Modulation of Skeletal Muscle Protein Synthesis by Amino Acids and Insulin During Sepsis

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Effects of different concentrations of insulin and amino acids on protein synthesis in skeletal muscle of young, fed septic rats were determined in the perfused rat hindlimb. Rates of protein synthesis in gastrocnemius were measured by incorporation of [^{3}H]-phenylalanine into protein. Perfusion of hindlimb muscles from young, fed control rats with medium containing either insulin and a complete mixture of amino acids at plasma concentration (1×) or a mixture of amino acids at 10-fold (10×) plasma concentration resulted in an approximately twofold stimulation of the rate of protein synthesis. The effect of amino acids on protein synthesis was partly accounted for by elevated concentrations of branched-chain amino acids ([BCAA] leucine, isoleucine, and valine). In young, fed septic rats, the rate of protein synthesis in muscle perfused with buffer containing the normal concentration of amino acids was reduced 40% as compared with control levels ($P < .05$). In contrast to controls, addition of insulin (1,000 μU/mL) did not augment protein synthesis in muscle from young, fed septic rats perfused with the complete mixture of amino acids. Addition of insulin 10,000 μU/mL stimulated protein synthesis approximately 80% in gastrocnemius of septic rats ($P < .05$). However, the rate of protein synthesis remained less than that observed in young, fed control rats at similar insulin concentrations. Perfusion with medium containing 10× plasma amino acids stimulated protein synthesis approximately fourfold in young, fed septic rats as compared with control animals. In contrast to controls, BCAA at 10× plasma concentration did not augment protein synthesis in young, fed septic rats. There were no significant differences in rates of protein synthesis between control and nonseptic-abscess rats in any of the conditions examined. The results suggest a relative resistance of protein synthesis to the stimulatory effects of insulin or BCAA in muscle from young, fed septic animals, whereas the response to total amino acid supplementation is unaffected by the septic process.

SEPSIS INITIATES a cascade of events that eventually lead to a negative nitrogen balance and loss of lean body mass. Because skeletal muscle comprises as much as 45% of body weight, much of this loss of nitrogen originates from protein in this tissue. This catabolic phase is an intrinsic response to trauma and sepsis, with the amount of muscle loss exceeding that resulting from bed rest alone. In trauma patients, the catabolic phase is short-lived, and is followed by restoration of positive nitrogen balance and lean body mass. In contrast to trauma, the catabolic phase continues unabated and accounts for the massive loss of body protein in sepsis. The loss of protein in skeletal muscle arises from an imbalance in protein turnover. Muscle protein dyshomeostasis in sepsis arises from an activation of protein degradation[1,2] and inhibition of protein synthesis.[3-6]

Amino acid nutrition improves nitrogen balance during trauma or infection. Negative nitrogen balance after severe trauma or sepsis can be minimized by infusion of amino acids, provided adequate energy substrates are also present.[7-9] Hence, nutritional support therapy involving amino acid supplementation has become an integral part of the management of the critically ill patient.[10] Despite the empirical finding of reduced urea excretion and negative nitrogen balance, little is known concerning the mechanisms responsible for improved nitrogen retention in critically ill patients after infusion of amino acids.

Amino acids have been shown to have a regulatory role in protein metabolism both in vivo[11-14] and in vitro.[15,16] During in vitro perfusion, inclusion of amino acids in the medium at five or 10 times normal plasma concentrations of amino acids stimulates protein synthesis in skeletal muscle of young (80 to 130 g) fed or fasted rats.[16] Similarly, *in vivo* infusion of amino acids to achieve a relative hyperaminoacidemia increases muscle protein synthetic rate in rats[17] and man.[13] However, in vivo studies are more difficult to interpret because infusion of amino acids also increases plasma insulin concentrations. The possibility that the effect of amino acids to stimulate protein synthesis *in vivo* results from a combination of increased amino acids and increased insulin concentrations cannot be excluded.

Like amino acids, insulin stimulates protein synthesis in isolated perfused skeletal muscle and heart preparations.[18,19] The effects of insulin on skeletal muscle protein synthesis in vivo remain unresolved. In young rats, infusion of insulin stimulates muscle protein synthesis, with maximal insulin effects occurring at approximately 150 μU/mL.[20] However, when amino acids are infused along with insulin, maximal stimulation of protein synthesis is observed at a much lower concentration of insulin (20 μU/mL). Despite the anabolic effect of insulin on protein synthesis in animal studies, similar findings in humans have proved elusive. The major reason for the lack of stimulatory effect of insulin on protein synthesis in human skeletal muscle may lie in the failure to maintain normal plasma and intracellular amino acid concentrations during hyperinsulinemia.[21-22] In this regard, infusion of amino acids concurrently with euglycemic hyperinsulinemia is associated with stimulation of protein synthesis across the leg.[23]

The purpose of the present set of investigations was to establish the effect of insulin and/or amino acid supplemen-

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tation on augmenting protein synthesis in gastrocnemius muscle during sepsis. The gastrocnemius was chosen because sepsis causes inhibition of protein synthesis in muscle composed primarily of fast-twitch fibers (ie, gastrocnemius and psoas), whereas in muscles composed of slow-twitch fibers (ie, soleus and heart), protein synthesis is unaffected.[5] Using the perfused hindlimb, effects of amino acids on protein synthesis can be examined independent of plasma insulin concentration. The results provide evidence that inclusion of amino acids at 10-fold (10×) normal plasma concentration in the perfusate stimulates protein synthesis approximately fourfold in gastrocnemius of septic rats. Furthermore, the studies provide evidence for a relative resistance to the effect of insulin to stimulate protein synthesis during sepsis.

MATERIALS AND METHODS

All animals were provided rat chow and water ad libitum throughout the study. Male Sprague-Dawley rats were anesthetized with a combination of ketamine 110 mg/kg body weight and acepromazine 1 mg/kg body weight. Sepsis was then induced by implanting a fecal-agar pellet inoculated with 10^3 colony-forming units *Escherichia coli* and 10^8 colony-forming units *Bacteroides fragilis* into the lower abdominal cavity.[5,24-27] A sterile, nonseptic abscess (sterile inflammation) was generated by replacing the bacterial inoculations with sterile saline.[5,24,27] After recovery from the surgical procedures, the intraabdominal abscess was allowed to develop for 5 days. Rats implanted with an infected pellet have a 10% to 20% mortality during the first 48 hours, while no deaths are observed in rats with sterile inflammation. After the first 48 hours, all septic animals survive and form a stable abscess. Septic animals examined 5 days after introduction of the fecal-agar pellet show an intraabdominal abscess with accumulation of pus surrounding the remainder of the pellet. The abscess cavity shows uniform sheets of polymorphonuclear leukocytes and rod-shaped gram-negative bacteria. Bacteremia is present in approximately 75% of animals inoculated with the bacteria, but is absent in rats with the sterile, nonseptic abscess.[27] Septic animals show an increased cardiac output and decreased total peripheral resistance indicative of a hyperdynamic cardiovascular state, leukocytosis, increased extracellular fluid volume, weight loss, muscle wasting, and low-grade fever. Metabolically, these animals exhibit euglycemia and hyperlactatemia with increased glucose production and utilization rates. All these are characteristic of patients with a stable hypermetabolic, hyperdynamic septic condition without evidence of hypoperfusion or end-stage organ failure.

Laparotomies were not performed in control rats, because previous studies provided evidence that skeletal muscle protein content and rates of protein synthesis are not altered 48 hours after laparotomy[28] or 5 days after formation of a sterile, nonseptic abscess[5] as compared with these parameters in nonoperated rats. Previous studies have provided evidence that nonseptic-abscess rats and/or septic rats consume equal amounts of rat chow over the course of 5 days.[24-26] However, neither nonseptic-abscess rats nor septic rats consume as much rat chow as control rats on days 1 and 2 postsurgery. By 72 hours postsurgery, food consumption in septic rats equals that of control animals.[24-26] In a previous report, we provided evidence indicating that pair-feeding control rats for 2 days followed by normal food intake for 3 days results in rates of protein synthesis indistinguishable from those of rats fed ad libitum for 5 days.[25] Therefore, reduced food intake for 2 days followed by ad libitum feeding for 3 days does not alter protein synthesis in control rats.

Hindlimb Perfusion Technique

Hindlimb perfusions were performed according to procedures described by Ruderman et al[29] as modified by Bylund-Felenius et al.[30] Five days after implantation of the fecal-agar pellet, rats were anesthetized with sodium pentobarbital 50 mg/kg body weight and the skin covering the right and left hindlimbs was removed. A midline incision was made, and both the inferior vena cava and abdominal aorta were exposed. The abdominal aorta was cannulated, and perfusate was delivered immediately via the abdominal aorta to the hindlimb musculature. The inferior vena cava was then cannulated. The first 50 mL perfusate passing through the hindlimb was discarded. The inferior vena cava cannula was then connected to the perfusion system, and recirculation of the perfusate was begun. After perfusion for an additional 5 minutes, [^{3}H]-phenylalanine was introduced into the perfusate to produce a final concentration of 1 μCi/mL, and perfusion continued for 30 to 120 minutes. Following perfusion, gastrocnemius muscles were frozen between aluminum blocks precooled to the temperature of liquid nitrogen. A perfusate sample was withdrawn and centrifuged to remove red blood cells. Plasma samples were stored at −20°C until analyzed for determination of phenylalanine specific radioactivity. All perfusions were begun between 8 and 11 AM.

The perfusate consisted of a modified Krebs-Henseleit bicarbonate buffer containing 30% (vol/vol) washed bovine erythrocytes, 4.5% (wt/vol) bovine serum albumin (Bovine serum albumin fraction V; ICN Biochemicals, Aurora, OH), 11 mmol/L glucose, 1.4 mmol/L phenylalanine, and all other amino acids at concentrations normally found in rat plasma, unless otherwise indicated. Concentrations of each amino acid in the different perfusates are listed in Table 1. Previous reports from our laboratory have provided evidence that plasma amino acid concentrations are not different between control and septic rats.[24] The medium was maintained at 37°C and gassed with humidified 95% O_2/5% CO_2.

Measurement of Protein Synthesis

Rates of protein synthesis were estimated by incorporation of [^{3}H]-phenylalanine into muscle proteins.[5,25,26] Phenylalanine is a suitable marker for measuring protein synthesis because it is neither synthesized nor degraded by skeletal muscle. Frozen tissue

Table 1. Composition of Amino Acids in the Perfusates (μmol/L)

Amino Acid	1x Amino Acid	10x Amino Acid	1x Amino Acid + 10x BCAA
Leucine	161	1,610	1,610
Isoleucine	90	900	900
Valine	173	1,730	1,730
Alanine	470	4,700	470
Glutamic acid	75	750	75
Aspartic acid	38	380	38
Threonine	270	2,700	270
Serine	243	2,430	243
Proline	186	1,860	186
Glycine	408	4,080	408
Cysteine	23	230	23
Histidine	63	630	63
Arginine	132	1,320	132
Tryptophan	69	690	69
Tyrosine	83	830	83
Lysine	418	4,180	418
Methionine	46	460	46
Asparagine	63	630	63
Glutamine	666	6,660	666
Phenylalanine	1,400	1,400	1,400

was powdered under liquid nitrogen, and a portion (0.5 g) was homogenized in 5 vol ice-cold 10% trichloroacetic acid (TCA) and centrifuged at 10,000 × *g* for 11 minutes at 4°C. The supernatant was decanted, and the pellet was washed five times with 10% TCA to remove any acid-soluble radioactivity. After a wash with acetone and then water, the pellet was dissolved in 0.1N NaOH. Aliquots were assayed for protein content using the Biuret method with crystalline bovine serum albumin as a standard. Another aliquot was assayed for radioactivity by liquid scintillation spectrometry using corrections for quenching (disintegrations per minute). The rate of protein synthesis, expressed as nanomoles of phenylalanine incorporated into protein per hour per gram of muscle, was determined by dividing disintegrations per hour incorporated in muscle by perfusate phenylalanine specific radioactivity.

Specific radioactivity of phenylalanine in perfusate samples was measured by high-performance liquid chromatography analysis using the method reported by Drnevich and Vary.[31] Briefly, perfusate samples were deproteinized by addition of an equal volume of 10% (wt/vol) TCA, mixed by vortexing, and centrifuged. The supernatant was decanted, and the pH of the supernatant was increased to 9.0 with NaOH. An aliquot (40 μL) of the plasma extract was dabsylated. Samples were chromatographed using an Alltech Econosphere C18 (5 μm) column (Alltech Associates, Deerfield, IL) with a Beckman Model 450 high-performance liquid chromatography apparatus equipped with a Beckman 171 Radioisotope Detector (Beckman Instruments, San Ramon, CA). Specific radioactivity of phenylalanine was then calculated by dividing disintegrations per minute of the phenylalanine peak by concentration of phenylalanine in the sample.[31] Bylund-Felenius et al[30] provided evidence that at perfusate concentrations above 0.8 mmol/L, specific radioactivity of tRNA-bound phenylalanine is equal to that of extracellular and intracellular pools of free phenyalanine. Therefore, specific radioactivity of perfusate phenylalanine provides an accurate estimate of specific radioactivity of phenylalanine tRNA.

Determination of Translational Efficiency

Translational efficiency was estimated by expressing the rate of protein synthesis relative to total RNA content in muscle. Total RNA content was measured from homogenates of muscle samples as described previously.[5,25,26] Briefly, 0.3 g frozen, powdered tissue was homogenized in 5 vol ice-cold 10% TCA. The homogenate was centrifuged at 10,000 × *g* for 11 minutes at 4°C. The supernatant was discarded, and the remaining pellet was mixed in 2.5 mL 6% perchloric acid. The sample was centrifuged at 10,000 × *g* for 6 minutes at 4°C, the supernatant discarded, and the procedure repeated. Then, 1.5 mL 0.3N KOH was added to the pellet, and the samples were placed in a 50°C water bath for 1 hour. Samples were then mixed with 5 mL 4N perchloric acid and centrifuged at 10,000 × *g* for 11 minutes. RNA concentration in the resulting supernatant was determined by absorbance at 260 nm corrected by absorbance at 232 nm.

Statistical Analysis

Statistical evaluation of data was performed using ANOVA (INSTAT Software, San Diego, CA) to test for overall differences among groups. This was followed by Student's *t* test for unpaired comparisons only when a significant difference by ANOVA was observed. Differences among means were considered significant when *P* was less than .05.

RESULTS

We attempted to determine whether inclusion of amino acids at 10× plasma concentration in the perfusate would augment the rate of protein synthesis in muscles measured during in vitro perfusion. Initially, effects of amino acids on protein synthesis in gastrocnemius were examined in fed control rats weighing between 250 and 300 g, a size comparable to that used in our previous studies.[5,32] When hindlimb muscle from fed control rats (n = 6) weighing between 250 and 300 g was perfused in vitro, the rate of protein synthesis was not significantly enhanced when the perfusate was supplemented with 10× plasma concentration of amino acids (Fig 1). This observation is consistent with studies in fasted animals, in which protein synthesis in muscles from older rats was not enhanced by addition of a mixture of amino acids to the perfusate.[16] Previous investigations providing evidence for a stimulatory effect of amino acids on skeletal muscle protein synthesis were performed on young rats weighing 60 to 130 g.[16] Therefore, effects of amino acids on protein synthesis were measured in fed younger rats (140 g) and compared with effects in fed older rats (300 g). When animals weighing between 140 and 160 g were used, rates of protein synthesis at 1× amino acid concentrations were greater in younger rats (n = 6) than in older rats (n = 6). Inclusion of amino acids at 10× plasma concentration resulted in a 2.5-fold stimulation of protein synthesis ($P < .01$) in younger rats (n = 5; Fig 1) as compared with younger fed rats perfused with buffer containing 1× amino acids. These data suggest that the stimulatory effects of amino acids on protein synthesis were observed in perfused skeletal muscle of fed young rats but not in fed older rats. Furthermore, the results suggest that age rather than nutritional state may influence the response of skeletal muscle to altered amino acid concentrations. Therefore, subsequent studies were performed in rats weighing between 150 and 200 g.

Because our previous studies in vivo were performed in

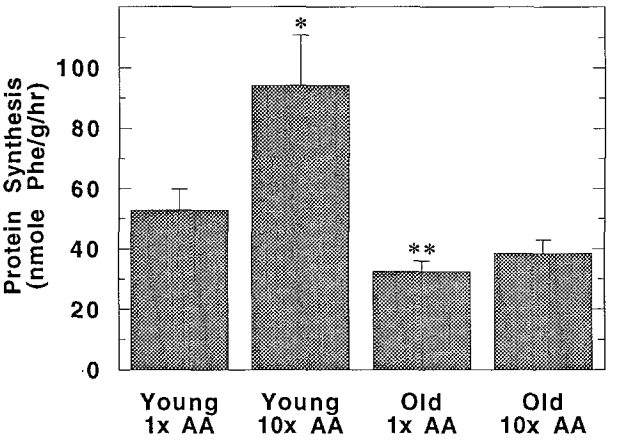

Fig 1. Effects of amino acids on muscle protein synthesis in young and old rats. Hindlimb preparations from either young (140 to 160 g) or old (250 to 300 g) fed rats were perfused in vitro. Amino acids were added to the perfusate at normal (1× AA) or 10 times (10× AA) plasma concentrations, with the exception of phenylalanine, which was present at 1.4 mmol/L in all perfusates. After washout of the first 50 mL perfusate, recirculation of buffer was initiated. After 5 minutes, [^{3}H]-phenylalanine (1 μCi/mL) was added, and perfusion continued for an additional 30 minutes. Rates of protein synthesis were measured by incorporation of [^{3}H]-phenylalanine in muscle protein. Each value represents the mean ± SE of 5 to 6 determinations, each involving a separate perfusion experiment. ANOVA $F_{3,20} = 9.3$, $P < .001$: *$P < .01$, **$P < .05$ *v* young 1× AA.

fed larger rats,[5,25,32] it was necessary to establish that the sepsis-induced inhibition of protein synthesis was observed in younger rats with a septic abscess. In these studies, rates of protein synthesis in gastrocnemius from control and septic rats weighing between 150 and 200 g were measured over a 2-hour period with addition of amino acids at normal (1×) plasma concentration to the perfusate. Results of these experiments are shown in Fig 2A. Compared with control rats (n = 15) perfused with amino acids at 1× plasma concentration, the rate of protein synthesis was not significantly altered in rats with a sterile abscess (n = 4) (40 ± 7 nmol Phe/g/h for sterile inflammation *v* 47 ± 7 for control). This observation is consistent with our previous investigations indicating no significant difference in the rate of protein synthesis in muscle between nonoperated control rats and rats with a sterile, nonseptic abscess.[5] Furthermore, the results are also consistent with reports by Preedy et al[28] demonstrating that rates of protein synthesis in skeletal muscle were unaffected in rats that underwent laparotomy as compared with nonanesthetized control rats.

In contrast to control rats or rats with a nonseptic abscess, the rate of protein synthesis was significantly reduced by approximately 55% in gastrocnemius of septic rats (n = 7) as compared with controls. The magnitude of the decrease in protein synthesis in our septic rats as compared with either control or sterile-inflammation rats is consistent with our previous measurements both during in vitro perfusion at similar perfusate amino acid concentrations[25] and in vivo.[5,26,32] Because sterile-inflammation and septic rats consume equal amounts of rat chow after implantation of the fecal-agar pellet,[24,26] food intake can be eliminated as a causative factor for the decrease in the rate of protein synthesis in muscles from septic rats.

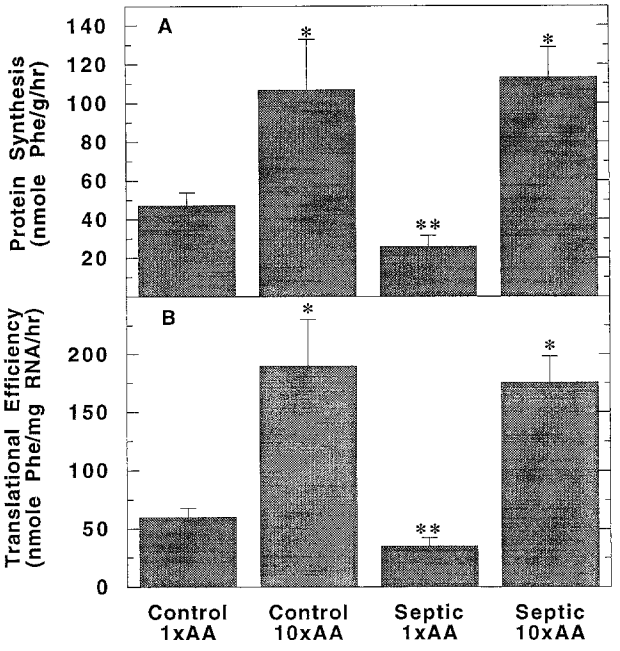

Fig 2. Effects of amino acids on protein synthesis and translational efficiency in gastrocnemius from control and septic rats. Hindlimb preparations from either control or septic rats were perfused in vitro. Amino acids were added to the perfusate at normal (1× AA) or 10 times (10× AA) plasma concentrations, with the exception of phenylalanine, which was present at 1.4 mmol/L in all perfusates. After washout of the first 50 mL perfusate, recirculation of buffer was initiated. After 5 minutes, [^{3}H]-phenylalanine (1 μCi/mL) was added, and perfusion continued for an additional 120 minutes. Rates of protein synthesis were measured by incorporation of [^{3}H]-phenylalanine in muscle protein (A). Translational efficiency (B) was calculated by expressing the rate of protein synthesis relative to total RNA content of muscle. Each value represents the mean ± SE of 7 to 15 determinations, each involving a separate perfusion experiment. (A) ANOVA $F_{3,38} = 9.3$, $P < .001$: *$P < .005$ *v* 1× AA; **$P < .05$ *v* control 1× AA. (B) ANOVA $F_{3,37} = 16.4$, $P < .001$: *$P < .005$ *v* 1× AA; **$P < .05$ *v* control 1× AA.

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As was observed in control rats (n = 7), addition of amino acids at 10× plasma concentration significantly increased the rate of protein synthesis in gastrocnemius of both sterile-inflammation rats (68 ± 13 nmol Phe/g/h) and septic rats (n = 8) as compared with rates obtained with 1× amino acids. Furthermore, muscle protein synthesis was stimulated by the mixture of amino acids to approximately the same extent in septic rats as in controls.

The protein synthetic rate depends on both the number of ribosomes and efficiency of the translational process. Previous results from our laboratory have provided evidence that inhibition of protein synthesis in muscle of septic rats results from a decreased translational efficiency rather than a decreased number of ribosomes.[5,25,26] Ribosomal RNA accounts for as much as 80% of the total tissue RNA content. Hence, changes in total tissue RNA content presumably represent alterations in the number of ribosomes. Consistent with our previous observations,[5,25] total muscle RNA content was not significantly altered in muscle from septic rats (0.72 ± 0.05 mg RNA/g) as compared with controls (0.71 ± 0.03). Therefore, we assessed whether the decrease in the rate of protein synthesis in muscles from younger septic rats perfused at 1× amino acids resulted from a reduced translational efficiency. Translational efficiency in gastrocnemius of control and septic rats, expressed as the rate of protein synthesis relative to RNA content, is shown in Fig 2B. Efficiency of translation was significantly ($P < .05$) decreased over 40% in gastrocnemius of young septic rats as compared with controls. Consistent with our previous findings,[5,25,26] inhibition of protein synthesis in younger septic rats also correlated with a reduced translational efficiency rather than with a change in the amount of ribosomes. Inclusion of amino acids at 10× plasma concentration did not alter total RNA content in either condition examined, but stimulated translational efficiency in both control and septic rats. The sepsis-induced decrease in translational efficiency of gastrocnemius was reversed by perfusion with buffer containing 10× amino acids.

Studies in perfused skeletal muscle,[16] incubated diaphragm,[33,34] and perfused heart[35] have provided evidence that a mixture of branched-chain amino acids ([BCAA] leucine, valine, and isoleucine) stimulate protein synthesis. This observation was confirmed for fed control rats in the present set of experiments. Inclusion of BCAA at 10× and other amino acids at 1× plasma concentration stimulated protein synthesis in gastrocnemius of control rats (n = 11) by approximately 40% as compared with muscle from control rats perfused with buffer containing 1× amino acids

(n = 5; Fig 3). There was no significant difference in the rate of protein synthesis in gastrocnemius between control and sterile-inflammation rats ($P > .4$) perfused in the presence of 10× BCAA. In contrast to controls, inclusion of BCAA at 10× plasma concentration did not stimulate protein synthesis in gastrocnemius of septic rats (n = 5) as compared with septic rats perfused with buffer containing 1× amino acids (n = 10).

Inhibition of protein synthesis in gastrocnemius during sepsis is caused by a block in peptide-chain initiation.[5,25] In other studies both in heart[36] and skeletal muscle,[18,19] decreased rates of protein synthesis secondary to inhibition of peptide-chain initiation were overcome by addition of insulin to the perfusate. Therefore, we investigated the effect of different concentrations of insulin on protein synthesis in gastrocnemius from control, sterile-inflammation, and septic rats. Because reports concerning the effects of insulin on muscle protein synthesis measured in vivo are conflicting, it was necessary to use an in vitro perfusion system, in which sensitivity to insulin can be compared in control and septic rats.[18,19] In the present experiments, the stimulatory effect of insulin on protein synthesis in skeletal muscle from control fed animals was confirmed (Fig 4). Insulin at the lowest concentration tested (100 μU/mL) stimulated protein synthesis (n = 8) approximately 50% as compared with muscle perfused in the absence of insulin (n = 18) when physiologic concentrations of amino acids were provided ($P < .005$). Increasing insulin concentration 10-fold (1,000 μU/mL, n = 13) further increased the rate of protein synthesis in muscles from control animals. There were no further significant increases in protein synthesis at higher insulin concentrations (10,000 μU/mL, n = 5). The concentration of insulin producing a 50% stimulation of protein synthesis was calculated to be 102 μmol/L. These findings are consistent with previous studies in skeletal muscle both in vitro[18,19] and in vivo.[20] In muscle from sterile-inflammation rats, when insulin was added to the perfusate at concentrations of 1,000 μU/mL, rates of protein synthesis (61 ± 4) were significantly ($P < .005$) stimulated 88% as compared with rates obtained in muscle from sterile-inflammation rats perfused in the absence of insulin (33 ± 4). There were no significant differences in the rate of protein synthesis between control and sterile-inflammation rats ($P > .2$).

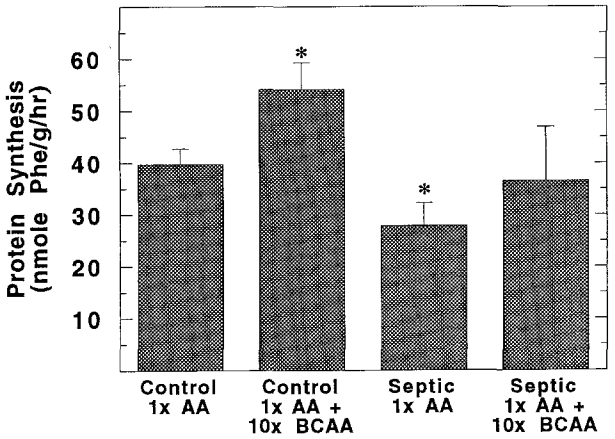

Fig 3. Effects of BCAA on rates of protein synthesis in gastrocnemius from control and septic rats. Rates were measured during in vitro perfusion as described in Fig 2. Amino acids were added to the perfusate at normal (1× AA) plasma concentrations, with the exception of phenylalanine, which was present at 1.4 mmol/L in all perfusates. In some experiments, BCAA (leucine, isoleucine, and valine) were added at 10 times (10× BCAA) normal plasma concentrations. Each value represents the mean ± SE of 5 to 11 determinations, each involving a separate perfusion experiment. ANOVA $F_{3,27} = 5.3$, $P < .01$: *$P < .05$ *v* control 1× AA.

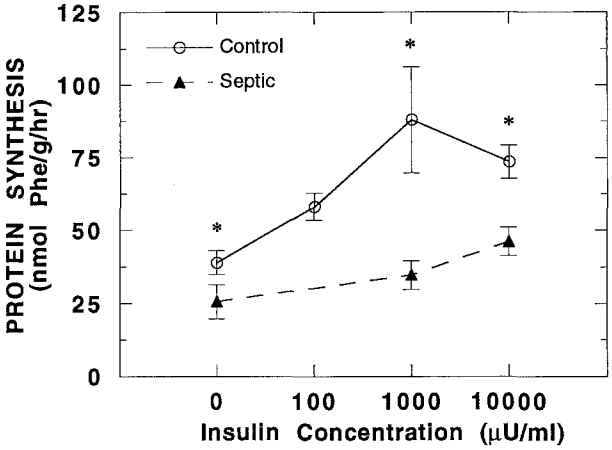

Fig 4. Effects of different insulin concentrations on rates of protein synthesis in gastrocnemius from control or septic rats. Rates were measured during in vitro perfusion as described in Fig 2. Amino acids were added to the perfusate at normal (1× AA) plasma concentrations, with the exception of phenylalanine, which was present at 1.4 mmol/L in all perfusates. Insulin at various concentrations (0 to 10,000 μU/mL) was added to the perfusate. Each value represents the mean ± SE of 4 to 18 determinations, each involving a separate perfusion experiment. *$P < .05$ *v* septic rats at corresponding insulin concentrations.

In contrast to controls, when insulin was added to the perfusate at concentrations of 1,000 μU/mL (n = 14), no significant stimulation of protein synthesis was observed in gastrocnemius of septic rats as compared with muscle perfused in the absence of insulin (n = 7; Fig 4). Increasing the perfusate insulin concentration to 10,000 μU/mL (n = 4) resulted in approximately 85% stimulation of protein synthesis as compared with rates in septic animals perfused in the absence of insulin ($P < .05$). However, the rate of protein synthesis remained significantly decreased as compared with control rates at each concentration of insulin examined (Fig 4). The concentration of insulin producing a 50% stimulation of protein synthesis was calculated to be 741 μmol/L in gastrocnemius of septic rats. Therefore, a relative resistance to the stimulatory effect of insulin on protein synthesis was observed in gastrocnemius of septic rats.

Garlick and Grant[20] and Preedy and Garlick[37] demonstrated that effects of amino acids are additive to or permissive with those of insulin to stimulate muscle protein synthesis. In the present study, the effect of simultaneously increasing amino acid and insulin concentrations on protein synthesis in muscles from control and septic rats was investigated. These data are presented in Fig 5. The rate of

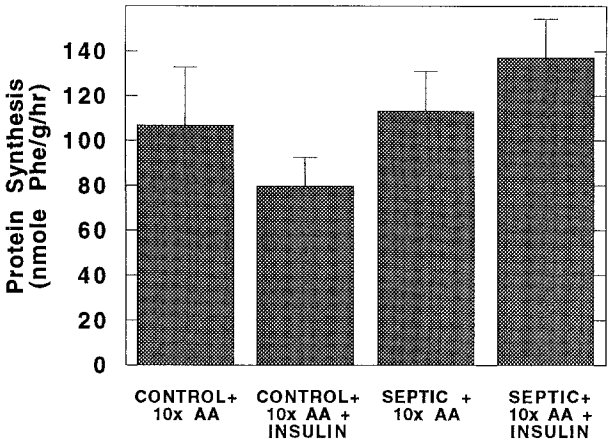

Fig 5. Effects of amino acids plus insulin on rates of protein synthesis in gastrocnemius from control and septic rats. Rates were measured during in vitro perfusion as described in Fig 2. Amino acids were added to perfusates at 10 times plasma concentrations (10× AA), with the exception of phenylalanine, which was present at 1 mmol/L. In some perfusions, insulin (1,000 μU/mL) was added to the perfusate. Each value represents the mean ± SE of 7 to 8 determinations, each involving a separate perfusion experiment. ANOVA $F_{3,29}$ = 1.5, P = .22.

protein synthesis was not significantly different in gastrocnemius perfused with buffer containing elevated concentrations of insulin plus amino acids (control, n = 7; septic, n = 8) as compared with buffer supplemented solely with amino acids (control, n = 7; septic, n = 8). Thus, no additional stimulation of protein synthesis was found if the perfusate contained both insulin (1,000 μU/mL) and amino acids at 10× concentrations as compared with amino acids alone. Thus, effects of insulin and amino acids to stimulate protein synthesis in control animals were not additive under conditions of elevated amino acid concentrations.

DISCUSSION

The experiments define conditions required for demonstrating a stimulatory effect of amino acids on protein synthesis in perfused skeletal muscle from septic rats. In fed septic rats, amino acid supplementation resulted in a greater than fourfold stimulation of muscle protein synthesis. Protein synthesis in skeletal muscle from septic rats responded to the increased availability of amino acids in much the same manner as perfused skeletal muscle from control rats. A complete mixture of amino acids at 10× normal plasma concentrations caused a greater than twofold stimulation of protein synthesis in gastrocnemius from controls. The magnitude of stimulation of protein synthesis evoked by perfusion with buffers supplemented with amino acids was such that the rate of protein synthesis in muscles of septic rats was not significantly different from that observed in control rats. These results are consistent with observations that a short period of infusion of a mixture of amino acids plus glucose in postabsorptive rats is sufficient to restore protein synthesis in gastrocnemius to a rate close to fed values,[37] and that addition of amino acids to the perfusate stimulates protein synthesis in skeletal muscle of fasted rats to fed values.[16]

There is general agreement regarding the biochemical locus in the protein synthetic pathway influenced by amino acid availability. Studies involving perfused liver[38] or hepatocytes in culture[39] have provided evidence that limiting amino acid availability results in the development of a block in peptide-chain initiation. Inhibition of peptide-chain initiation can be prevented by addition of a mixture of amino acids to the perfusate.[38] In perfused heart, the stimulatory effect of amino acids on protein synthesis is also mediated by stimulation of peptide-chain initiation.[15,35] The increased rate of protein synthesis in perfused skeletal muscle by amino acids, BCAA, or leucine alone occurs through a facilitated rate of peptide-chain initiation.[16] The results of the present study are also consistent with a restoration of translational efficiency and hence peptide-chain initiation to control values by perfusion of skeletal muscle from septic rats with amino acids. In the present study, inclusion of the complete mixture of amino acids at elevated plasma concentrations acutely reversed sepsis-induced alterations in translational efficiency in gastrocnemius of septic rats to values observed in control rats.

A mixture of leucine, isoleucine, and valine stimulated protein synthesis in gastrocnemius from control rats by 40%. The response was not as great as that produced by increasing the concentration of all amino acids. The magnitude of stimulation of skeletal muscle protein synthesis by BCAA in muscle of control rats in the present study is consistent with previous findings in skeletal muscle,[16] heart,[35] and diaphragm.[33,34] A mixture of leucine, isoleucine, and valine at 10× plasma concentration did not stimulate protein synthesis in muscle from septic rats. Therefore, unlike control rats, BCAA could not by themselves substitute for the complete mixture of amino acids in stimulating protein synthesis in muscles from septic rats. Thus, stimulatory effects of amino acids on protein synthesis in gastrocnemius of septic rats were not mediated in part by BCAA alone. These findings suggest that sepsis induces changes in the pathway for protein synthesis that make skeletal muscle resistant to stimulatory effects of BCAA.

Hasselgren et al[40] showed that protein synthesis in soleus from septic rats was stimulated 30% to 40% by leucine, an effect comparable to that observed in gastrocnemius of control rats. However, stimulation of muscle protein synthesis by BCAA in septic rats occurred at a concentration of leucine twofold higher than that needed to effect similar increases in muscle protein synthesis in control animals. These results suggested that there was a decreased sensitivity but not a maximal responsiveness to BCAA-induced stimulation of protein synthesis in soleus from septic rats. There are several potential reasons for the apparent discrepancy between results of the present study and those of Hasselgren et al.[40] It is not the result of differences in leucine concentrations. In the present study, perfusate leucine concentration was 1.6 mmol/L, which is greater than the leucine concentration used in studies reported by Hasselgren et al.[40] One difference between the two studies is the presence of other amino acids at normal plasma concentration in the present experiments, whereas in the other study,[40] only leucine and phenylalanine were added to

the incubation medium. A second difference between the two studies relates to the time after induction of sepsis when rates of protein synthesis were assessed. In studies reported by Hasselgren et al,[40] the response of protein synthesis to leucine was measured 16 hours after ligation and puncture of the cecum to initiate sepsis. In the present study, the effect of BCAA on protein synthesis was investigated 5 days after induction of sepsis. Thus, the difference in time (4 days) may allow for the full compensatory effects to the septic insult to induce a complete resistance to the stimulatory effects of BCAA on skeletal muscle protein synthesis, rather than a decreased sensitivity. These findings are important because they may help explain why BCAA administration in septic patients has shown only marginal effects at best.

Insulin is an important regulator of protein metabolism at both the transcriptional and translational level (for review, see Kimball et al[41]). In general, insulin-induced transcriptional regulation results in synthesis of specific proteins, whereas stimulation of translation by insulin usually causes an increase in synthesis of mixed total proteins. Insulin enhances the global rate of protein synthesis in skeletal muscle through an acceleration of the translational phase of protein synthesis.[18,19,41,42] Therefore, one goal of the present studies was to establish whether the sepsis-induced block in protein synthesis could be reversed by increasing the insulin concentration. To answer this question, we used the protocol originally described by Jefferson et al[18,19] to examine the effect of insulin on protein synthesis in muscles of the perfused hemicorpus. In this perfusion system, rates of protein synthesis in muscle decline during the second and third hours of perfusion in the absence of insulin.[18,19] Hence, a relative insulin deficiency can be induced by in vitro perfusion after 1 hour if no insulin is present in the perfusate. Reduced rates of protein synthesis in perfused hindlimb after perfusion with medium lacking insulin result from a restraint on peptide-chain initiation. Inclusion of sufficient insulin in the perfusate prevents this decrease in protein synthesis by maintaining peptide-chain initiation. In the present study, the stimulatory effect of insulin on protein synthesis in skeletal muscle from fed control animals was further established.

Insulin administration has previously been demonstrated to reduce net protein catabolism in injured[43] or septic[44] patients, and in nonseptic traumatized animals.[45,46] We have previously shown that insulin attenuated release of amino acids from the periphery in septic animals.[47] However, these types of studies do not provide evidence as to whether insulin modified protein synthesis or degradation in sepsis. The results of the present study delineate the response of protein synthesis in gastrocnemius from septic rats to increasing insulin concentrations. Only when insulin was increased to pharmacologic concentrations (10,000 μU/mL) was the rate of protein synthesis enhanced in muscle from septic rats. Furthermore, the maximal rate of protein synthesis in gastrocnemius achieved by high concentrations of insulin was significantly less in septic rats versus controls. The response of protein synthesis to increasing insulin concentrations in gastrocnemius of septic rats was characterized by both a decreased sensitivity and decreased maximal responsiveness as compared with control levels. Therefore, a relative resistance to the stimulatory effect of insulin on muscle protein synthesis during sepsis but not during sterile inflammation was observed. The failure to demonstrate an effect of insulin on protein metabolism is not related to the perfusion system, because inhibition of peptide-chain initiation in gastrocnemius muscle of starved or diabetic rats is corrected during in vitro perfusion with buffer containing insulin.[42] These results are consistent with a postreceptor insulin resistance[48] of protein synthesis in muscles from septic rats. The mechanism(s) responsible for the observed sepsis-induced resistance of protein synthesis to stimulation by insulin remains to be elucidated.

Skeletal muscle is heterogeneous with respect to fiber composition, containing variable proportions of slow- and fast-twitch oxidative fibers and fast-twitch glycolytic fibers. We have previously demonstrated that sepsis preferentially inhibits protein synthesis in muscles composed of mixed fast-twitch fibers.[5,26] In muscles composed of slow-twitch fibers, sepsis is without affect on rates of protein synthesis.[5,26,49] The effect of insulin to stimulate protein synthesis in skeletal muscle during sepsis may also depend on fiber type of the muscle investigated. Hasselgren et al[49] showed that insulin caused a similar stimulation of protein synthesis in soleus from septic rats as compared with controls. No differences in either sensitivity or maximal responsiveness to insulin were observed in soleus from septic rats versus controls. However, results of the present study provide evidence that protein synthesis in gastrocnemius from septic rats does not respond to insulin in a manner similar to that of controls. At each insulin concentration tested, protein synthesis in gastrocnemius of septic rats was reduced relative to control levels. One potential explanation for the difference in results between our study and that reported by Hasselgren et al[49] is that only muscles composed primarily of fast-twitch fibers develop a resistance to the effects of insulin on protein synthesis in sepsis. This suggestion is supported by observations in acutely starved animals, in which the stimulatory effects of insulin on protein synthesis are seen in muscles composed of fast-twitch fibers, but not in muscles composed of slow-twitch fibers.[50,51] Therefore, the apparent discrepancy for the effect of insulin on protein synthesis in sepsis between the two studies may reflect differences in the composition of muscles examined (fast-twitch *v* slow-twitch). This finding of an insulin resistance in gastrocnemius of septic rats is particularly important, since insulin has been proposed as a treatment modality for postinjury/postsurgical catabolism.[43]

In summary, we showed that sepsis leads to inhibition of protein synthesis in gastrocnemius of young rats by a reduction in translational efficiency. The magnitude of reduction in protein synthesis and translational efficiency in younger septic rats was similar to that of our previous reports using older rats.[5,25,26] Inclusion of amino acids in the perfusate stimulated protein synthesis in muscle from septic rats. Amino acids stimulated protein synthesis to the same degree (~100 nmol Phe/g/h) in both control and septic rats. Furthermore, stimulation of protein synthesis in

septic rats perfused with high concentrations of amino acids resulted from an enhanced translational efficiency. However, in contrast to controls, the response to BCAA or insulin was impaired or absent in gastrocnemius of septic rats. Although the biochemical mechanisms involved in stimulation of protein synthesis by BCAA or insulin remain unknown, it is tempting to speculate that sepsis interferes with a common final pathway by which both these compounds enhance translational efficiency and hence protein synthesis in muscle.

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