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Acute Response of Human Muscle Protein to Catabolic Hormones

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Objective

The purpose of this study was to determine the acute *in vivo* response of human muscle protein to stress.

Summary Background Data

Prior animal and human *in vitro* studies have suggested that physiologic stress increases muscle protein turnover. In contrast, recent publications using a polyribosomal methodology have demonstrated a reduction in human muscle protein synthesis *in vivo* after surgery.

Methods

Five healthy volunteers were given a stable isotopic infusion of 1,2^{13}C leucine that allowed for determination of the fractional rate of muscle protein synthesis by measuring the rate of incorporation of ^{13}C label into vastus lateralis muscle biopsies. Simultaneous infusion of ^{15}N lysine and quantitation of leg blood flow by indocyanine green dye dilution allowed for estimation of leg muscle protein breakdown rate (Lys Ra) and synthesis rate (Lys Rd). These measurements were performed before and then at the conclusion of a 4-hour femoral arterial infusion of the catabolic hormones epinephrine, cortisol, and glucagon.

Results

The catabolic hormone infusion elicited a significant (65%) increase in the leg muscle protein breakdown rate and a significant but less marked increase in the rate of muscle protein synthesis, as assessed by both an increase in the fractional rate of muscle protein synthesis of 48.5% and in lysine uptake within the leg of 32%.

Conclusions

This study conclusively demonstrates that a hormonally induced stress results in a net catabolism of human muscle protein by increasing the rate of protein breakdown in excess of an increased protein synthetic rate.

During critical illness, amino acids are released from storage as muscle for use in tissue wound repair and by the liver in the acute phase protein response.[1] The resulting increase in protein catabolism can be deleterious.[1] Although it has been shown that this wasting of lean body mass results from an excess of protein breakdown, the specific pattern of protein turnover remains in debate. Determining the rates of protein synthesis and breakdown and their response to injury may be extremely helpful in directing future therapies aimed at protein sparing. For, if in catabolism, protein synthesis is

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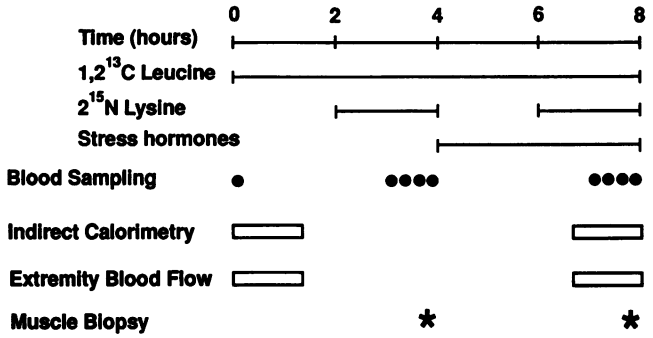

Figure 1. Study protocol flow diagram. Flow diagram of experimental design and procedure.

decreased, then attempts at blocking the inhibitory component are likely to be beneficial. However, if protein synthesis is increased (but to a lesser extent than breakdown), then pinpointing the responsible mechanism may eventually provide ways to augment this response and override the increased rate of protein breakdown.

Several investigations have attempted to discern the etiologic mechanism for protein catabolism. Studies by Bessey et al. have placed a strong suspicion that the alterations in the hormonal milieu seen with severe disease may play a significant role in mediating protein catabolism.[2] In these studies many of the metabolic responses observed during catabolic conditions, including a negative nitrogen balance, were reproduced by an infusion of the stress hormones epinephrine, cortisol, and glucagon. Based on these findings, we have attempted to assess the response in the rates of muscle protein synthesis and breakdown during critical illness by simulating the hormonal milieu with an infusion of these stress hormones.

MATERIALS AND METHODS

Five male subjects (age range, 21 to 29 years) participated in this study, which was approved by the Institutional Review Board of the University of Texas Medical Branch. Subjects were considered to be healthy according to medical history, physical examination, and screening laboratory profile. They had normal heights and weights and were not taking any medications.

Study Protocol

The study began in the morning after an overnight fast. The study lasted 8 hours and consisted of a 4-hour basal period followed by a 4-hour hormonal infusion period (Fig. 1). Placement of femoral arterial and venous catheters allowed for pressure monitoring and for serial blood sampling. After baseline blood sampling, a peripheral intravenous infusion of $1,2^{13}C$ leucine (infusion, 0.12 μM/kg/min; prime, 7.2 μM/kg) was begun at 8:00 A.M. and continued for 8 hours. At 10:00 A.M. a peripheral intravenous infusion of ^{15}N lysine (infusion, 0.06 uM/kg/min; prime, 4.8 uM/kg) was begun and continued with the $1,2^{13}C$ leucine infusion for the next 2 hours (Fig. 1). The infusion of $1,2^{13}C$ leucine was continued for 4 hours in each study period to ensure adequate incorporation of $1,2^{13}C$ into muscle protein.[3] The ^{15}N lysine infusion did not last as long since previous studies have demonstrated that a steady state equilibrium could be achieved with only 2 hours at this prime, constant infusion dosage.[4] During the 4-hour basal period, extremity blood flow was determined by bolus (5 mg) injection of indocyanine green dye into the femoral artery with spectrophotometric analysis of aspirated blood from the femoral vein.[5] The depreciation of the spectrophotometric measurement then reflected extremity perfusion as quantitated by a pediatric cardiac output computer and confirmed graphically (Lyons Medical Instruments Corp., Van Nuys, CA). Blood flow measurements were standardized for leg volume as assessed by the integration of several circumference measurements at 5-cm intervals with the length of the calf, thigh, and foot. Oxygen consumption and carbon dioxide production were also determined during the basal period by indirect calorimetry using a metabolic cart (Sensor Medics MMC Horizon System 4400, Anaheim, CA). At the completion of the 4-hour basal period, repeated blood samples were drawn simultaneously from the femoral artery and vein for determination of lysine net balance across the leg. At the end of this basal period, a muscle biopsy from the vastus lateralis muscle was obtained with a Bergström needle. Muscle specimens were immediately frozen with liquid nitrogen.

After basal measurements were obtained, the ^{15}N lysine infusion was stopped. Epinephrine (15 ng/kg/min), cortisol (6 μg/kg/min), and glucagon (3 ng/kg/min) was then infused into the femoral artery and continued for the next 4 hours. The peripheral infusion of $1,2^{13}C$ leucine continued uninterrupted during this 4-hour stress hormone infusion period. The ^{15}N lysine was restarted after 2 hours of hormone infusion by repeating the priming bolus followed by continuous infusion for the subsequent 2 hours. During the last hour of the stress hormone period, extremity blood flow and indirect calorimetry measurements were repeated. At the completion of the 4-hour stress hormone infusion, simultaneous femoral arterial and venous blood sampling was repeated and an adjacent muscle biopsy was taken to complete the study.

Analysis of Samples

Whole blood was collected in ice cold heparinized tubes, and an aliquot was deproteinized immediately

with ice cold 15% sulfosalicylic acid for determination of amino acid concentration (121 M auto analyzer, Beckman Instruments, Inc., Fullerton, CA). The remaining whole blood was stored in an ice bath until completion of the study, when plasma was separated and stored at −20 C. Hormone concentrations (catecholamines, cortisol, glucagon, and insulin) were measured by an independent laboratory using radioimmunoassay techniques (SmithKline Beecham Clinical Laboratories, Van Nuys, CA). Enrichment of plasma lysine was determined from its N- acetylpropyl ester derivative with a gas chromatography mass spectrometry system (5985B Hewlett Packard, Palo Alto, CA) using chemical ionization and monitoring ions at m/e 273.2 and 274.2. The isotopic enrichment of plasma αKIC (α-ketoisocaproic acid) was measured on its silylquinoxalinol derivative using electron impact ionization and monitoring ions at m/e 232.2 and 234.2. For each study, an aliquot of the infusate was analyzed for the exact isotope concentration to calculate the actual infusion rate.

Frozen muscle tissue was homogenized in ice cold 5% perchloric acid ($HClO_4$) and centrifuged to precipitate proteins in order to separate leucine for isotopic analysis. The protein precipitate was washed several times with perchloric acid, reprecipitated, and hydrolyzed in distilled 6 molar hydrochloric acid for 48 hours at 110 C. The hydrolysate was evaporated in vacuum to remove the hydrochloric acid and the amino acid precipitate was then assessed for the isotopic enrichment of the protein bound leucine. Because a muscle sample was not taken before starting the isotopic infusion, background enrichment of leucine was determined from proteins precipitated from a baseline plasma sample. These measurements were used as an estimate of the background enrichment of leucine in muscle protein. Proteins were precipitated from 1 mL of plasma with 5% perchloric acid and then the precipitate was washed several times with perchloric acid to ensure no contamination with plasma-free leucine. The precipitated plasma protein was next treated exactly as described for the muscle protein analysis. The enrichment of leucine from muscle and plasma proteins was measured as previously described.[3] Pure leucine was separated by ion exchange chromatography on an HPLC system (LKB, Gaithersburg, MD) using a sodium phosphate buffer system. The leucine fractions were dried in a quartz tube. After cupric oxide was added, the tube was sealed under high vacuum and the sample was combusted in a furnace at 590 C. The resulting CO_2 gas was analyzed in a SIRA II isotope ratio mass spectrometer (V.G. Isotech, Chashire, UK). This measures the isotopic enrichment of all the carbons in leucine. Because only two of the six leucine carbons were labeled with ^{13}C, the actual isotopic enrichment of leucine was obtained by multiplying the CO_2 enrichment by a factor of 3.

Calculations

The fractional muscle protein synthesis rate (%/day) was calculated according to the following formula:

$$\text{FMPS rate} = \frac{PE_{t1} - PE_{tO}}{E\alpha KIC} \times \frac{24}{t1 - tO} \times 100$$

where FMPS rate is the fractional muscle protein synthesis rate (%/day), PE_{t1}–PE_{tO} is the increase in enrichment of protein-bound leucine over the 4-hour period of tracer infusion t1–tO, and E αKIC is plateau enrichment of plasma αKIC (APE).

The net balance of lysine across the isolated limb was expressed per unit (100 mL) leg volume and calculated using the following equation:

$$NB = F([Art] - [Ven])$$

where NB is the net balance (μm/min/100 mL leg volume), F is the extremity blood flow (L/min/100 mL leg volume), and [Art], [Ven] is the femoral arterial, venous lysine concentration (μm).

Thus, a negative net balance reflects net release of lysine from the limb.

Because lysine is not known to be metabolized by peripheral tissues,[6] the rate of its appearance (Ra) from the limb reflects protein breakdown (μm Lysine/min/100 mL leg volume) and the rate of its disappearance (Rd) in the limb reflects protein synthesis (μm lysine/min/100 mL leg volume). These two rates are determined as previously described by Cheng et al.[7] from the following equations:

$$Ra = F[Art][(^{Ea}/Ev) - 1]$$

$$NB = Ra - Rd$$

where F is the extremity blood flow (L/min/100 mL leg volume), [Art] is the femoral arterial lysine concentration (μm), and Ea, Ev is the femoral artery, vein enrichment of lysine (APE).

A Student's paired t test was employed for statistical comparisons between basal and stress hormone conditions. Results are presented as mean ± SEM and $p < 0.05$ was accepted as significant.

RESULTS

This study was performed only on males similar in age and body habitus (see Table 1). The simultaneous infusion of the three stress hormones epinephrine, cortisol, and glucagon into the femoral artery resulted in significant elevations of the concentration of these hormones

Table 1. VOLUNTEER CHARACTERISTICS

Age (yr)	28 ± 1*
Body surface area (m^2)	1.95 ± 0.06
Weight (kg)	76.5 ± 4.2
Leg volume (100 mL)	149 ± 9

* Mean ± SEM.

in the femoral vein (Table 2) and are similar to elevations observed in severely injured patients.[8] Hemodynamically, this simulated stress resulted in a significant increase in the heart rate and leg blood flow in these volunteers without an alteration in mean arterial blood pressure (Table 3). There was only a slight, insignificant increase in total body energy expenditure with the stress hormone infusion into the leg (Table 3).

Isotopically labeled lysine served as the tracer amino acid for evaluation of the isolated limb protein kinetics. Because most of the metabolically active protein in extremities is muscle, studies of amino acid balance across the leg imply an assessment of muscle protein kinetics. In the basal fasting state, the rates of protein breakdown and synthesis were similar and resulted in a near equilibrium in the net protein balance in the leg (Fig. 2). The stress hormone infusion produced a significant increase (65%) in lysine Ra (protein breakdown rate), but only a 32% increase in lysine Rd (protein synthesis rate). Although this increased rate of protein synthesis was significantly greater than the basal value, the resultant negative net protein balance was equivalent to a loss of 5.7 g nitrogen/day from the leg assuming 3.4 um of lysine per gram of nitrogen.[9]

The rate of incorporation into muscle protein of isotopically labeled leucine served as an index of the fractional rate of muscle protein synthesis. The fractional muscle protein synthesis rate was 1.34 ± 0.12% per day, which is similar to that reported by other investigators using this method.[10,11] The stress hormone infusion induced a significant (49%) increase in the muscle protein synthesis rate without a significant change in the precursor pool (αKIC) enrichment (Table 4).

Table 2. HORMONE CONCENTRATIONS (FEMORAL VEIN)

Hormone	Basal	Hormone Infusion
Epinephrine (pg/mL) (normal, <60)	142 ± 59	1204 ± 649*
Cortisol (μg/dL) (normal, 7–27)	17 ± 3	92 ± 9*
Glucagon (pg/mL) (normal, 50–200)	145 ± 45	498 ± 102*
Insulin (μU/mL) (normal, 5–25)	13 ± 3	15 ± 2

Mean ± SEM.
* $p < 0.05$ compared to basal.

Table 3. HEMODYNAMIC RESPONSE

	Basal	Hormone Infusion
Mean arterial blood pressure (torr)	95 ± 4	96 ± 5
Heart rate (beats/min)	54 ± 3	68 ± 4*
Resting energy expenditure (kcal/24°)	839 ± 71	954 ± 42
REE/predicted basal REE†	0.93 ± 0.06	1.02 ± 0.04
Leg blood flow (mL/min/100 mL leg volume)	2.96 ± 0.16	13.11 ± 1.59*

Mean ± SEM.
* $p < 0.05$ compared to basal.
† Predicted basal REE calculated from Harris-Benedict Eq.

DISCUSSION

This study demonstrates that the skeletal muscle in humans responds to stress as simulated by catabolic hormones by increasing both the rates of protein synthesis and breakdown with net muscle protein catabolism resulting from the greater increase in protein breakdown. Documentation of the increased rate of muscle protein synthesis induced by stress hormones is corroborated by two separate methodologies. One method assessed the rate of incorporation of isotopically label leucine into muscle and demonstrated that the rate of muscle protein synthesis increased by 49% with the acute infusion of stress hormones. The rate of protein synthesis was also determined by the isolated leg amino acid balance of ^{15}N lysine. This methodology demonstrated a similar per cent increase (32%) in the protein synthesis rate with the

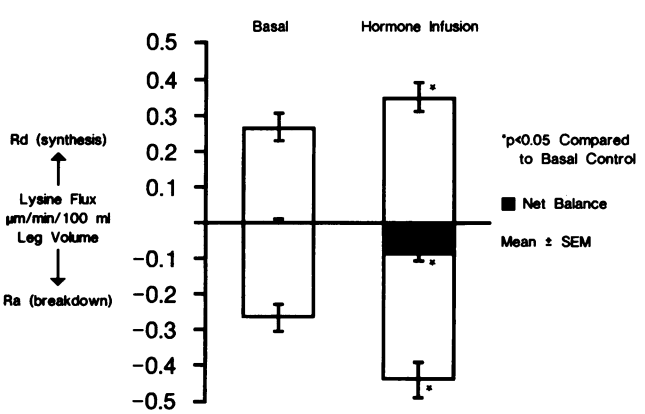

Figure 2. Leg protein kinetics. The changes in the rates of muscle protein synthesis and breakdown induced by catabolic hormones.

Table 4. FRACTIONAL RATE OF MUSCLE PROTEIN SYNTHESIS

	Basal	Hormone Infusion
Fractional muscle protein synthesis rate (%/day)	1.34 ± 0.12	1.99 ± 0.39*
Plateau enrichment of plasma αKIC (APE)	8.1 ± 0.6	9.2 ± 0.7

Mean ± SEM.
* $p < 0.05$ compared to basal.

hormonally simulated stress. This correlation of response by different methodologies strongly suggests that stress hormones stimulate the rate of human muscle protein synthesis. Muscle protein breakdown was also determined by the isolated leg ^{15}N lysine balance method and demonstrated a 65% increase in response to the stress hormone infusion. The 33% greater increase in protein breakdown over synthesis represents the net muscle protein catabolism induced by catabolic hormones and is consistent with the overall net protein catabolism seen during critical illness.

Several studies in animals and *in vitro* studies with human muscle support the findings of this study,[12-14] which are consistent with the hypothesis of Newsholme et al. that increasing substrate turnover increases metabolic control and sensitivity during physiologic stress.[15] However, the current results conflict with the results of another study by Wernerman et al. in which the rate of human muscle protein synthesis was estimated from alterations in the concentration and configuration of ribosomes in skeletal muscle. In a series of similar investigations, Wernerman et al. suggested that the rate of human muscle protein synthesis actually decreased 3 days after surgery[16] and after six hours of catabolic hormone infusion in normal volunteers.[17] One possible reason for the discrepancy in the results obtained by the polyribosome methodology compared to the current stable isotopic methodologies is that the polyribosome method merely reflects the capacity of DNA/RNA to synthesize bulk muscle proteins, which may not be reflective of the actual rate of skeletal muscle protein synthesis. Supporting this argument Wernerman et al. have demonstrated that the function of several enzymes within skeletal muscle remains intact after surgical trauma despite a documented decrease in polyribosome concentration.[18] Thus, it may be that muscle enzyme function remains normal and supportive of hormonally induced increases of muscle protein synthesis. Yet the peak capacity for muscle protein synthesis and enzyme function, as estimated by the alteration of polyribosomes, is decreased. Previous studies using the stable isotope methodology have demonstrated that the rate of whole body muscle protein synthesis correlates with the fractional muscle protein synthesis rate.[11] This supports the contention that stable isotope methodology accurately reflects alterations in muscle protein kinetics.

The findings of this study demonstrate that the alterations of protein turnover during critical illness appear to be mediated and regulated to a significant degree by acute changes in the endocrine environment. While to extrapolate the observed changes in muscle protein kinetics induced by this short 4-hour hormonal infusion to encompass the muscle protein metabolism during the entire prolonged hypermetabolic response to severe injury cannot be verified, this acute hormone infusion and the response to severe injury both result in a characteristic net protein catabolism and thus connote a similarity in kinetics. In this acute study, insulin levels are not significantly altered by the stress hormone infusion. This implies that the alterations in muscle protein kinetics observed with the stress hormones are not the result of any counter-regulatory affect mediated by insulin. However, other potential modifiers of this muscle protein response have yet to be quantitated. For example, the muscle protein response to stress may be partially mediated by the presence of a wound. Several studies have demonstrated that activated mononuclear cell preparations significantly increase muscle protein breakdown supposedly by a liberation of cytokines.[19,20] Moldawer et al. have recently shown that the activating mediator is not interleukin-1 or tumor necrosis factor.[21] Thus, mediators released from macrophages within damaged tissue may possibly further augment protein catabolism but by a yet unidentified mechanism. Furthermore, the effect of any neurologic component to protein catabolism cannot be assessed in this study. These healthy volunteers did have placement of intravenous and arterial catheters using local anesthesia, yet their discomfort and anxiety with study participation could not be totally eliminated. However, it is reasonable to assume that the neurologic response in the volunteers is minimal compared to severely injured patients.

In this study the three stress hormones were infused simultaneously, hence it is not possible to ascertain their individual effects on muscle protein metabolism. It is highly probable that each of these hormones infused individually would produce distinctly different muscle protein kinetic responses. There have been suggestions that epinephrine alone may be anabolic by mobilizing free fatty acids and increasing glucose production with a resultant increased energy turnover.[22,23] However, epinephrine's anabolic effect on muscle protein metabolism, as inferred from amino acid balance studies, appears to be transient and thus prolonged continuous epi-

nephrine infusions may not be an effective anabolic agent.[24] In contrast to epinephrine, cortisol induces muscle protein catabolism.[25] While elevations in glucocorticoids after severe trauma have been shown to be essential for survival,[26] there may be potential therapeutic value in antagonizing the muscle catabolic action of cortisol if such an effect could be isolated. Glucagon alone also appears to induce muscle protein catabolism (as demonstrated by Pacy et al.), where hyperglucagonemia during somatostatin induced insulin resistance, increased leucine oxidation, and decreased synthesis both at the whole body and across the forearm musculature.[27] This implies a potential therapeutic value in a glucagon antagonist. Such a cortisol or glucagon antagonist may be therapeutic only in the face of extensive energy substrate availability and amino acid supplementation.

The hormonal alterations that accompany critical illness have a major impact on modulating muscle protein kinetics. Net protein catabolism results from increases in muscle breakdown that is only partially negated by increases in muscle protein synthesis. Therefore, attempts at pharmacologically altering the hormonal milieu may hold promise for future therapeutic interventions aimed at minimizing protein catabolism.

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