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THOMAS C. VARY, DOMINIQUE DARDEVET, JEAN GRIZARD, LAURE VOISIN, CAROLINE BUFFIERE, PHILLIPE DENIS, DENIS BREUILLE, AND CHRISTIANE OBLED
Laboratoire d'Etude du Metabolisme Azote, Institut National de la Recherche Agronomique, Theix 63122 Ceyrat; Clintec Technologies, 78148 Velizy Cedex, France; and Department of Cellular and Molecular Physiology, Penn State University College of Medicine, Hershey, Pennsylvania 10733

Vary, Thomas C., Dominique Dardevet, Jean Grizard, Laure Voisin, Caroline Buffiere, Phillipe Denis, Denis Breuille, and Christiane Obled. Differential regulation of skeletal muscle protein turnover by insulin and IGF-I after bacteremia. *Am. J. Physiol.* 275 (*Endocrinol. Metab.* 38): E584–E593, 1998.—Skeletal muscle catabolism is a characteristic metabolic response to sepsis. We investigated the ability of physiological insulin (2 nM) or insulin-like growth factor I (IGF-I, 10 nM) concentrations to modify protein metabolism during incubation of epitrochlearis 2, 6, or 15 days after injection of live *Escherichia coli*. On *days 2* and *6* postinfection, skeletal muscle exhibited an exacerbated negative protein balance resulting from both an inhibition in protein synthesis (25%) and an enhanced proteolysis (90%) compared with controls. By *day 15* postinfection, protein balance in infected rats was significantly improved compared with either *day 2* or *6*. At this time, protein synthesis was augmented and protein degradation was decreased in infected rats relative to *day 6*. Insulin or IGF-I stimulated protein synthesis in muscles from septic and control rats in vitro to the same extent at each time point examined. The ability of insulin or IGF-I to limit protein degradation was severely blunted 48 h after infection. On *day 6* postinfection, the effect of insulin or IGF-I to inhibit proteolysis was more pronounced than on *day 2*. Incubation with IGF-I limited proteolysis to a greater extent than insulin on both days in infected but not control rats. By *day 15*, insulin diminished proteolysis to the same extent as in controls. The results suggest that injection of bacteria causes fundamental derangements in protein metabolism that persist for days after infection.

epitrochlearis; protein synthesis; proteolysis; insulin-like growth factor I; sepsis

SEVERE INFECTION initiates a cascade of events that leads to a loss of body proteins, reflected by massive excretion of urea and large negative nitrogen balances. Nitrogen losses equivalent to 5–17% of the total body protein are observed in septic patients despite adequate nutritional support. Much of this loss of body protein originates from skeletal muscle. Catabolism of skeletal muscle proteins results in muscle wasting and fatigue, which are well-recognized features of the host's response to sepsis. The loss of protein in skeletal muscle is a consequence of an imbalance between protein synthesis and protein degradation. Ultimately, the negative protein balance in skeletal muscle results from the complex interactions among hormones, amino acid fluxes, and mediators of the septic response that shift the dynamic balance between protein synthesis and proteolysis. The ability of physiological concentrations of anabolic hormones to modulate protein balances in skeletal muscle during chronic sepsis remains unresolved.

Although parenteral nutrition has become an important therapy in septic patients, it is insufficient alone to prevent the loss of skeletal muscle protein. Thus additional regimens, such as use of anabolic agents, have been proposed to attenuate the loss of muscle protein during sepsis (34). The major anabolic hormones promoting accretion of skeletal muscle proteins include insulin, growth hormone (GH), and insulin-like growth factor I (IGF-I). Insulin enhances the global rate of protein synthesis and decreases the rate of protein degradation in skeletal muscle under a variety of conditions. Administration of insulin to injured (35) or septic patients (14) reduces whole body net protein catabolism. However, the ability of insulin to enhance protein synthesis is variable in skeletal muscle during sepsis. During the immediate postinfection period (up to 48 h), insulin stimulates protein synthesis (11, 12, 29). However, during chronic (5 day) intra-abdominal sepsis, insulin was without effect in augmenting skeletal muscle protein synthesis (16). In contrast to protein synthesis, protein degradation in skeletal muscle consistently shows a relative resistance to insulin during the immediate posttrauma period (29) or during acute (16 h) peritonitis (11, 12).

Like insulin, GH promotes nitrogen retention and improves nitrogen balance in a variety of catabolic conditions, including burn and postoperative surgical patients (for review, see Ref. 34). However, septic patients show a relative GH resistance (5). The anabolic actions of GH on protein metabolism in skeletal muscle are believed to be mediated indirectly through GH-stimulated secretion of another hormone, IGF-I. In sepsis, there is an uncoupling of the normal GH/IGF-I axis, so that although GH concentrations are elevated, IGF-I concentrations are generally depressed (3, 5, 19). The suppression of IGF-I concentrations is thought to limit the anabolic actions of GH in sepsis.

Consequently, IGF-I may be of more importance than GH in improving nitrogen balance in skeletal muscle during sepsis. In this regard, systemically administered IGF-I stimulates weight gain and protein synthesis in normal rats (13), increases lean body mass during starvation (20) or diabetes (28), and attenuates protein loss during glucocorticoid-induced cachectic states (27).

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Chronic IGF-I administration has been reported to improve nitrogen balance in rats injected with endotoxin (8). The mechanisms responsible for the improvement in nitrogen balance after IGF-I in endotoxin-treated rats have not been elucidated. Jurasinski and Vary (18) reported that IGF-I stimulates protein synthesis in skeletal muscle from chronic septic rats during in vitro perfusion, suggesting that some of the protein-sparing effect of IGF-I may be mediated through enhanced rates of protein synthesis. Likewise, IGF-I is thought to enhance protein accretion in muscle by enhancing protein synthesis in normal humans (10, 22). In contrast to protein synthesis, there are no reports concerning the effect of IGF-I on protein degradation in skeletal muscle during sepsis. However, IGF-I attenuates the stimulation of proteolysis induced by thermal injury (9).

The purpose of the present study was to compare and contrast the ability of insulin or IGF-I to modulate protein synthesis and degradation in the epitrochlearis after injection of live *Escherichia coli*. By use of the incubated muscle preparation, effects of insulin or IGF-I on protein synthesis and degradation can be examined independently of alterations in plasma concentrations of substrates or other hormones induced by in vivo injection of either hormone (24, 25). We established the effects of these two anabolic hormones on skeletal muscle protein metabolism during the anorexic (2 days postinfection), hypermetabolic (6 days postinfection), and recovery (15 days postinfection) phases of the response to a severe infection.

MATERIALS AND METHODS

Experimental design. Male Sprague-Dawley rats (250–300 g) were individually housed in wire-bottom cages in a temperature-controlled environment (22–23°C) with a 12:12-h light-dark cycle. During a 6-day period of acclimatization before injection of bacteria, all rats had free access to water and food ad libitum. The diet consisted of a semisynthetic laboratory diet containing 12% protein and previously shown to sustain normal growth in rats (1).

Two groups of animals, control (*group 1*) and infected (*group 2*), were used to investigate the effect of sepsis on protein synthesis and protein degradation. The infected group was produced by an injection of live *E. coli* ($0.6–0.9 \times 10^9$ colony forming units/rat) into the tail vein as previously described (1, 2, 33). Control rats received an equal volume of saline. After injection of bacteria, a hypermetabolic, sepsis-like condition develops as evidenced by leukocytosis, an acute phase response, hyperlactatemia, low-grade fever, weight loss, and muscle atrophy (1, 2, 31, 33). Bacteremia is maintained for at least 48 h after injection of *E. coli* (33). The mortality rate was 20% for animals injected with bacteria. No animals injected with saline expired. Because injection of live *E. coli* causes anorexia in this model, control rats were pair fed to the septic rats by estimating food intake based on previous studies and correcting the daily intake with the actual food consumption of the septic group (1, 33). Animals were offered food twice daily at 9:00 AM and 5:00 PM. All rats were fasted overnight before sampling of muscles for in vitro incubations. Animals were weighed on a daily basis. The experiments were carried out with the approval of animal care and use committees at both institutions.

Incubations of epitrochlearis muscles. Epitrochlearis muscles were incubated in vitro as described previously (6, 7, 24, 25, 29, 31, 33). The epitrochlearis was chosen for examination of protein turnover after injection of bacteria because previous reports have provided evidence that sepsis preferentially affects protein metabolism in muscles composed of mixed fast-twitch fibers (4, 26, 32). On the day of the experiment, rats were anesthetized with pentobarbital sodium (50 mg/kg body wt). The skin on each of the forearms was removed. The epitrochlearis muscles were excised intact and immediately placed in Krebs-Henseleit bicarbonate buffer. The muscles were quickly rinsed and transferred to plastic tubes containing 2 ml of buffer. The tubes were capped and immediately oxygenated. One muscle from each rat was incubated under basal conditions (no hormones) while the contralateral muscle was incubated in the presence of insulin or IGF-I. Because incubations were performed on different days, muscles from control and infected rats incubated under basal conditions were always performed to allow for comparisons from one experiment to the next.

Epitrochlearis muscles were first preincubated for 30 min. After the preincubation period, muscles were transferred to fresh medium (2 ml) and incubated for a further 180 min, with a change of buffer every 60 min. During the final 60 min of the incubation period, the buffer was supplemented with 0.5 mM L-[^{14}C]phenylalanine (0.15 μCi/ml). At the end of the incubation, muscles were removed from the incubation buffer, trimmed of connective tissue, immersed into 2 ml of ice-cold 10% (wt/vol) TCA, and weighed. The incubation medium was frozen and stored at −20°C for analysis of tyrosine and the specific radioactivity of phenylalanine.

The Krebs-Henseleit bicarbonate buffer consisted of 120 mM NaCl, 4.8 mM KCl, 25 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, and 1.2 mM $MgSO_4$ (pH 7.4) supplemented with 5 mM glucose, 5 mM HEPES, 0.1% (wt/vol) BSA, 0.17 mM leucine, 0.20 mM valine, and 0.10 mM isoleucine. Muscles were incubated at 37°C under an atmosphere of 95%O_2-5%CO_2. In some experiments, insulin (Novo Pharmaceuticals, Denmark) or IGF-I (Preprotech, France) was added at the concentrations described in the figures and tables. Insulin or IGF-I was diluted fresh the day of the experiment in Krebs-Henseleit buffer.

Protein synthesis. Rates of protein synthesis were estimated by the incorporation of radioactive phenylalanine into muscle protein. Muscles were homogenized in 2 ml of 10% TCA using a Polytron PT10 set at 60% of maximal speed. The homogenate was centrifuged at 10,000 *g* for 10 min at 4°C. The supernatant was decanted, and the pellet was washed three additional times with 10% TCA to remove any acid-soluble radioactivity. The resulting pellet was dissolved in 1 N NaOH and incubated at 37°C for a minimum of 30 min. Aliquots were assayed for protein using the bicinchoninic acid procedure (Pierce Chemicals, Rockford, IL) per the manufacturer's procedure with crystalline BSA as a protein standard. Another aliquot was assayed for radioactivity by liquid scintillation spectrophotometry using corrections for quenching (disintegrations/min). Rates of protein synthesis, expressed as nanomoles of phenylalanine incorporated per hour per milligram of protein, were calculated by dividing the amount of radioactivity incorporated into muscle protein over a 1-h period by the specific radioactivity of the phenylalanine in the incubation medium.

Protein degradation. Rates of protein degradation were measured by the accumulation of tyrosine in the incubation medium as described previously (6, 7, 29, 33). Because tyrosine is neither synthesized nor metabolized by muscle, except for use by protein synthesis, the release of tyrosine

from muscle into the incubation medium reflects net protein balance. Total protein degradation was estimated simultaneously with the rate of protein synthesis as the sum of the accumulation of tyrosine in the incubation buffer over a 1-h period plus the amount of tyrosine equivalents incorporated into protein via protein synthesis during the same time interval. To obtain the amount of tyrosine incorporated into mixed muscle proteins, we multiplied the values for incorporation of radioactive phenylalanine into protein by the molar ratio of tyrosine to phenylalanine in mixed proteins from skeletal muscle (0.77) (6, 7, 29). Thus values for tyrosine equivalents incorporated into mixed muscle protein could be estimated for each individual muscle. Tyrosine in the incubation medium was measured fluorometrically as described previously (6, 7).

Statistics. The experimental data for each condition are summarized as means ± SE. The statistical evaluation of the data was performed using ANOVA to test for overall differences among groups, followed by the Sidak test for multiple comparisons to determine significance between means only when the ANOVA indicated a significant difference among the group means (31). Differences among means were considered significant when $P < 0.05$.

RESULTS

Incubation conditions for assessing effects of insulin or IGF-I on rates of protein synthesis and degradation. Previous studies examining control of protein metabolism by hormones in epitrochlearis muscle incubated in vitro have utilized either young, rapidly growing rats (24, 25) or old rats that display retarded growth rates and a resistance to the effects of insulin or IGF-I on protein metabolism (6). It was therefore necessary to establish the responsiveness of incubated epitrochlearis muscle to insulin or IGF-I from 250- to 300-g rats used in the present study. Epitrochlearis muscles from 300-g rats were incubated for various periods of time with buffer supplemented with 30 nM insulin to establish a time course for studying the effects of insulin on protein turnover (Table 1). Protein synthesis was increased, although not significantly during the 1st h of incubation with buffer containing insulin. Thereafter, the rate of protein synthesis remained significantly elevated compared with values obtained in muscles incubated in the absence of the hormone. In contrast to protein synthesis, only during the 3rd h of incubation was a significant difference in protein degradation between muscles incubated with and without insulin observed. The effect of insulin was to prevent the enhanced rates of proteolysis observed during the 3rd h of incubation in the absence of the hormone. Because 3 h of incubation were required for an effect of insulin on protein degradation to be observed, all subsequent studies were performed during the 3rd h of incubation. Over the same time interval, rates of protein synthesis remained constant whether in the presence or absence of insulin. These observations are consistent with previous reports using epitrochlearis muscles weighing less than the ones used in the present study (24, 25).

Table 1. *Effect of time of incubation on rates of protein synthesis and protein degradation in rat epitrochlearis muscle incubated in vitro in presence or absence of insulin*

Incubation Interval, min	Protein Synthesis, nmol Phe incorp·h^{-1}·mg protein^{-1}		Proteolysis, nmol Tyr released·h^{-1}·mg protein^{-1}	
	−Insulin	+Insulin	−Insulin	+Insulin
30–90	0.318 ± 0.019	0.420 ± 0.039	1.33 ± 0.06	1.33 ± 0.19
90–150	0.266 ± 0.025	0.429 ± 0.017*	1.39 ± 0.04	1.25 ± 0.03
150–210	0.262 ± 0.019	0.469 ± 0.023†	1.64 ± 0.09	1.29 ± 0.08‡

Values are means ± SE for 8 muscles in each group at each time interval. Muscles were incubated for intervals up to 3.5 h. [^{14}C]phenylalanine was included during final hour of incubation. Insulin was included in buffer at a concentration of 30 nM. Rates of protein synthesis and protein degradation were calculated as described in METHODS. *$P < 0.001$; †$P < 0.005$; ‡$P < 0.05$ vs. −insulin.

The sensitivity of protein synthesis and protein degradation to various concentrations of insulin or IGF-I was determined to establish the minimal concentration of insulin or IGF-I necessary to elicit both a maximal stimulation of protein synthesis and a maximal inhibition of protein degradation. Protein synthesis was significantly increased at the lowest insulin concentration (0.5 nM) tested and was maximally stimulated at an insulin concentration of 2 nM (Fig. 1, *top*). The rate of protein degradation was maximally inhibited at 2 nM as well. Therefore the maximal responsiveness of both protein synthesis and protein degradation was achieved at 2 nM insulin. All subsequent studies involving insulin were performed with this concentration.

A similar approach was used to establish the minimal concentration of IGF-I necessary to elicit its maximal effect on protein synthesis and protein degradation (Fig. 1, *bottom*). Protein synthesis was stimulated with a concentration of 3 nM IGF-I, with maximal stimulation occurring at 10 nM. Likewise, protein degradation was maximally decreased by 10 nM IGF-I. The concentration of IGF-I (10 nM) necessary to elicit the maximal responsiveness for protein synthesis was similar to that observed for rapidly growing young rats (6, 18). All subsequent studies involving IGF-I were performed using 10 nM IGF-I.

Effect of infection on protein catabolism measured in incubated epitrochlearis. Injection of *E. coli* caused a profound anorexia that lasted ~2 days (Fig. 2). Thereafter, food consumption increased in infected rats and returned to values observed before injection of bacteria. On *day 14* postinfection, food was removed at ~5:00 PM. Therefore, food consumption on that day reflected what the animals consumed during the day. Also shown is the food consumption in control animals pair fed to match the food intake of the infected rats.

Growth rates were similar in both groups before infection (data not shown). Figure 3 shows the growth rates of control and infected rats over the course of the experimental period. Control rats lost weight for 72 h after beginning the pair-feeding regimen. Then, as food consumption returned toward normal, control rats began to gain weight and continued to grow throughout the remainder of the experimental period. Infected rats lost weight during the first 72 h after injection of the bacteria. In contrast to pair-fed control rats, infected rats did not gain weight from 3 to 7 days postinfection,

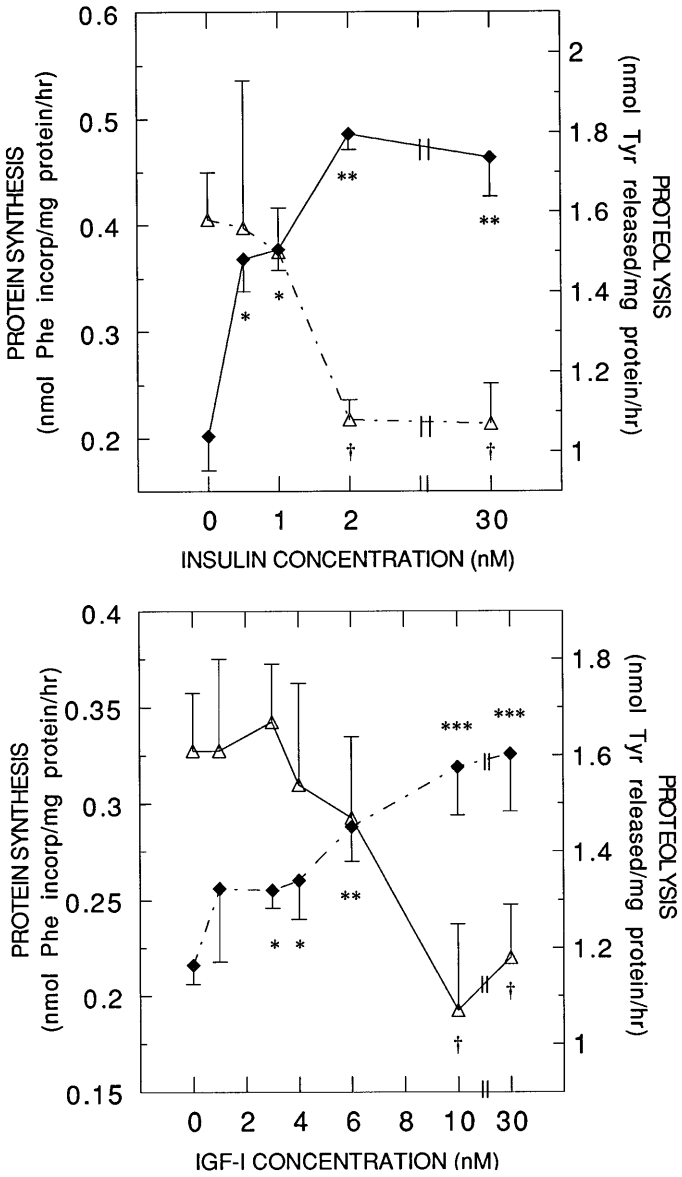

Fig. 1. Effect of insulin (*top*) or insulin-like growth factor I (IGF-I; *bottom*) on rates of protein synthesis and protein degradation in rat epitrochlearis muscle incubated in vitro. Epitrochlearis muscles from control rats were excised and incubated in vitro as described in MATERIALS AND METHODS. Rates of protein synthesis were measured by incorporation of [^{14}C]phenylalanine (Phe) into muscle proteins. Protein degradation was estimated simultaneously with rates of protein synthesis as sum of net change in amount of tyrosine (Tyr) in incubation buffer over 1 h + amount of tyrosine equivalents incorporated by protein synthesis during same time interval. The latter was calculated from measured incorporation of radioactive phenylalanine into protein, by using a conversion factor of 0.77 nmol of tyrosine incorporated into muscle proteins per nanomole of phenylalanine. Values shown are means ± SE. Insulin, n = 3–16 muscles at each concentration; IGF-I, n = 4–5 at each concentration. Protein synthesis: *$P < 0.05$; **$P < 0.005$; ***$P < 0.001$ vs. 0 insulin or IGF-I. Protein degradation: †$P < 0.05$ vs. 0 insulin or IGF-I.

even though food consumption increased. Then, beginning approximately on *day 8*, the body weight of the infected rats began to rise and continued to do so for the remainder of the experimental period. However, the weight of the infected rats never reached that of the pair-fed control rats. Thus, despite equal food intake,

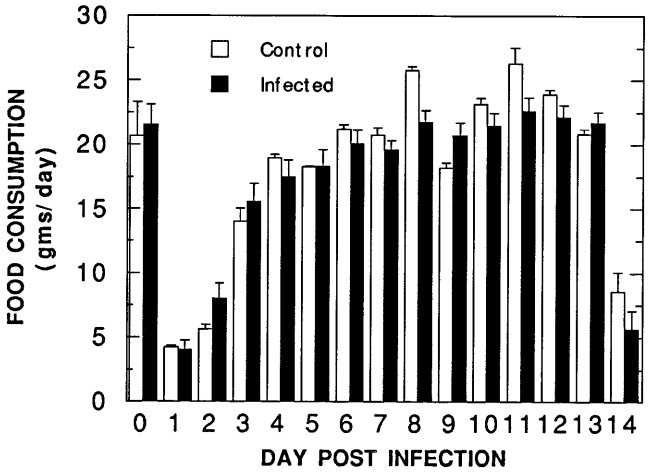

Fig. 2. Food consumption after injection of *E. coli*. Amount of food consumed by control and septic rats was measured twice (9:00 AM and 5:00 PM) daily. Amount of food consumed was taken as difference between weight of food presented and amount remaining. Septic group was offered food ad libitum throughout experimental period. Amount of laboratory food presented to pair-fed control group was adjusted accordingly, so that food intake was limited to equalize consumption relative to sepsis group. On last day (*day 14*), food was withdrawn at 3:00 PM. Therefore food consumption for that day represents only the amount of food eaten during day. Values shown are means ± SE for 9–25 animals in each group at each time.

the body weight of the infected rats remained depressed relative to control rats over the entire course of the experimental period.

The weight of the epitrochlearis muscle in pair-fed control and infected rats is shown in Fig. 4 (*top*). The weight of the epitrochlearis muscle was significantly decreased 24, 31, and 20% in the infected rats compared with muscles from control rats on *days 2*, *6*, and *15* postinfection, respectively. Over the period from *days 6* to *15* postinfection, the weight of the epitrochlearis increased significantly by 50% ($P < 0.01$) in infected rats, corresponding with the increase in the body weight over the same time period. Similar find-

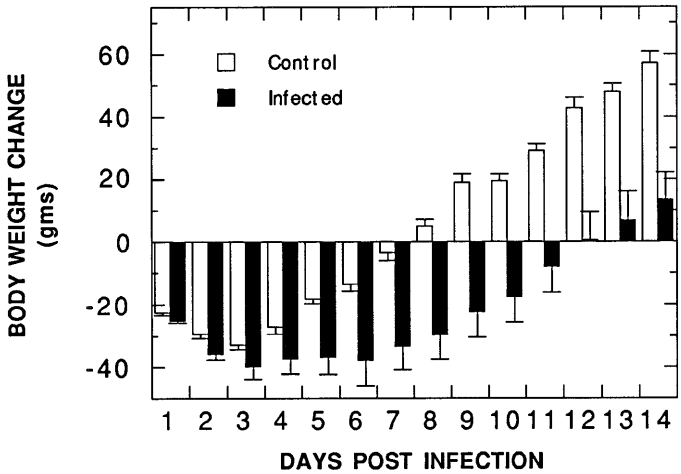

Fig. 3. Cumulative weight changes in infected and pair-fed control rats. Infected and pair-fed control rats described in Fig. 2 were weighed daily before morning meal. Differences in weight from *day 0* are plotted. Values shown are means ± SE for 9–17 animals in each group at each time.

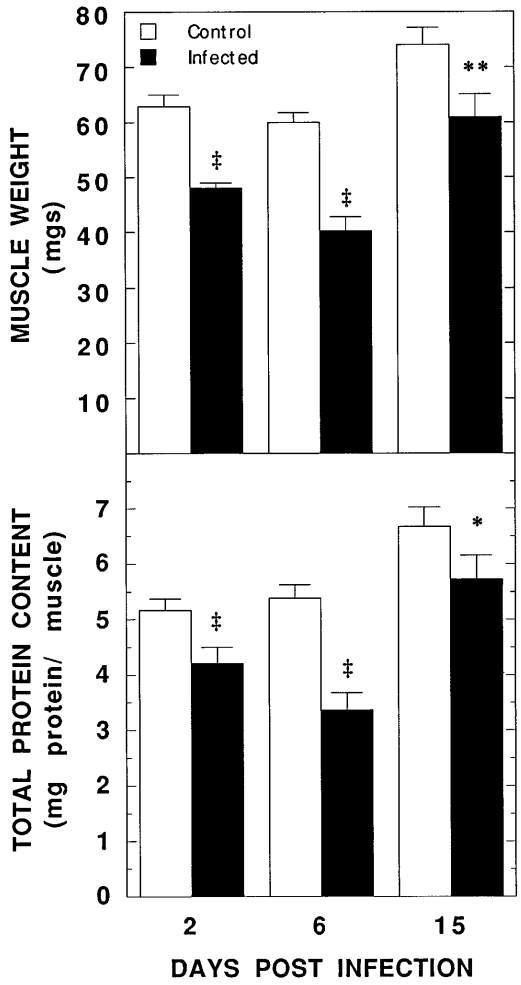

Fig. 4. Weight (*top*) and total protein content (*bottom*) of epitrochlearis muscle in infected and pair-fed control rats. Values shown are means ± SE for 7–25 animals in each group at each time. *$P < 0.05$; **$P < 0.01$; ‡$P < 0.001$ vs. control at same day postinfection.

ings were observed in the total protein content of the epitrochlearis (Fig. 4, *bottom*). The total protein content/muscle was reduced 24, 37, and 14% in the infected rats compared with muscles from control rats on *days 2*, *6*, and *15* postinfection, respectively. Over the period from *days 6* to *15* postinfection, the total protein content/muscle was significantly increased from 3.4 ± 0.3 to 5.7 ± 0.4 mg/muscle ($P < 0.001$) in infected rats. During the same time period, total protein content/muscle in control rats increased from 5.4 ± 0.3 to 6.7 ± 4 mg/muscle ($P < 0.005$).

The negative protein balance (basal condition; Table 2), as measured by the net release of tyrosine from incubated muscles, was significantly increased 2.4-fold 2 days after infection (2.73 ± 0.28 nmol Tyr·mg protein^{-1}·h^{-1}) compared with control (1.13 ± 0.07 nmol Tyr·mg protein^{-1}·h^{-1}). Protein balance improved by 25% on *day 6* postinfection (2.04 ± 0.34 nmol Tyr·mg protein^{-1}·h^{-1}) but remained twofold more negative in muscles from infected rats compared with controls (1.05 ± 0.06 nmol Tyr·mg protein^{-1}·h^{-1}). By *day 15* postinfection, protein balance continued to improve in muscles from infected rats, returning to values observed in control animals (1.27 ± 0.09 vs. 1.5 ± 0.14 nmol Tyr·mg protein^{-1}·h^{-1}).

To examine the possible mechanisms responsible for the enhanced negative protein balance of epitrochlearis after injection of bacteria, rates of protein synthesis and protein degradation were measured 2, 6, and 15 days postinfection (Figs. 5–7). The rate of muscle protein synthesis in infected rats 2 days postinfection was significantly decreased ($P < 0.05$) by ~30% compared with their pair-fed controls (Fig. 5, basal). Six days after injection of bacteria, the rate of protein synthesis remained inhibited by 22% ($P < 0.005$) compared with control rats (Fig. 6, basal). In contrast to *day 2* or *6* postinfection, the rate of protein synthesis was significantly increased on *day 15* postinfection ($P < 0.05$) compared with pair-fed control rats (Fig. 7, basal).

The rate of protein degradation was significantly increased approximately twofold ($P < 0.001$) 2 days after infection compared with pair-fed control rats (Fig. 5, basal). The rate of degradation was not significantly decreased in infected rats on *day 6* postinfection and remained elevated (70%; $P < 0.001$) above values observed in muscles from control rats (Fig. 6, basal). By 15 days postinfection, the rate of protein degradation decreased relative to that observed at *day 6* in infected rats. On *day 15* postinfection, rates of protein degradation continued to diminish (Fig. 7, basal). There were no significant differences between control and infected rats at this time point.

Effect of insulin or IGF-I on protein turnover during infection. Table 2 shows the effects of addition of insulin (2 nM) or IGF-I (10 nM) to the incubation medium on net protein balance in muscles from infected or pair-fed

Table 2. *Effect of injection of bacteria on protein balance in epitrochlearis muscle during in vitro incubation: modulation by insulin or IGF-I*

Day Postinfection	Control			Infection		
	Basal	+Insulin	+IGF-I	Basal	+Insulin	+IGF-I
2	1.13 ± 0.07 (12)	0.77 ± 0.06[a] (12)	0.71 ± 0.04[b] (6)	2.73 ± 0.28[a] (4)	2.12 ± 0.25[c,d] (4)	1.31 ± 0.09[e] (7)
6	1.05 ± 0.06 (14)	0.50 ± 0.05[a] (9)	0.46 ± 0.02[b] (6)	2.04 ± 0.34[a] (9)	1.16 ± 0.16[c,f] (9)	0.65 ± 0.06[e,g] (6)
15	1.5 ± 0.14 (10)	1.03 ± 0.10[b] (10)	ND	1.27 ± 0.09[h] (9)	0.99 ± 0.06[f] (8)	ND

Values are means ± SE for no. in each group at each time given in parentheses. Protein balance is measured by net nanomoles of tyrosine released per milligram of protein per hour. ND, not determined. Muscles were excised and incubated as described in METHODS. Basal protein balance results were obtained in absence of hormones in incubation medium. Insulin or insulin-like growth factor I (IGF-I) was added to give a final concentration of 2 or 10 nM in the medium, respectively. Basal: (ANOVA $F = 10.85$, $P < 0.001$); +Insulin: (ANOVA $F = 19.76$, $P < 0.001$); +IGF-I: (ANOVA $F = 16.447$, $P < 0.001$). [a]$P < 0.01$; [b]$P < 0.05$ vs. control, basal at same time point. [c]$P < 0.01$ vs. control, +insulin at same time point. [d]$P < 0.001$ vs. *day 6* or *15* infection, +insulin. [e]$P < 0.05$ vs. infection, basal or infection, +insulin at same time point; [f]$P < 0.05$ vs. infection, basal at same time; [g]$P < 0.05$ vs. *day 2* infection, +IGF-I. [h]$P < 0.05$ vs. *day 2* or *6* infection, basal.

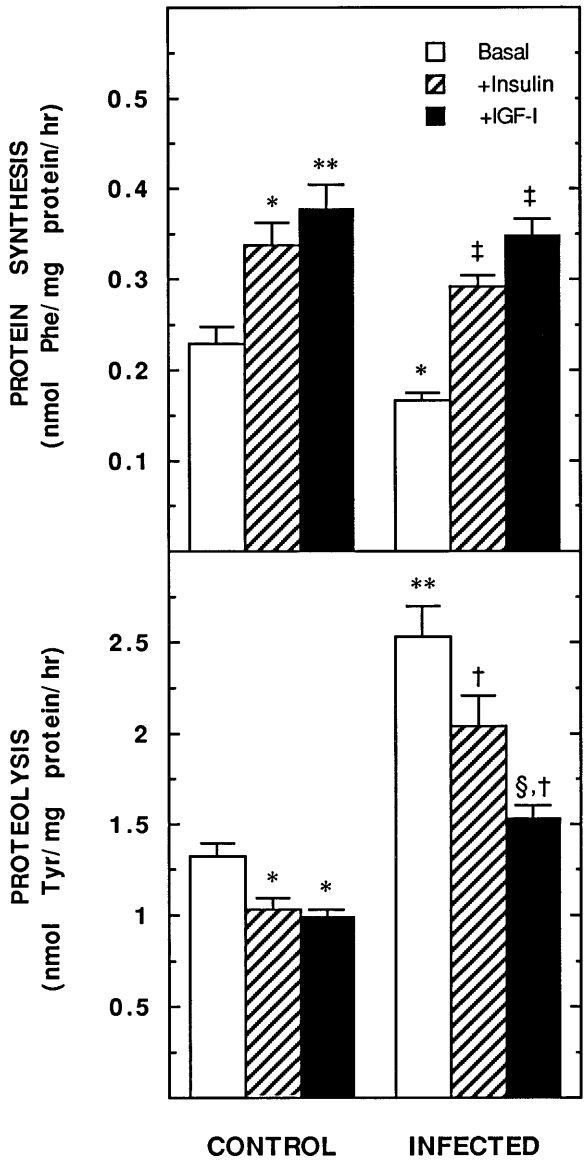

Fig. 5. Rates of protein synthesis (*top*) and proteolysis (*bottom*) 2 days after injection of *E. coli* in infected and pair-fed control rats: effects of insulin and IGF-I. Epitrochlearis muscles from infected and pair-fed control rats (as indicated) were excised and incubated in vitro 2 days after injection of bacteria as described in MATERIALS AND METHODS in presence of insulin or IGF-I or in their absence. Insulin or IGF-I was added to give final concentrations of 2 and 10 nM, respectively, in incubation medium. Rates of protein synthesis and protein degradation were measured as described Fig. 1. Values shown are means ± SE for 5–14 muscles in each group. Protein synthesis: ANOVA $F = 25.68$, $P < 0.001$. Protein degradation: ANOVA $F = 23.58$, $P < 0.001$. *$P < 0.05$; **$P < 0.005$ vs. control, basal; †$P < 0.001$ vs. infected, basal; ‡$P < 0.001$ vs. control, +insulin or control, +IGF-I; §$P < 0.05$ vs. infected, basal and infected, +insulin.

control rats. In control rats, addition of either insulin or IGF-I to the incubation medium significantly depressed the net release of tyrosine from epithrochlearis by 30–50% compared with basal conditions at each of the days investigated. Both hormones were equally effective in limiting the negative protein balance. There were no significant differences between insulin and IGF-I with respect to their ability to inhibit net tyrosine release.

In contrast to control, muscles from infected rats responded differently to insulin or IGF-I (Table 2). The negative protein balance (2.12 ± 0.25 nmol Tyr·mg protein^{-1}·h^{-1}) in the presence of insulin was not significantly reduced on *day 2* postinfection compared with rates obtained under basal conditions (2.78 ± 0.28 nmol Tyr·mg protein^{-1}·h^{-1}). By 6 days postinfection, the inhibition of proteolysis by insulin was greater than on *day 2* postinfection. The negative protein balance in

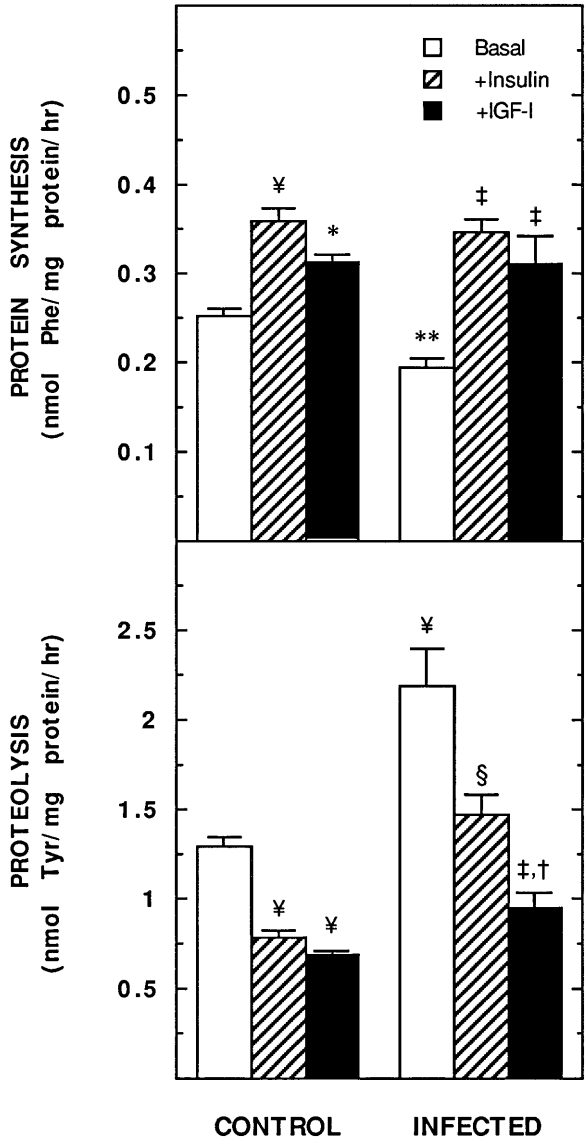

Fig. 6. Rates of protein (*top*) synthesis and proteolysis (*bottom*) 6 days after injection of *E. coli* in infected and pair-fed control rats: effects of insulin and IGF-I. Epitrochlearis muscles from infected and pair-fed control rats (as indicated) were excised and incubated in vitro 6 days after injection of bacteria as described in MATERIALS AND METHODS in presence of insulin or IGF-I or in their absence. Insulin or IGF-I was added to give a final concentration of 2 and 10 nM, respectively, in incubation medium. Rates of protein synthesis and protein degradation were measured as described Fig. 1. Values shown are means ± SE for 5–18 muscles in each group. Protein synthesis: ANOVA $F = 17.57$, $P < 0.001$. Protein degradation: ANOVA $F = 23.25$, $P < 0.001$. *$P < 0.05$; **$P < 0.005$; ¥$P < 0.001$ vs. control, basal; ‡$P < 0.005$ vs. infected, basal; †$P < 0.05$ vs. infected, +insulin; §$P < 0.005$ vs. infected, basal, and control, +insulin

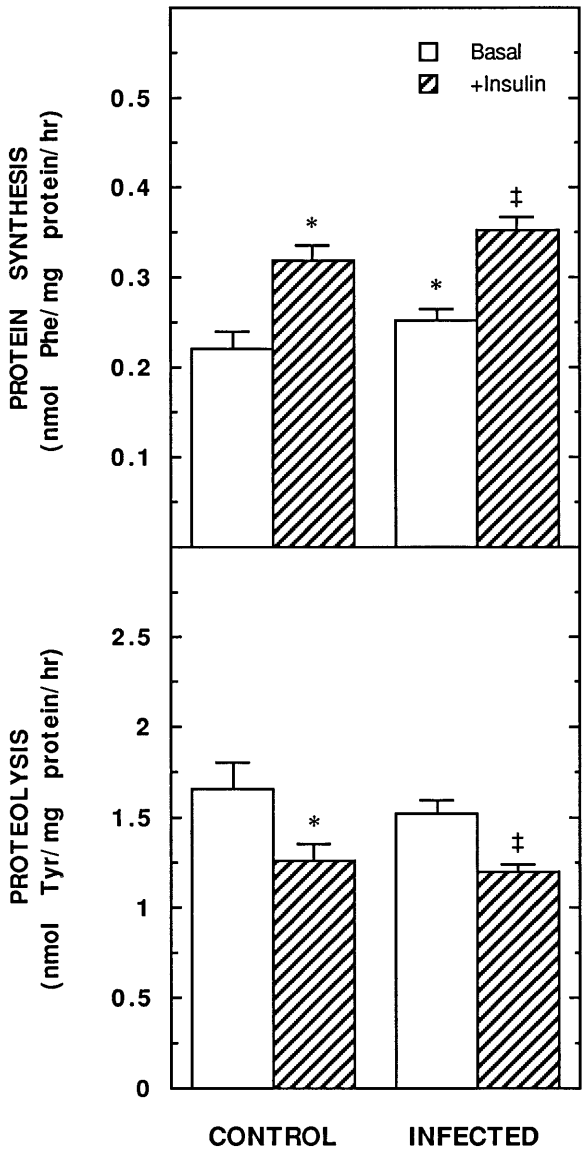

Fig. 7. Rates of protein synthesis (*top*) and proteolysis (*bottom*) 15 days after injection of *E. coli* in infected and pair-fed control rats: effects of insulin. Epitrochlearis muscles from infected and pair-fed control rats (as indicated) were excised and incubated in vitro 15 days after injection of bacteria as described in MATERIALS AND METHODS in presence or absence of insulin. Insulin was added to give a final concentration of 2 nM in incubation medium. Rates of protein synthesis and protein degradation were measured as described Fig. 1. Values shown are means ± SE for 8–10 muscles in each group. Protein synthesis: ANOVA $F = 22.22$, $P < 0.001$. Protein degradation: ANOVA $F = 4.49$, $P < 0.01$. *$P < 0.05$, control, basal; ‡$P < 0.05$ vs. infected, basal.

presence of insulin (1.16 ± 0.16 nmol Tyr·mg protein^{-1}·h^{-1}) was reduced 43% relative to the basal state in infected rats (2.04 ± 0.34 nmol Tyr·mg protein^{-1}·h^{-1}). At both days, the negative protein balance remained elevated twofold relative to control muscle incubated in presence of insulin. By 15 days postinfection, insulin significantly reduced net protein balance by 22%. Furthermore, no differences between control and infected rats were observed after incubation with insulin. In contrast to the effects of insulin, addition of IGF-I to incubation medium resulted in a significant 50% reduction in the negative protein balance 2 days postinfection. However, net protein balance remained elevated relative to muscles from control rats incubated in the presence of IGF-I. By 6 days postinfection, the protein balance was further reduced in muscles incubated with IGF-I compared with *day 2*. The release of tyrosine was not significantly different from that observed in controls.

To further characterize the processes responsible for changes in the protein balances, rates of protein synthesis and proteolysis in the presence of insulin or IGF-I were measured. After infection, the rate of protein synthesis in muscles from either control or infected rats was significantly stimulated by addition of insulin or IGF-I to the medium at each of the time points investigated (Figs. 5–7). There were no significant differences between control and infected rats with respect to the rate of protein synthesis in the presence of insulin or IGF-I. Furthermore, there were no significant differences in the maximal rate of protein synthesis after incubation with either insulin or IGF-I. Thus muscles from each group responded in a similar manner to addition of insulin or IGF-I.

Rates of proteolysis were significantly reduced after incubation of muscles with insulin or IGF-I in control rats at each of the time points examined (Figs. 5–7). There were no apparent differences between insulin and IGF-I with respect to their ability to inhibit protein degradation in epitrochlearis from control rats.

We next examined whether insulin and IGF-I were equally effective in reducing proteolysis in muscle from rats after injection with live bacteria. Unlike muscles from control rats, protein degradation was not significantly decreased in muscle from infected rats 2 days postinfection incubated in the presence of insulin (Fig. 5). The rate of protein degradation in muscle of infected rats incubated with insulin remained twofold higher than in pair-fed control rats. In contrast to insulin, IGF-I caused an ~40% decrease in the rate of protein degradation in muscles from infected rats ($P < 0.01$) 2 days after infection (Fig. 5). However, the rate of protein degradation in muscles from infected rats remained significantly elevated (54%) compared with pair-fed control rats incubated with IGF-I ($P < 0.001$).

On *day 6* postinfection, the rate of protein degradation was significantly decreased 33% ($P < 0.05$) in epitrochlearis incubated in the presence of insulin compared with muscles from infected rats incubated in the hormone's absence (Fig. 6). However, the rate of protein degradation in muscles from infected rats remained approximately twofold higher than that observed in muscles from control rats incubated under identical conditions. On the other hand, IGF-I reduced protein degradation ~55% in muscles from infected rats (Fig. 6). The rate of protein degradation in presence of IGF-I was significantly reduced 35% compared with muscles incubated in the presence of insulin. The diminution of proteolysis in the presence of IGF-I was sufficient, so that no significant differences in the rate of protein degradation were observed between infected and control rats.

By *day 15* postinfection, proteolysis in muscles from rats injected with bacteria was reduced to the same extent as control in the presence of insulin (Fig. 7). No significant differences between control and infected rats were observed. Because IGF-I completely restored protein balance to values observed in control rats by 6 days postinfection, the effect of IGF-I on protein synthesis and degradation was not tested at 15 days after injection of bacteria.

DISCUSSION

Results from the present studies indicate that fundamental defects in the regulation of protein synthesis and protein degradation in skeletal muscle occur days after injection of live *E. coli*. The dyshomeostasis was manifested by an inhibition of protein synthesis and stimulation of protein degradation that persisted for up to 1 wk postinfection. Protein synthesis was stimulated by insulin or IGF-I to the same extent in infected rats compared with controls. In contrast, proteolysis in infected rats was limited to a lesser degree by insulin compared with IGF-I. Comparisons between control and infected rats were made under similar nutritional conditions so that the differences observed can be ascribed to the host's response to the bacterial insult rather than nutritional deficiency. Because noninfected, control animals consumed the same amount of food as septic rats, the derangements in protein synthesis and proteolysis after injection of live bacteria are independent of and in addition to those associated with reduced food intake. The persistence of these infection-induced changes in protein synthesis and protein degradation compared with pair-fed control rats demonstrates the response to be a direct consequence of the infection process rather than an indirect effect via alterations in food intake.

The host's response to sepsis is characterized by three phases (3, 4, 17). The first phase, which lasts for up to 2 days, is characterized by initiation of infection. A predominant feature during this period is anorexia (1, 3, 17, 33), with rats injected with bacteria consuming only ~30% of the normal food intake during this period. Moreover, infected rats exhibited a greater loss of muscle mass than pair-fed control rats. Therefore, although anorexia may contribute, it is not sufficient by itself to account for the loss of skeletal muscle mass during sepsis. The loss of protein from skeletal muscle in infected rats results from a 27% reduction in protein synthesis and a 91% acceleration of protein degradation relative to pair-fed control rats.

The second phase of the host's response to sepsis is associated with a stable, hypermetabolic condition whereby despite adequate nutrient intake, normal growth or positive nitrogen balance is not achieved (1, 4, 17, 32, 33). In the present set of investigations, the skeletal muscle mass is reduced relative to pair-fed controls 6 days after injection of bacteria. Protein balance improves relative to the anorexic phase but remains twofold elevated compared with noninfected rats. During the hypermetabolic phase, protein synthesis is inhibited by 22% and protein degradation is stimulated by 70%. An inhibition of protein synthesis of a similar magnitude has also been reported in muscles of rats with a chronic septic abscess (4, 16–18, 32) and septic patients in vivo (3, 23). Proteolysis is accelerated in muscles from septic patients, indicating that protein degradation remains elevated during the chronic, hypermetabolic phase of sepsis (23). The results obtained in the present study are consistent with these reports concerning the long-term effect of sepsis on protein turnover both in septic patients and animals.

During the recovery phase (*day 15* postinfection), body weight and muscle mass increase compared with the hypermetabolic phase. From *days 6* to *15* postinfection, the total protein content/muscle was significantly increased by 70% (2.36 mg protein/muscle) in infected rats. During the same time period, total protein content/muscle in control rats increased by 24% (1.29 mg protein/muscle). Thus, by *day 15* postinfection, the protein balance in incubated muscles was not significantly different between control and infected rats. Rates of protein synthesis were actually augmented in infected animals compared with control, whereas protein degradation returned to values observed in noninfected animals. Accretion of muscle mass during the recovery phase resulted from a decreased rate of protein degradation and an increase in protein synthesis relative to the hypermetabolic phase of sepsis (*day 6*).

Insulin resistance is defined as a less than normal response to a dose of insulin (21). Muscles from infected rats showed a markedly reduced response to the anabolic actions of insulin on net protein balance during the anorexic phase of sepsis. During the hypermetabolic phase, the ability of insulin to affect protein balance was partially restored, but the negative protein balance remained elevated twofold relative to controls. Improvement in the protein balance was greater in muscles incubated in the presence of IGF-I than insulin after infection. During the anorexic phase, negative protein balance was reduced 50% in the presence of IGF-I. This observation is in contrast to insulin, for which no significant effect on negative protein balance was observed. On *day 6* postinfection, protein balance in muscles incubated with IGF-I was improved 40% relative to muscles from septic rats incubated in the presence of insulin. Furthermore, the protein balance was not different in infected rats incubated with IGF-I compared with controls.

The changes in the protein balance are a consequence of infection-induced derangements in protein synthesis and degradation. Protein synthesis in muscles from infected rats incubated in the presence of insulin was enhanced during each of the phases examined. The maximal responsiveness of protein synthesis to stimulation by insulin was not altered in infected rats compared with controls. Like insulin, IGF-I stimulated protein synthesis at each of the time points examined. Furthermore, the maximal stimulation of protein synthesis by IGF-I was not different between control and infected rats. Therefore no evidence of a resistance to insulin- or IGF-I-induced stimulation of protein synthesis was observed after injection of a single strain of

bacteria. Similar conclusions concerning the responsiveness of protein synthesis to stimulation by insulin were observed after acute (16 h) peritonitis by Hasselgren and co-workers (11, 12). However, a chronic (5 days), hypermetabolic intra-abdominal abscess sepsis was associated with a profound insulin resistance, even at pharmacological doses of the hormone (16). The reasons for this apparent discrepancy are unclear but may reflect differences in the host's response to intra-abdominal polyclonal septic abscess vs. the intravenous injection of a single bacterial strain.

In contrast to protein synthesis, the ability of insulin to decrease proteolysis was severely curtailed for periods up to 48 h after injection of bacteria. This observation suggests that the relative resistance of proteolysis to insulin, described in extensor digitorum longus during acute (16 h) peritonitis (11, 12), persists for at least 48 h after injection of bacteria. In the present studies, the ability of insulin to reduce proteolysis was augmented in infected rats during the chronic hypermetabolic phase relative to the anorexic period. However, proteolysis remained elevated relative to noninfected animals. Caution must be exercised in extrapolating our results to conclude that protein degradation was totally insensitive to insulin during the anorexic and hypermetabolic phases in the response to infection. Although we have not defined a dose-response relationship between protein degradation and insulin in incubated muscles from infected rats, previous studies in fed, infected animals have shown that proteolysis is less sensitive to the antiproteolytic effects of insulin even at pharmacological (100 nM) doses of insulin (33).

There are no other reports concerning the effect of IGF-I on muscle protein degradation during sepsis. In the present study, incubation with IGF-I was able to partially inhibit proteolysis during the anorexic phase. Despite the ability of IGF-I to reduce proteolysis, the rate of protein degradation remained 50% higher than values observed in muscles from control animals. IGF-I caused a further inhibition of proteolysis during the hypermetabolic phase after infection. During this period, proteolysis in the muscles from infected and control rats did not differ. Hence, muscle from infected rats appeared more responsive to the antiproteolytic effects of IGF-I compared with insulin. The mechanisms responsible for the differential responses to insulin and IGF-I in muscles from infected rats remain unknown. Unlike insulin, the plasma concentrations of IGF-I are reduced during sepsis (19) in animals or after infusion of GH in septic patients (5). Maintenance of the plasma IGF-I concentrations during chronic sepsis prevented the inhibition in muscle protein synthesis (19).

Several lines of evidence argue against the differences in skeletal muscle protein synthesis and degradation between control and infected rats being a consequence of decreased viability of the preparation over the course of the incubation period. First, protein synthesis in muscles from infected rats is stimulated to the same extent by insulin or IGF-I as muscles from control rats. Moreover, the magnitude of the stimulation of protein synthesis in control rats by insulin is similar to previous reports (24, 25). Second, lactate production, although elevated in muscles from infected rats, remains constant over the 3-h incubation period in epitrochlearis muscle from either control or infected rats (31). Third, the high-energy phosphate contents (ATP + creatine phosphate) in muscle are not significantly reduced after incubation for periods up to 240 min in epitrochlearis muscle weighing ~150 mg (twice mass of muscles used in present study) under similar conditions (Savary, I., D. Dardevet, and J. Grizard, unpublished data). Fourth, several investigators have suggested that the stimulation of proteolysis after infection occurs by an energy-dependent pathway (26, 33). Thus decreased viability and loss of energy would lead to a diminution, not a stimulation of proteolysis in muscles from infected rats.

In summary, infection causes fundamental defects in skeletal muscle protein synthesis and proteolysis independent of the nutritional state of the animal. The impairment in protein synthesis is observed as early as 2 days postinfection and is maintained for at least 6 days. However, by 15 days postinfection, protein synthesis was actually enhanced in septic rats compared with control. Inclusion of insulin or IGF-I during in vitro incubation can overcome the infection-induced inhibition in protein synthesis. On the other hand, protein degradation was greatly enhanced after injection of bacteria. The stimulated rate of proteolysis after infection persisted for at least 6 days but returned to values observed in control rats by *day 15* postinfection. The ability of insulin to inhibit proteolysis was severely blunted, indicating that this process exhibits an insulin resistance after infection. However, proteolysis in muscles from infected rats appeared more responsive to IGF-I than insulin during both the anorexic and hypermetabolic phases of the host response to injection of bacteria. The ability of insulin or IGF-I to suppress proteolysis becomes enhanced with time as the effects of infection wane. Because the present experiments were performed in incubated muscles, the effects of insulin or IGF-I on protein synthesis or proteolysis are the direct effect of these hormones on epitrochlearis and are not related to systemic perturbations secondary to infusion of the hormones in vivo. The differential response to IGF-I vs. insulin may reflect how infection alters the dose-response curves for each individual hormone. Furthermore, it is unknown whether the component of proteolysis dysregulated by infection is the same component that responds to the hormones. However, the defect does not appear to be a generalized phenomenon in insulin or IGF-I signaling pathways, because rates of protein synthesis measured in the same muscles are responsive to stimulation by these hormones.

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