge from the present study. First, after fasts of brief duration (16 or 36 hours), heart protein balance is negative, as indicated by the negative balance for the essential amino acid phenylalanine. Second, physiological increments in plasma insulin inhibit myocardial proteolysis and stop the net loss of heart protein observed after a 16-hour fast, when plasma concentrations of amino acids are maintained near basal levels. Finally, the generalized hypoaminoacidemia that accompanies systemic insulin administration blunts insulin’s antiproteolytic action on the heart.

Unlike other methods, the technique of measuring the steady-state exchange of unlabeled and labeled
phenylalanine across the heart allowed us to calculate the rates of both protein synthesis and breakdown in each animal before and after intervention. The net balance of phenylalanine also permits a measure of net protein balance that is independent of many of the assumptions inherent in tracer measurements of protein turnover and can provide a sensitive temporal indicator of alterations in heart protein metabolism. In addition, the measurement of heart protein degradation by the phenylalanine isotopic approach is much more direct than other in vivo estimates based on the differences between rates of synthesis (obtained isotopically) and changes in net heart protein mass over time. As such, it can be expected to be more sensitive to acute changes in protein degradation. In interpreting these results, it should also be noted that the steady state observed with respect to plasma phenylalanine does not necessarily guarantee the constancy of the tissue free phenylalanine pool during the experiment. However, available data suggest that rapid equilibration would occur between plasma and heart cellular phenylalanine pools in vivo. The 50% increase in plasma phenylalanine concentration in the animals infused with insulin and amino acids would increase the intracellular free phenylalanine pool size. Expansion of this pool, however, would account for less than 20% of the change in phenylalanine balance observed in these animals.

The finding of a negative phenylalanine, and therefore negative protein, balance for heart muscle after both a 16-hour and 36-hour fast might be surprising if heart muscle were preserved during fasting. This question has been carefully studied in the rat; in this species, heart and skeletal muscle mass are lost at comparable rates with fasting. We have not carefully compared the rates of net protein loss from heart and skeletal muscle in the fasted dog; however, we have observed that like heart, skeletal muscle protein exhibits a negative balance after an overnight fast. In addition, we recently reported that in postabsorptive humans, heart phenylalanine balance is negative, indicating a net protein catabolic state in the heart parallel to that in skeletal muscle. These observations suggest that heart muscle, like skeletal muscle, protein is also mobilized in larger animals during periods of dietary restriction.

The observation that infusion of insulin with maintenance of basal concentrations of amino acids inhibits heart muscle proteolysis is quite similar to findings described for forearm skeletal muscle and whole-body protein turnover in humans after an overnight fast. Since hyperinsulinemia comparable to that seen here occurs postprandially in large animals and humans, these findings indicate a physiological role for insulin in the homeostasis of heart muscle protein. The mechanism by which insulin acts to restrict whole-body or muscle proteolysis is not known. In liver, data suggest that the lysosomal pathway of proteolysis is sensitive to inhibition by insulin as well as by several amino acids. Whole-body studies in humans suggest that a more general effect may occur at very high insulin concentrations, where total body protein degradation declines to zero. In accord with the present results, studies with the isolated perfused heart and heart muscle have previously shown that insulin inhibits proteolysis. The physiological significance of the latter observations has been uncertain due to the very high insulin concentrations used and the highly catabolic nature of these in vitro preparations. Similarly, in vivo studies in diabetic rats have suggested that insulin replacement for several days with correction of hyperglycemia slows catabolism of myocardial protein in general and may be particularly effective in decreasing ribosomal protein degradation.

The lack of effect of insulin on heart protein turnover in the dogs fasted 36 hours and not given replacement amino acids could be due to several factors. Hypoaminoacidaemia, resulting from insulin infusion, may have blunted insulin’s antiproteolytic action. In humans, circulating amino acids have an important role in modulating insulin’s antiproteolytic action: insulin’s suppression of total body protein degradation is significantly enhanced by either replacing or supplementing amino acids during insulin infusion. An alternative possibility, that insulin resistance developed in animals fasted 36 hours, seems less likely since both total-body and myocardial glucose utilization were comparable in the 16- and 36-hour-fasted animals. In addition, we have recently observed that human forearm skeletal muscle remains sensitive to relatively low doses of insulin even after 60 hours of fasting, a time sufficient to develop resistance to insulin’s action to promote skeletal muscle glucose uptake.

Heart phenylalanine uptake did not increase during the infusion of insulin, even in the 16-hour-fasted animals receiving amino acid replacement during the euglycemic clamp, suggesting that heart protein synthesis was not stimulated by hyperinsulinemia. In the growing rat, short-term glucose infusion (and the assumed resultant hyperinsulinemia) increases myocardial protein synthesis as measured by incorporation of radiolabeled amino acid into protein with the bolus phenylalanine method introduced by Garlick and colleagues. Higher rates of heart protein synthesis are also reported following insulin therapy in young rats with streptozotocin-induced diabetes. Recent studies from our laboratory in more mature rats have demonstrated an increase in heart protein synthesis during the euglycemic clamp only when plasma amino acids were significantly elevated. In vivo, variable results have likewise been found in rat skeletal muscle, whereas skeletal muscle protein synthesis is little affected by insulin in normal postabsorptive humans when plasma amino acid concentrations are not increased.

The differences in animal species and age and techniques used to measure protein synthesis may account for some of the disparities among these studies. In adult dogs and humans, however, physiological increments in plasma insulin (hyperinsulinemia) do not appear to significantly increase total protein synthesis in heart or skeletal muscle. Although insulin’s effect on muscle protein turnover generally occurs within 30–120 minutes, we cannot exclude that a longer insulin infusion might have some effect on heart protein synthesis or that fasting levels of insulin (~10 μU/ml) are important for heart protein synthesis.

Infusion of insulin locally, as has been done in the human forearm to study insulin’s effect on skeletal muscle protein turnover, avoids the changes in plasma amino acid concentrations that occur during the sys-
We attempted to maintain plasma amino acids during the euglycemic clamp by infusing a balanced amino acid mixture. Although changes in individual amino acids did occur, e.g., alanine and glycine increased and leucine fell from initial levels (Table 3), we were successful overall in maintaining the concentrations of essential, branched chain, and total measured amino acids (Figure 2). The changes in amino acid concentrations that occurred (Table 3) were much less than the threefold to fivefold changes that are known to influence heart and skeletal muscle protein turnover.14,17,20

In the 16-hour-fasted animals, we observed a 10–15% increase in systolic blood pressure during the course of the combined euglycemic clamp and amino acid infusion. Although much greater increases in arterial pressure (from 60 to 120 mm Hg) inhibit protein degradation and stimulate synthesis in the Langendorff perfused rat heart,29 they have no effect on protein degradation in the more physiological isolated working rat heart.51 In addition, in the present experiments, the lower rate of heart protein degradation during insulin and amino acid infusion compared with saline infusion was not associated with either a higher arterial blood pressure or cardiac double product.

The comparison of phenylalanine balance or flux rates between animals or groups of animals requires that similar amounts of myocardium are represented by the venous blood sampled in the mid coronary sinus position. These measurements are not normalized per gram of tissue because accurately measuring blood flow per gram of heart is not feasible in the acute closed-chest animal model. This is not an issue when assessing the effect of an intervention within an individual animal, since the venous anatomy is constant and the catheter position is fixed and rechecked at the end of each experiment. However, it warrants consideration when comparing rates of protein turnover in 16- versus 36-hour-fasted animals. Nonetheless, coronary blood flow was essentially identical in all groups, and the amount of myocardial mass sampled was likely similar to the extent that flow per gram of tissue was the same in both groups. With this caveat in mind, rates of basal heart protein degradation in the two 16-hour-fasted groups were quite similar and when combined (121 ±12 nmol/min, n = 19) were higher (p <0.05) than in animals after a 36-hour fast (81 ±13 nmol/min). Previous studies in the rat1 and rabbit2 have indicated that fasting inhibits myocardial protein synthesis in vivo, based on measurements of radiolabeled amino acid incorporation into heart protein. However, fasting had no significant effect on myocardial protein degradation when assessed indirectly from the difference between changes in heart mass and measured rates of protein synthesis.5 Our findings would suggest that heart muscle protein loss might decline as fasting proceeds.

In summary, these studies demonstrate that increasing insulin from postabsorptive levels to high physiological levels has an anabolic effect on heart protein metabolism, returning the heart to a neutral protein balance in the fasted dog. This effect appears to be caused by regulation of protein turnover at the level of protein degradation rather than protein synthesis. These results indicate the dynamic nature of myocardial protein turnover in large animals associated with physiological changes in circulating insulin concentrations.

Acknowledgments

We express our appreciation to Rosa Hendler, MD, John Alderman, Jill Seres, George Harnish, Christophe Baron, and Ralph Jacob for their assistance and Drs. Patrick McNulty and David Fryburg for their suggestions.

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Circ Res. 1992;71:393-400
doi: 10.1161/01.RES.71.2.393

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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