Insulin-Like Growth Factor I Reduces Ubiquitin and Ubiquitin-Conjugating Enzyme Gene Expression but Does Not Inhibit Muscle Proteolysis in Septic Rats*

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ABSTRACT

We examined the effect of insulin-like growth factor I (IGF-I), administered in vivo, on protein turnover rates and gene expression of the ubiquitin-proteasome proteolytic pathway in skeletal muscle of septic rats. Sepsis was induced by cecal ligation and puncture. Other rats were sham-operated. Miniosmotic pumps were implanted sc, and groups of rats received IGF-I (7 mg/kg/24 h) or saline. Protein synthesis and breakdown rates were determined in incubated extensor digitorum longus muscles. Messenger RNA levels for ubiquitin and the ubiquitin-conjugating enzyme E2_14k were determined by Northern blot analysis. Sepsis resulted in an approximately 30% reduction of muscle protein synthesis, and this effect of sepsis was blunted in rats treated with IGF-I. In contrast, IGF-I did not prevent the sepsis-induced increase in total and myofibrillar muscle protein breakdown. Ubiquitin and E2_14k messenger RNA levels were increased several fold in muscle from septic rats, and this effect of sepsis was abolished in IGF-I treated rats. The results suggest that administration of IGF-I may improve sepsis-induced muscle cachexia by stimulating protein synthesis. However, because muscles were resistant to IGF-I, with regard to regulation of protein breakdown, the use of IGF-I to treat muscle cachexia during sepsis remains unclear. An additional important implication of the present study is that changes in messenger RNA levels for ubiquitin and the ubiquitin-conjugating enzyme E2_14k do not always reflect changes in muscle protein breakdown rates.

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ONE OF THE most pronounced metabolic changes during sepsis is the catabolic response in skeletal muscle, characterized by increased protein breakdown and reduced protein synthesis (1–3). Recent studies from our and other laboratories provided evidence that sepsis-induced muscle cachexia mainly reflects increased ubiquitin-proteasome-dependent proteolysis and is associated with up-regulated gene expression of ubiquitin and the ubiquitin-conjugating enzyme E2_14k in muscle tissue (4–8). Some of the consequences of muscle cachexia during sepsis include muscle weakness and fatigue that may delay recovery and increase the risks for thromboembolic and pulmonary complications when respiratory muscles are affected. Methods to reduce the catabolic response in skeletal muscle during sepsis, therefore, have great clinical significance.

Previous studies suggest that insulin-like growth factor I (IGF-I) exerts an anabolic effect in muscle tissue. For example, treatment of cultured muscle cells with the hormone resulted in increased protein synthesis and reduced protein degradation (9, 10). In recent studies in our laboratory, IGF-I blocked the catabolic response in skeletal muscle after burn injury, both when incubated muscles were treated in vitro (11) and when burned rats were treated with the hormone in vivo (12).

In contrast, when incubated muscles from septic rats were treated with IGF-I, protein degradation was unaffected, even at high hormone concentrations, suggesting that muscle becomes resistant to IGF-I during sepsis (13). Because the muscles were treated with the hormone in vitro in those experiments and because the metabolic and hormonal milieu is much more complex in vivo than in vitro, the results observed in vitro do not necessarily mean that muscle becomes resistant to treatment with IGF-I in vivo during sepsis. In the present study, we tested the hypothesis that administration of IGF-I in vivo stimulates protein synthesis and inhibits protein breakdown in skeletal muscle during sepsis. Because, in several previous reports, sepsis-induced muscle proteolysis was associated with increased gene expression of ubiquitin (6, 7) and other components of the ubiquitin-proteasome proteolytic pathway, including the ubiquitin-conjugating enzyme E2_14k (5), messenger RNA (mRNA) levels for ubiquitin and E2_14k were determined, as well.

Materials and Methods

Experimental animals

Male Sprague Dawley rats, weighing 50–60 g, were anesthetized with pentobarbital (35 mg/kg ip), and miniosmotic pumps (model 2001 D, Alzet, Palo Alto, CA) were implanted sc on the neck of the rats. The pumps were filled with normal saline or recombinant human IGF-I (kindly provided by Genentech, Inc., South San Francisco, CA) dissolved in saline at a concentration (2.12 mg/ml) sufficient to deliver 7 mg/kg/24 h. The rate of infusion from the miniosmotic pumps was 8 µl/h, as specified by the manufacturer. Because the hormone concentration in the miniosmotic pumps was high, the influence of adhesion of IGF-I to the pump was probably negligible. To ensure immediate delivery of the hormone at the target rate, the miniosmotic pumps were primed in vitro in sterile normal saline, at 37 C, for 3 h before the implantation. The dose of IGF-I used here was based on a recent study in which administration of 7 mg/kg/24 h blocked the burn-induced increase in muscle proteolysis in rats (12).
Immediately after the sc implantation of the miniosmotic pumps, groups of rats underwent induction of sepsis or sham-operation. Sepsis was induced by cecal ligation and puncture (CLP), as described previously (4, 5, 7, 13). Sham-operated rats underwent laparotomy and manipulation but no ligation or puncture of the cecum. All rats were resuscitated with saline (10 ml/100 g BW), administered sc on the back, at the time of surgery. The rats were randomly assigned to one of four groups: 1) sham-operation + saline; 2) sham-operation + IGF-I; 3) CLP + saline; or 4) CLP + IGF-I. After the procedures, the rats were housed individually and were allowed free access to drinking water. Food was withheld after surgery to avoid any influence on metabolic changes of different food intake between the groups of rats. Metabolic studies were performed 16 h after CLP or sham-operation. In previous studies, rats were in a hyperdynamic phase of sepsis (14, 15), and ubiquitin-proteasome-dependent muscle proteolysis was increased 16 h after CLP (7). The experimental model of sepsis used here is clinically relevant because it resembles the situation in patients with sepsis caused by intraabdominal abscesses and devitalized tissue. The model was characterized in previous reports from our (15) and other laboratories (14), with regard to mortality rates and hemodynamic and metabolic changes. Rats weighing 50–60 g were used because lower extremity muscles from rats of this size are thin enough to allow for adequate tissue oxygenation and viability during in vitro incubation (2, 16). All experiments were conducted, and animals were cared for, in accordance with the National Research Council’s Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of Cincinnati.

**Muscle incubations**

Sixteen hours after CLP or sham-operation, rats were anesthetized with pentobarbital (35 mg/kg ip). The extensor digitorum longus (EDL) muscles were dissected with intact tendons and mounted on stainless steel supports at resting length and preincubated for 30 min at 37°C in 3 ml oxygenated (O2:CO2 = 95:5) Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 10 mM glucose. The muscles were incubated at resting length (rather than flaccid) because, in previous studies, energy levels and metabolic rates were better maintained in muscles at resting length (16, 17).

For measurement of protein synthesis rate, muscles were transferred to 3 ml fresh medium of the same composition as described above, containing U-14C-phenylalanine (0.5 mM, 0.05 μCi/ml). After incubation for 2 h, the amount of phenylalanine incorporated into trichloroacetic acid (10%)-precipitated proteins was determined as described previously (11, 13).

For measurement of protein breakdown rates, muscles were preincubated in oxygenated medium for 30 min, as described above. After preincubation, one muscle was rinsed with fresh medium, blotted, weighed, and placed in ice-cold 0.4 M perchloric acid for determination of tissue-free tyrosine and 3-methylhistidine (3-MH). The contralateral muscle was transferred to fresh medium containing cycloheximide (0.5 mM) and incubated for 2 h. Cycloheximide was present in the medium to prevent reincorporation into protein of amino acids released during proteolysis. After incubation for 2 h, the muscle was rinsed, blotted, weighed, and placed in ice-cold 0.4 M perchloric acid. Muscles and media were stored at −20°C until tyrosine and 3-MH were assayed by HPLC. Total and myofibrillar protein breakdown rates were determined as net release of tyrosine and 3-MH, respectively, taking changes in tissue levels of the amino acids during incubation into account, as previously described in detail (2, 16).

**Northern blot analysis**

Sixteen hours after CLP or sham-operation, rats were anesthetized with pentobarbital (35 mg/kg ip). The extensor digitorum longus muscles were harvested, immediately frozen in liquid nitrogen, and then stored at −70°C until analysis. Messenger RNA levels for ubiquitin and the 14-kDa ubiquitin-conjugating enzyme E214a were determined by Northern blot analysis, as previously described in detail, using [32P] labeled cDNA probes (5, 6). Blots were quantitated on a Phosphoimager using the Image Quant Program (Molecular Dynamics, Inc., Sunnyvale, CA), and the relative mRNA abundance was determined by using a rat 18S ribosomal probe to control for equal loading of the lanes.

**Plasma glucose and amino acids**

Blood was collected, by heart puncture, at the time of muscle dissection. Plasma glucose was determined by a colorimetric assay using Vitros GLU slides (Johnson & Johnson Clinical Diagnostics, Rochester, NY). Plasma amino acids were measured in an amino acid analyzer (Beckman Coulter, Inc. 6300; Beckman Coulter, Inc., Palo Alto, CA).

**Statistics**

Results are presented as mean ± SEM. Statistical comparisons were done by Student’s t test or ANOVA followed by Duncan’s test.

**Results**

Septic rats showed signs of illness in the form of piloerection, secretion around the eyes and nostrils, and moderate diarrhea. Mortality rate was almost identical in septic rats treated with IGF-I or saline (Table 1). There was no mortality among sham-operated rats treated with saline or IGF-I.

Muscle protein synthesis was reduced by approximately 30% in septic rats. The sepsis-induced inhibition of protein synthesis was significantly (P < 0.05) blunted in rats treated with IGF-I; and, in this group of rats, muscle protein synthesis rates were not significantly different from those in sham-operated rats (Fig. 1). Administration of IGF-I did not influence muscle protein synthesis rates in sham-operated rats.

Total muscle protein breakdown, measured as net release of tyrosine from incubated EDL muscles, was approximately 50% higher in septic than in sham-operated rats (Fig. 2), which is in line with previous reports from this laboratory (2, 4, 7, 13). Total muscle protein breakdown was not influenced by treatment with IGF-I in septic or sham-operated rats. Similar results were observed in three repeated experiments.

We have previously shown that the increase in total muscle protein breakdown during sepsis mainly reflects myofibrillar protein breakdown, measured as net release of 3-MH from incubated muscles (2, 6). Thus, the lack of effect of IGF-I on total muscle protein breakdown described above most likely reflected unchanged myofibrillar protein breakdown. To more specifically test the influence of IGF-I on myofibrillar proteolysis, we next measured 3-MH release in incubated muscles from sham-operated and septic rats treated with saline or IGF-I. Administration of IGF-I did not influence myofibrillar muscle protein degradation in sham-operated or septic rats (Fig. 3).

Total and myofibrillar protein breakdown rates were determined by measuring net release of tyrosine and 3-MH, respectively. For these calculations, changes in tissue levels of tyrosine and 3-MH during incubation were determined by measuring tissue levels at the start (0 h time point) and end of the 2-h incubation. In a previous study, we found that tissue levels of tyrosine increased somewhat during 2-h incubation, and tissue 3-MH levels decreased to a greater ex-

**TABLE 1. Mortality rates in sham-operated and septic (CLP) rats treated with saline or IGF-I**

<table>
<thead>
<tr>
<th></th>
<th>Sham Saline</th>
<th>IGF-I Saline</th>
<th>CLP Saline</th>
<th>IGF-I CLP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0/39</td>
<td>0/38</td>
<td>26/63 (41%)</td>
<td>28/66 (42%)</td>
</tr>
</tbody>
</table>


Similar changes were seen during incubation of muscles in the present study (Table 2). In previous studies from our laboratory, the gene expression of ubiquitin and the ubiquitin-conjugating enzyme E2\textsubscript{14k} was up-regulated in muscle from septic rats (5, 6). A similar response to sepsis was observed in the present experiments. Thus, ubiquitin mRNA levels were more than doubled in muscles from septic rats, compared with the levels in sham-operated rats (Fig. 4). The sizes of the ubiquitin transcripts noted here (2.6 and 1.2 kb) are similar to previous reports (6, 18, 19). Sepsis influenced the expression of both these transcripts to a similar degree, and both were therefore included in the quantitation. A smaller ubiquitin transcript (approximately 0.5 kb) was also present but was not influenced by sepsis (not shown). Because in several previous reports, parallel changes in muscle protein breakdown rates and ubiquitin mRNA levels occurred, we did not expect ubiquitin mRNA to be influenced by IGF-I treatment in the present experiments. Surprisingly, however, ubiquitin mRNA levels were normalized in muscle from septic rats treated with IGF-I (Fig. 4). Identical results were observed in three repeated experiments.

Sepsis resulted in an approximately 3-fold increase in muscle levels of the 1.2-kb E2\textsubscript{14k} transcript but did not influence the 1.8-kb transcript (Fig. 5). A similar selective increase in the concentration of the small E2\textsubscript{14k} transcript was observed during sepsis, in a recent study from our laboratory (5), and was reported in other catabolic conditions, as well (20, 21). Treatment with IGF-I blocked the sepsis-induced increase in muscle E2\textsubscript{14k} mRNA levels (Fig. 5).

The changes in mRNA levels noticed here need to be interpreted with caution, for several reasons. First, it is not known whether the changes (increased mRNA levels during sepsis with normalization during IGF-I treatment) reflect changes in gene transcription or mRNA stability. Second, changes in mRNA levels are not always accompanied by changes in protein levels and, importantly, it is not known
which specific ubiquitin or E2_{14k} transcript is most important for the synthesis of the proteins under the present experimental conditions. The main reason why ubiquitin and E2_{14k} mRNA levels were measured in the present study was that changes in muscle protein breakdown rates are typically associated with changes in these transcripts.

Although changes in plasma amino acids are a rather nonspecific indication of changes in whole-body protein metabolism, changes in the plasma concentrations of certain amino acids (including phenylalanine, tyrosine, histidine, and the branched chain amino acids) have been considered to reflect changes in muscle protein breakdown rates (22–24). Sepsis resulted in increased plasma concentrations of most of these amino acids, and most of the sepsis-induced changes in plasma amino acids were reversed by treatment with IGF-I (Table 3). Indeed, the majority of plasma amino acids were reduced during treatment with IGF-I, both in sham-operated and septic rats, and total amino acids were reduced by the hormone in both groups of rats.

One of the side-effects of treatment with IGF-I is hypoglycemia (25); and because hypoglycemia has a catabolic effect in skeletal muscle, either as a direct effect or secondary to release of catabolic hormones [in particular, epinephrine and glucagon (26, 27)], it is possible that the lack of effect of the hormone on muscle total and myofibrillar protein breakdown noted here reflected IGF-I-induced hypoglycemia. To test the potential role of hypoglycemia in the present results, we next determined plasma glucose levels in sham-operated and septic rats treated with saline or IGF-I. Treatment with IGF-I resulted in pronounced hypoglycemia, both in sham-operated and septic rats (Table 4).

To compensate for the hormone-induced hypoglycemia, we next treated septic rats, receiving IGF-I, with various amounts of glucose administered sc as a 5% or 10% solution immediately after CLP. Administration of 0.3 or 0.6 g glucose per rat did not prevent the hypoglycemia caused by the hormone (not shown). When 1.2 g glucose was given, the hormone-induced hypoglycemia was significantly blunted, and plasma glucose was raised to 80 ± 4 mg/dl, similar to the glucose level in septic rats treated with saline (compare with Table 4). We next measured muscle protein breakdown rates after administration of 1.2 g glucose to septic rats receiving IGF-I. Also, in this experiment, muscle protein breakdown rates were not influenced by the hormone (Fig. 6). The lower protein breakdown rates noted in septic rats in this, rather than our first experiment (compare with protein breakdown rates in septic rats in Fig. 2), may reflect an inhibitory effect of glucose on muscle protein degradation.
**Discussion**

In the present study, treatment of septic rats with IGF-I *in vivo* blocked the sepsis-induced inhibition of muscle protein synthesis but did not prevent the increase in muscle protein breakdown rates. Taken together with results in recent *in vitro* experiments in which treatment of incubated muscles from septic rats with IGF-I stimulated protein synthesis but did not inhibit protein degradation (13), the observations suggest that muscle becomes resistant to IGF-I during sepsis, with regard to the hormonal regulation of protein degradation. Because the stimulation of protein synthesis by IGF-I was not prevented by sepsis (Ref. 13 and present study) and because, in other studies, IGF-I stimulated muscle glucose uptake during sepsis (28), the lack of regulation of protein breakdown is consistent with a postreceptor defect.

A differential postreceptor regulation of protein synthesis...
TABLE 4. Plasma glucose levels in sham-operated and septic rats treated with saline or IGF-I

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>IGF-I</th>
<th>CLP</th>
<th>IGF-I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>IGF-I</td>
<td>Saline</td>
<td>IGF-I</td>
</tr>
<tr>
<td>Valine</td>
<td>130 ± 16</td>
<td>55 ± 8*</td>
<td>204 ± 32b</td>
<td>110 ± 20a</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>82 ± 8</td>
<td>32 ± 3*</td>
<td>100 ± 12</td>
<td>58 ± 9**</td>
</tr>
<tr>
<td>Leucine</td>
<td>118 ± 12</td>
<td>53 ± 6**</td>
<td>167 ± 21a</td>
<td>97 ± 15**</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>42 ± 4</td>
<td>21 ± 3*</td>
<td>65 ± 15a</td>
<td>37 ± 7**</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>61 ± 4</td>
<td>59 ± 7</td>
<td>95 ± 10b</td>
<td>69 ± 9*</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>69 ± 4</td>
<td>46 ± 4**</td>
<td>68 ± 7</td>
<td>47 ± 5**</td>
</tr>
<tr>
<td>Glutamate</td>
<td>744 ± 33</td>
<td>369 ± 48a</td>
<td>785 ± 64</td>
<td>410 ± 29a</td>
</tr>
<tr>
<td>Glutamine</td>
<td>68 ± 5</td>
<td>40 ± 3*</td>
<td>99 ± 5b</td>
<td>57 ± 6**</td>
</tr>
<tr>
<td>Threonine</td>
<td>243 ± 12</td>
<td>90 ± 14**</td>
<td>196 ± 29</td>
<td>130 ± 19a</td>
</tr>
<tr>
<td>Glycine</td>
<td>559 ± 46</td>
<td>271 ± 25a</td>
<td>422 ± 35b</td>
<td>283 ± 22a</td>
</tr>
<tr>
<td>Lysine</td>
<td>347 ± 25</td>
<td>201 ± 17a</td>
<td>295 ± 59</td>
<td>219 ± 27</td>
</tr>
<tr>
<td>Alanine</td>
<td>383 ± 19</td>
<td>148 ± 20a</td>
<td>476 ± 111</td>
<td>200 ± 42a</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>15 ± 1</td>
<td>12 ± 0.4</td>
<td>39 ± 7b</td>
<td>26 ± 4**</td>
</tr>
<tr>
<td>Asparagine</td>
<td>50 ± 3</td>
<td>18 ± 2*</td>
<td>41 ± 4</td>
<td>16 ± 5**</td>
</tr>
<tr>
<td>Histidine</td>
<td>76 ± 4</td>
<td>62 ± 6</td>
<td>98 ± 12b</td>
<td>71 ± 9**</td>
</tr>
<tr>
<td>Arginine</td>
<td>105 ± 8</td>
<td>49 ± 3**</td>
<td>74 ± 10b</td>
<td>49 ± 8**</td>
</tr>
<tr>
<td>Cysteine</td>
<td>24 ± 2</td>
<td>17 ± 1*</td>
<td>13 ± 2b</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Citrulline</td>
<td>128 ± 6</td>
<td>67 ± 5*</td>
<td>68 ± 12a</td>
<td>53 ± 6</td>
</tr>
<tr>
<td>Ornithine</td>
<td>67 ± 6</td>
<td>30 ± 3*</td>
<td>56 ± 12</td>
<td>29 ± 9*</td>
</tr>
<tr>
<td>Serine</td>
<td>277 ± 11</td>
<td>118 ± 8a</td>
<td>198 ± 22b</td>
<td>135 ± 11</td>
</tr>
<tr>
<td>Proline</td>
<td>133 ± 17</td>
<td>36 ± 7b</td>
<td>62 ± 9b</td>
<td>42 ± 6</td>
</tr>
<tr>
<td>Methionine</td>
<td>69 ± 4</td>
<td>39 ± 3**</td>
<td>37 ± 3b</td>
<td>25 ± 5*</td>
</tr>
<tr>
<td>Total amino acids</td>
<td>3620 ± 136</td>
<td>1831 ± 65a</td>
<td>3895 ± 482</td>
<td>2254 ± 202a</td>
</tr>
</tbody>
</table>

Results are given as μmol/liter (mean ± se). n = 5 in both sham groups; n = 7 in both CLP groups.

a P < 0.05 vs. saline.
b P < 0.05 vs. corresponding sham group.

The present finding, that administration of IGF-I to sham-operated rats did not stimulate muscle protein synthesis or inhibit protein breakdown, was surprising and is in conflict with several previous studies in rats. It should be noted, however, that in most previous experiments in which IGF-I stimulated basal muscle protein synthesis (including using studies from our laboratory), the tissue was treated with the hormone in vitro (11, 13, 30, 31). Where rats were infused in vivo with IGF-I, using an identical protocol as used here, basal muscle protein synthesis rates were not affected (12). In contrast, acute iv administration of IGF-I (60 min) in rats resulted in increased muscle protein synthesis determined by a flooding dose of phenylalanine (32). Although plasma amino acids were not measured in that study, it may be speculated that the treatment period was too short to result in hypoaminoacidemia, whereas in the present and in our previous report (12), infusion of the hormone reduced plasma amino acids. A recent study by Fryburg et al. (33) suggests that the anabolic effects of IGF-I may be prevented by hypoaminoacidemia.

Another reason why the present and some of the previous reports, with regard to the effect of IGF-I on basal muscle protein turnover rates, are apparently conflicting may be the potential role of IGF-I binding proteins (34). Although binding proteins may be produced by muscles in vivo, it is possible that long-term administration of IGF-I in vivo results in greater changes in IGF-I binding proteins than short-term treatment in vivo or in vitro.

It may be argued that the lack of effect of IGF-I on muscle protein breakdown noted here in septic rats reflected an insufficient amount of the hormone administered, rather than hormone resistance. Though this may be true, it should be noted that an identical regimen of hormone administra-

TABLE 3. Plasma amino acids in sham-operated and septic rats treated with saline or IGF-I

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>IGF-I</th>
<th>CLP</th>
<th>IGF-I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>IGF-I</td>
<td>Saline</td>
<td>IGF-I</td>
</tr>
<tr>
<td>Valine</td>
<td>102 ± 2</td>
<td>34 ± 2a</td>
<td>87 ± 4b</td>
<td>45 ± 3a</td>
</tr>
</tbody>
</table>

Results are given as mg/dl. n = 7 in each group.

a P < 0.05 vs. saline.
b P < 0.05 vs. corresponding sham group.

and degradation by IGF-I, as indicated by the present and a recent study (13), is consistent with the concept that different intracellular signaling pathways mediate the hormonal effects on muscle protein synthesis and breakdown. A similar conclusion was reached in a previous report in which we found that the stimulation of protein synthesis by insulin was not affected by sepsis, whereas inhibition of protein breakdown in septic muscle required higher concentrations of insulin than in nonseptic muscle (2, 29). The results in the present study and in a recent in vitro study (13) suggest that muscles become completely unresponsive to IGF-I during sepsis. This differs from other experiments in which insulin inhibited protein breakdown in septic muscle, although to a much smaller degree than in nonseptic muscle (2, 29). Thus, the degree of resistance to IGF-I and insulin in septic muscle may differ between the two hormones. The mechanism of the sepsis-induced resistance in skeletal muscle to IGF-I is not known from the present study, and it is not known whether the same mechanism accounts for the postreceptor IGF-I and insulin resistance. It should be noted that insulin resistance and IGF-I resistance do not always occur simultaneously. For example, muscle glucose uptake during sepsis is resistant to insulin but not to IGF-I (28), suggesting that different mechanisms account for the resistance to the two hormones.
Immediately after CLP, to prevent hypoglycemia.

The septic rats treated with IGF-I received glucose (1.2 g/rat), immediately after dissection. It should be noted, however, that different catabolic conditions may influence the responsiveness to IGF-I in skeletal muscle differently, with sepsis giving rise to hormone resistance of protein degradation (Ref. 13 and present study), renal failure resulting in resistance of both protein synthesis and breakdown (35), and burn injury not giving rise to hormone resistance at all (11, 12). These observations suggest that treatment with IGF-I of patients with muscle cachexia needs to be tailored specifically to the cause of the catabolic condition. The results may also explain why, in some clinical studies, administration of IGF-I improved protein balance (39, 40); whereas, in other studies, the hormone had no beneficial effect (41, 42).

The reduced mRNA levels for ubiquitin and the ubiquitin-conjugating enzyme E214k, in muscles of septic rats treated with IGF-I, were surprising, in light of the unchanged protein breakdown rates in the same muscles. In several previous studies, muscle protein breakdown rates and mRNA levels for ubiquitin and other components of the ubiquitin-proteasome proteolytic pathway were up- or down-regulated in parallel (43). It should be noted, however, that there is not an absolute correlation between steady-state levels of mRNA for proteolytic enzymes or other components of proteolytic pathways and the actual proteolytic activity in that pathway (44). The present observations of unchanged protein breakdown rates and reduced mRNA levels for ubiquitin and E214k in muscles from septic hormone-treated rats, therefore, are not necessarily contradictory. The mechanism(s) of reduced mRNA levels for ubiquitin and E214k in hormone-treated septic rats is not known from the present study, but the results may reflect increased breakdown of mRNA (45), in addition to inhibited gene transcription. Regardless of the mechanism, the observations are important because they suggest that changes in mRNA levels for ubiquitin and E214k do not always reflect changes in muscle protein breakdown rates.

In contrast to the situation in sepsis and chronic renal failure, burn injury [another condition characterized by muscle cachexia (37, 38)] is not associated with resistance to IGF-I. Thus, in recent studies, we found that treatment of burned rats in vivo (12) or of muscles from burned rats in vitro (11) with IGF-I stimulated protein synthesis and inhibited protein breakdown in a dose-dependent fashion and the effect of the hormone on protein synthesis were even more pronounced in muscle from burned, than from nonburned, rats.

Because hypoglycemia stimulates the secretion of glucagon and epinephrine (26, 27), it is possible that hypoglycemia-associated hormonal changes offset the anabolic effects of IGF-I. However, the present observation that IGF-I did not reduce muscle protein breakdown in septic rats, even after supplementation with a large amount of glucose, suggests that the lack of effect of IGF-I on muscle proteolysis during sepsis does not reflect an insufficient amount of the hormone. More important, the hypoglycemia that is associated with administration of IGF-I is a limiting factor (25). Severe hypoglycemia developed with the rate of IGF-I in vitro, protein degradation was not inhibited by the hormone in septic muscle, even at hormone concentrations approximately five times normal plasma concentrations (13), further supporting the concept that the lack of effect of IGF-I on muscle proteolysis during sepsis does not reflect an insufficient amount of the hormone. More important, the hypoglycemia that is associated with administration of IGF-I is a limiting factor (25). Severe hypoglycemia developed with the rate of IGF-I administration used in the present experiments and administration of an even larger amount of the hormone would probably not be clinically relevant.

Because hypoglycemia stimulates the secretion of glucagon and epinephrine (26, 27), it is possible that hypoglycemia-associated hormonal changes offset the anabolic effects of IGF-I. However, the present observation that IGF-I did not reduce muscle protein breakdown in septic rats, even after supplementation with a large amount of glucose, suggests that the lack of effect of IGF-I on muscle proteolysis was not caused by hypoglycemia alone.

In addition to sepsis, the effect of IGF-I on muscle protein metabolism has been tested in other catabolic conditions, as well. In an experimental model of chronic renal failure, muscles were resistant to IGF-I, with respect to both protein synthesis and degradation (35). In the same report, evidence was found for impaired autophosphorylation of the IGF-I receptor β-subunit and decreased activity of the IGF-I receptor tyrosine kinase toward insulin receptor substrate-1. The same group reported impaired metabolic response to IGF-I in patients with chronic renal failure (36).

In contrast to the situation in sepsis and chronic renal failure, burn injury [another condition characterized by muscle cachexia (37, 38)] is not associated with resistance to IGF-I. Thus, in recent studies, we found that treatment of burned rats in vivo (12) or of muscles from burned rats in vitro (11) with IGF-I stimulated protein synthesis and inhibited protein breakdown in a dose-dependent fashion and the effect of the hormone on protein synthesis were even more pronounced in muscle from burned, than from nonburned, rats.

It is obvious, then, that different catabolic conditions may influence the responsiveness to IGF-I in skeletal muscle differently, with sepsis giving rise to hormone resistance of protein degradation (Ref. 13 and present study), renal failure resulting in resistance of both protein synthesis and breakdown (35), and burn injury not giving rise to hormone resistance at all (11, 12). These observations suggest that treatment with IGF-I of patients with muscle cachexia needs to be tailored specifically to the cause of the catabolic condition. The results may also explain why, in some clinical studies, administration of IGF-I improved protein balance (39, 40); whereas, in other studies, the hormone had no beneficial effect (41, 42).

The reduced mRNA levels for ubiquitin and the ubiquitin-conjugating enzyme E214k, in muscles of septic rats treated with IGF-I, were surprising, in light of the unchanged protein breakdown rates in the same muscles. In several previous studies, muscle protein breakdown rates and mRNA levels for ubiquitin and other components of the ubiquitin-proteasome proteolytic pathway were up- or down-regulated in parallel (43). It should be noted, however, that there is not an absolute correlation between steady-state levels of mRNA for proteolytic enzymes or other components of proteolytic pathways and the actual proteolytic activity in that pathway (44). The present observations of unchanged protein breakdown rates and reduced mRNA levels for ubiquitin and E214k in muscles from septic hormone-treated rats, therefore, are not necessarily contradictory. The mechanism(s) of reduced mRNA levels for ubiquitin and E214k in hormone-treated septic rats is not known from the present study, but the results may reflect increased breakdown of mRNA (45), in addition to inhibited gene transcription. Regardless of the mechanism, the observations are important because they suggest that changes in mRNA levels for ubiquitin and E214k do not always reflect changes in muscle protein breakdown rates.

It may be argued that comparisons between protein breakdown rates and mRNA levels cannot be done under the present experimental conditions because protein breakdown rates were determined in muscles incubated for a total of 2.5 h (30 min preincubation and 2 h incubation), whereas mRNA levels were measured in muscles that were frozen immediately after dissection. It should be noted, however, that although muscles were incubated for a total of 2.5 h, the protein turnover rates did not reflect the metabolic activity only at the end of incubation but reflected the protein synthesis and breakdown rates during the 2-h incubation period. With the present in vitro technique, protein turnover rates are constant during incubation for at least 2 h in most conditions (16). Thus, the protein breakdown rate is basically the same at the start and end of incubation, and comparisons between
protein breakdown rates (determined with the present in vitro technique) and mRNA levels for ubiquitin and E2 14k, therefore, are valid.

Changes in plasma amino acids have been frequently used as an indicator of changes in muscle protein breakdown rates during sepsis and other catabolic conditions (22–24). The present results, however, strongly suggest that changes in plasma amino acids do not only reflect changes in muscle proteolysis, at least not under the present experimental conditions. Although it may be argued that the changes in plasma amino acids reflected protein turnover rates in muscles other than the extensor digitorum longus muscle, this is less likely because we have found previously that sepsis mainly induces a catabolic response in white, fast-twitch skeletal muscle (e.g. extensor digitorum longus muscle), with only minor changes noted in other types of muscle (2, 6).

Changes in plasma amino acids may be caused by a number of different factors in addition to changes in muscle protein turnover rates, such as changes in metabolism of the individual amino acids, tissue uptake or release or urinary excretion of amino acids, and changes in protein or amino acid metabolism in organs and tissues other than skeletal muscle. Despite the fact that changes in plasma amino acids may be rather nonspecific, the present observations are important because they suggest that the amount of hormone administered was sufficient to block some of the sepsis-induced changes in protein or amino acid metabolism, resulting in changes in plasma amino acid levels. Further studies are needed to define which metabolic alteration(s) caused the changes in plasma amino acids noted here.

In summary, the present results suggest that administration of IGF-I may improve sepsis-induced muscle cachexia by stimulating protein synthesis. However, because muscles were resistant to IGF-I, with regard to the regulation of protein breakdown, the use of IGF-I to treat muscle cachexia during sepsis remains unclear. Because at least some of the mechanisms of sepsis-induced muscle proteolysis are similar in rats and humans (7), it is possible that muscle proteolysis becomes resistant to IGF-I in septic patients also, although further studies are needed to test that notion. It will be important, in the future, to determine the intracellular mechanisms of the unresponsiveness to IGF-I in skeletal muscle during sepsis.

References

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