Treatment of burned rats with insulin-like growth factor I inhibits the catabolic response in skeletal muscle

CHENG-HUI FANG, BING-GUO LI, JING JING WANG, JOSEF E. FISCHER, AND PER-OLOF HASSELGREN
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Fang, Cheng-Hui, Bing-Guo Li, Jing-Jing Wang, Josef E. Fischer, and Per-Olof Hasselgren. Treatment of burned rats with insulin-like growth factor I inhibits the catabolic response in skeletal muscle. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1091–R1098, 1998.—Thermal injury is associated with a pronounced catabolic response in skeletal muscle, reflecting both reduced protein synthesis and stimulated protein breakdown. Administration of insulin-like growth factor I (IGF-I) has a nitrogen-sparing effect after burn injury, but the influence of this treatment on protein turnover rates in skeletal muscle is not known. In the present study, we examined the effect of IGF-I on muscle protein synthesis and breakdown rates following burn injury in rats. After a 30% total body surface area burn injury or sham procedure, rats were treated with a continuous infusion of IGF-I (3.5 or 7 mg·kg⁻¹·24 h⁻¹) for 24 h. Protein synthesis and breakdown rates were determined in incubated extensor digitorum longus muscles. Burn injury resulted in increased total and myofibrillar protein breakdown rates and reduced protein synthesis in muscle. The increase in protein breakdown rates was blocked by both doses of IGF-I and the burn-induced inhibition of muscle protein synthesis was partially reversed by the higher dose of the hormone. IGF-I did not influence muscle protein turnover rates in nonburned rats. The results suggest that the catabolic response to burn injury in skeletal muscle can be inhibited by IGF-I.

BURN INJURY IS ASSOCIATED with a pronounced catabolic response in skeletal muscle, reflecting both reduced protein synthesis and stimulated protein breakdown, in particular myofibrillar protein breakdown (7, 15, 18). Because current nutritional support regimens have not been able to completely reverse net protein catabolism or block the accelerated rate of protein breakdown in patients with thermal injury, additional treatment modalities have been tested. In early studies, evidence was found that growth hormone may improve nitrogen balance in patients with thermal injury (35, 45). Because there is evidence that growth hormone exerts its anabolic effects by inducing release of insulin-like growth factor I (IGF-I) from the liver (2) and that critically ill patients may become resistant to growth hormone (9), IGF-I may be a more attractive candidate to prevent the catabolic response to burn injury. The potential use of IGF-I was further supported by previous reports of decreased circulating IGF-I levels in animals (42) and humans (6, 30) after burn injury. In addition, in previous studies, IGF-I exerted an anabolic effect in cultured muscle cells (38, 44), improved wound healing, and reduced gut atrophy and bacterial translocation following burn injury in rats (29, 42), consistent with a role of IGF-I in the treatment of burn-induced catabolism.

In a recent report by Cioffi et al. (6), administration of IGF-I to patients with thermal injury reduced whole body protein oxidation, indicating a nitrogen-sparing effect, but the specific influence of IGF-I on protein synthesis and breakdown rates in muscle was not determined in that study. We recently found that IGF-I in vitro stimulated protein synthesis and inhibited protein breakdown in incubated muscles from burned rats (20). Although these reports suggest that IGF-I may inhibit the catabolic response in skeletal muscle following thermal injury, the effects of IGF-I administered in vivo on muscle protein synthesis and breakdown rates have not been reported. In the present study, burned rats were treated with a continuous infusion of IGF-I, whereafter muscle protein synthesis and breakdown rates were measured. The burn-induced inhibition of protein synthesis and increase in total and myofibrillar protein breakdown rates were reversed in IGF-I-treated rats, supporting the concept that IGF-I can block the catabolic response in skeletal muscle following thermal injury.

MATERIALS AND METHODS

A 30% total body surface area full-thickness flame burn injury was inflicted on the back of male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 50–60 g as described in detail previously (18). Other rats underwent sham procedure; i.e., they were anesthetized with pentobarbital sodium (35 mg/kg ip) and the hair on the back was shaved but no burn injury was inflicted. All rats were resuscitated with 10 ml/100 g body wt of normal saline administered intraperitoneally immediately before burn or sham procedure. Small rats were used because muscles from rats of this size are thin enough to allow for in vitro incubation and measurement of protein synthesis and breakdown rates (18, 23, 25). This experimental burn model has been used in several previous reports from our laboratory and typically results in reduced protein synthesis and increased total and myofibrillar protein breakdown in muscle tissue (18, 20, 21), similar to the catabolic response seen in patients with burn injury.

Immediately after burn or sham procedure, miniosmotic pumps (model 2001D; Alzet, Palo Alto, CA) were implanted subcutaneously on the neck of the rats. The pumps were filled with saline or recombinant human IGF-I (kindly provided by Genentech, South San Francisco, CA, and Pharmacia, Stockholm, Sweden) dissolved in saline at concentrations sufficient...
to deliver 3.5 or 7 mg·kg⁻¹·24 h⁻¹. 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 (1.06 or 2.12 mg/ml), the influence of adherence 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 saline at 37°C for 2 h before the subcutaneous implantation. The rats were randomly assigned to one of four groups: 1) sham + saline, 2) sham + IGF-I, 3) burn + saline, or 4) burn + IGF-I. The rats were housed individually with free access to water. All rats were fed an equal amount of chow (10 g/100 g body wt) based on a previous study in which rats consumed 10 g/100 g body wt during the first 24 h after burn injury using an experimental protocol identical to the present one (18). This experimental design resulted in groups of sham-burned rats that were "pair fed" with groups of burned rats although individual sham-burned rats were not pair fed with individual burned rats. All food was consumed in all groups, and food intake was not influenced by administration of IGF-I. Twenty-four hours after burn injury or sham procedure, the rats were anesthetized with pentobarbital sodium (35 mg/kg ip) and the extensor digitorum longus muscles were harvested for measurement of protein synthesis and breakdown rates and ubiquitin mRNA levels (see below). Blood was collected by heart puncture in a heparinized syringe for determination of plasma glucose, amino acids, and IGF-I levels. Plasma glucose was measured by a colorimetric assay using Vitros GLU Slides (J. Johnson & J. Johnson Clinical Diagnostics, Rochester, NY) and amino acids in an amino acid analyzer (Beckman 6300; Beckman Instruments, Palo Alto, CA). Plasma levels of human and rat IGF-I were measured separately by an immunoradiometric assay (DSL-5600 ACTIVE Insulin-Like Growth Factor-I coated-Tube IRMA Kit; Diagnostic Systems Laboratories, Webster, TX) and radioimmunoassay (DSL-2900 Rat IGF-I Radioimmunoassay Kit, Diagnostic Systems Laboratories, respectively).

All rats were cared for in accordance with the National Research Council’s Guide for the Care and Use of Laboratory Animals, and the experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of Cincinnati.

Muscle incubations. The extensor digitorum longus 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 of oxygenated (95% O₂-5% CO₂) 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 (1, 25).

For measurement of protein synthesis rate, muscles were transferred to 3 ml of 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%) precipitable amino acids during incubation into account as described in detail previously (18).

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 N perchloric acid (PCA) 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 N PCA.

Muscles and media were stored at −20°C until tyrosine and 3-MH were assayed by high-performance liquid chromatography. Total and myofibrillar protein breakdown rates were determined as net production of free tyrosine and 3-MH, respectively, taking changes in tissue levels of the amino acids during incubation into account as described in detail previously (18, 20, 21, 25).

Northern blot analysis. Because in a recent study the burn-induced muscle proteolysis reflected increased ubiquitin-dependent protein breakdown and was associated with increased muscle levels of ubiquitin mRNA (21), we examined the expression of ubiquitin mRNA by Northern blot analysis. Extensor digitorum longus muscles were harvested from burned rats treated with saline or IGF-I as described above and were immediately frozen in liquid nitrogen and stored at −70°C until analysis. Total RNA was extracted by the guanidinium thiocyanate-phenol-chloroform method (5) using a RNA STAT-60 kit (Tel-Test, Friendswood, TX). Total RNA, 10 µg from each sample, was denatured and separated by electrophoresis on 1% agarose gel containing formaldehyde. The RNA was transferred from the gel to nylon membranes (MSI, Westboro, MA) by capillary action in 20× SSC (1× SSC = 0.15 M NaCl, 15 mM Na-citrate) overnight. RNA was immobilized either by baking in an 80°C oven for 2 h or by ultraviolet cross-linking. The blots were prehybridized at 42°C for 4 h in 50% formaldehyde and 6× SSPE (1× SSPE = 0.15 M NaCl, 10 mM Na₂HPO₄, 1 M EDTA, 5× Denhardt’s solution, 0.5% SDS, and 100 µg/ml salmon sperm DNA) and the 32P-labeled cDNA probes for ubiquitin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were labeled by random priming with [α-32P]dATP or [γ-32P]dCTP (Stratagene, LaJolla, CA). After the 4-h prehybridization, the 32P-labeled cDNA probe was added and the blots were hybridized at 42°C overnight. The blots were then washed twice in 1× SSC, 0.1% SDS, and once in 0.1× SSC, 0.1% SDS, at room temperature and autoradiographed at −70°C. All blots were rehybridized with 32P-labeled GAPDH cDNA probe to control for equal loading of RNA. Blots were quantitated on a Phosphoimager using the Image Quant Program (Molecular Dynamics, Sunnyvale, CA), and the relative mRNA abundance was expressed as the ratio between ubiquitin mRNA and GAPDH mRNA. The ubiquitin probe used here was obtained as described previously from our laboratory (19). The GAPDH probe was purchased from American Type Culture Collection, Rockville, MD.

Statistics. Results are presented as means ± SE. Statistical comparisons were done by Student’s t-test or ANOVA followed by Duncan’s test as indicated in the legends to Tables 1–3 and Figs. 1–5.

RESULTS

Muscle protein turnover. Two series of experiments were performed. In the first series of experiments, rats were treated with 3.5 mg·kg⁻¹·24 h⁻¹ of IGF-I. This regimen was based on previous reports in which IGF-I improved wound healing and mucosal integrity following burn injury (29, 42). Treatment of rats with 3.5 mg·kg⁻¹·24 h⁻¹ of IGF-I did not influence mortality rate following burn injury, which was 12 of 65 (18%) in saline-treated burned rats and 12 of 69 (17%) in IGF-I-treated burned rats. Except for one anesthesia-related death each in saline- and IGF-I-treated ani-
mals, there was no mortality in rats undergoing sham procedure.

As expected (18, 20, 21), burn injury resulted in increased total and myofibrillar muscle protein breakdown (Fig. 1). This increase in muscle proteolysis was blocked in rats treated with 3.5 mg·kg⁻¹·24 h⁻¹ of IGF-I (Fig. 1). Muscle protein breakdown rates in nonburned rats were not affected by administration of the hormone.

In addition to stimulated muscle protein breakdown, the catabolic response in skeletal muscle following burn injury reflects inhibited protein synthesis as well (18). We next examined the effect of IGF-I on the burn-induced inhibition of muscle protein synthesis. Treatment of rats with 3.5 mg·kg⁻¹·24 h⁻¹ of IGF-I did not significantly influence muscle protein synthesis in nonburned rats but were increased almost threefold when the rate of IGF-I infusion was increased from 3.5 to 7 mg·kg⁻¹·24 h⁻¹ (Table 1). Interestingly, plasma levels of rat IGF-I were reduced in animals treated with human IGF-I and total IGF-I levels were increased only in rats treated with the hormone at a rate of 7 mg·kg⁻¹·24 h⁻¹. With this regimen, the burn-induced increase in total and myofibrillar protein breakdown rates was again inhibited (Fig. 3). Protein synthesis in muscles from burned rats was significantly increased following treatment with 7 mg·kg⁻¹·24 h⁻¹ of IGF-I but did not reach the synthesis rate seen in muscles from nonburned rats (Fig. 4). Similar to the first series of experiments, protein synthesis and breakdown rates were not affected by IGF-I in muscles from nonburned rats (Figs. 3 and 4).

Ubiquitin mRNA. Burn-induced muscle proteolysis is at least in part caused by increased ubiquitin-dependent protein breakdown and is associated with upregulated expression of ubiquitin mRNA (21). Northern blot analysis showed that ubiquitin mRNA levels in muscles from burned rats were reduced by 7 mg·kg⁻¹·24 h⁻¹ of IGF-I (Fig. 5). The effect of 3.5 mg·kg⁻¹·24 h⁻¹ of IGF-I on ubiquitin mRNA levels was not tested in the present study.

Table 1. Plasma levels of rat and human IGF-I in groups of burned rats infused with saline or recombinant human IGF-I at a rate of 3.5 or 7 mg·kg⁻¹·24 h⁻¹

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rat IGF-I</th>
<th>Human IGF-I</th>
<th>Total IGF-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>386 ± 31</td>
<td>ND</td>
<td>386 ± 31</td>
</tr>
<tr>
<td>IGF-I (3.5 mg·kg⁻¹·24 h⁻¹)</td>
<td>167 ± 7*</td>
<td>132 ± 15</td>
<td>299 ± 17</td>
</tr>
<tr>
<td>IGF-I (7 mg·kg⁻¹·24 h⁻¹)</td>
<td>163 ± 3*</td>
<td>341 ± 33†</td>
<td>504 ± 33†</td>
</tr>
</tbody>
</table>

Results are means ± SE in nanograms per milliliter; n = 7 for each group. IGF-I, insulin-like growth factor I; ND, not detectable (the lower limit for detection of human IGF-I was 5 ng/ml). *P < 0.05 vs. saline. †P < 0.05 vs. IGF-I, 3.5 mg·kg⁻¹·24 h⁻¹.
Plasma amino acids. Burn injury is typically associated with changes in plasma amino acids, some of which reflect the catabolic response in skeletal muscle (41). Changes in plasma amino acids following burn injury and the influence of treatment with 7 mg·kg⁻¹·24 h⁻¹ of IGF-I are shown in Table 2. Burn resulted in decreased total plasma amino acid concentrations caused by a substantial reduction of glutamine and reduced levels of other amino acids as well, including glycine, lysine, asparagine, arginine, citrulline, and serine. The plasma concentrations of several amino acids that are usually considered to reflect muscle protein breakdown (27, 41) were elevated in burned rats, including phenylalanine, tyrosine, histidine, and

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Sham (n=7)</th>
<th>Burn (n=8)</th>
<th>Saline (n=5)</th>
<th>IGF-I (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valine</td>
<td>103±7</td>
<td>88±5</td>
<td>183±14</td>
<td>111±15</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>69±5</td>
<td>46±4*</td>
<td>93±11</td>
<td>56±7*</td>
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<tr>
<td>Leucine</td>
<td>95±6</td>
<td>92±6</td>
<td>162±14</td>
<td>97±10*</td>
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<tr>
<td>Tyrosine</td>
<td>43±2</td>
<td>29±2*</td>
<td>52±3</td>
<td>40±4*</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>44±2</td>
<td>60±2</td>
<td>80±4</td>
<td>67±4*</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>66±3</td>
<td>42±2*</td>
<td>69±4</td>
<td>69±6</td>
</tr>
<tr>
<td>Glutamine</td>
<td>707±30</td>
<td>371±22*</td>
<td>190±52</td>
<td>343±31*</td>
</tr>
<tr>
<td>Glutamate</td>
<td>73±9</td>
<td>25±3*</td>
<td>59±7</td>
<td>34±5*</td>
</tr>
<tr>
<td>Threonine</td>
<td>191±12</td>
<td>132±6*</td>
<td>155±18</td>
<td>135±11</td>
</tr>
<tr>
<td>Glycine</td>
<td>606±29</td>
<td>342±9*</td>
<td>324±40</td>
<td>267±22</td>
</tr>
<tr>
<td>Lysine</td>
<td>422±22</td>
<td>358±29</td>
<td>261±23</td>
<td>211±13</td>
</tr>
<tr>
<td>Alanine</td>
<td>452±21</td>
<td>163±12*</td>
<td>356±56</td>
<td>381±67</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>16±1</td>
<td>9±0.3*</td>
<td>14±1</td>
<td>10±1*</td>
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<tr>
<td>Asparagine</td>
<td>40±4</td>
<td>14±1*</td>
<td>16±7</td>
<td>25±4</td>
</tr>
<tr>
<td>Histidine</td>
<td>87±2</td>
<td>98±5</td>
<td>120±7</td>
<td>101±5</td>
</tr>
<tr>
<td>Arginine</td>
<td>144±6</td>
<td>65±6*</td>
<td>93±20</td>
<td>74±21</td>
</tr>
<tr>
<td>Cysteine</td>
<td>12±1</td>
<td>6±1*</td>
<td>15±1</td>
<td>13±1</td>
</tr>
<tr>
<td>Citrulline</td>
<td>140±4</td>
<td>96±4*</td>
<td>66±3</td>
<td>66±5</td>
</tr>
<tr>
<td>Ornithine</td>
<td>57±3</td>
<td>38±3</td>
<td>81±13</td>
<td>78±13</td>
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<tr>
<td>Serine</td>
<td>282±12</td>
<td>156±4*</td>
<td>202±34</td>
<td>172±16</td>
</tr>
<tr>
<td>Proline</td>
<td>158±15</td>
<td>53±13*</td>
<td>99±40</td>
<td>110±30</td>
</tr>
<tr>
<td>Methionine</td>
<td>45±2</td>
<td>27±1*</td>
<td>61±9</td>
<td>62±5</td>
</tr>
</tbody>
</table>

Results are means ± SE in micromoles per liter. *P < 0.05 vs. saline within the same group. †P < 0.05 vs. saline in sham group by ANOVA.
that IGF-I-induced hypoglycemia did not cause any
between saline- and IGF-I-treated burned rats suggests
burned rats and no difference in mortality rates be-
two nonburned rats as described above) among non-
was no mortality (except anesthesia-related deaths in
any rats died from hypoglycemia. The fact that there
sham procedure or burn injury, it is not known whether
any rats died from hypoglycemia. The fact that there
was no mortality (except anesthesia-related deaths in
two nonburned rats as described above) among non-
burned rats and no difference in mortality rates be-
tween saline- and IGF-I-treated burned rats suggests
that IGF-I-induced hypoglycemia did not cause any
deaths.

DISCUSSION

In the present study, treatment of rats with IGF-I
blocked the burn-induced increase in muscle total
and myofibrillar protein breakdown and partially reversed
the reduction of muscle protein synthesis seen follow-
ing burn injury. The burn-induced increases in plasma
levels of tyrosine, phenylalanine, and branched-chain
amino acids were also reversed by treatment with
IGF-I, lending further support to the concept that
muscle protein breakdown after burn injury may be
blocked by this hormone. The results suggest that
IGF-I may be useful to reduce the catabolic response to
thermal injury, although potential side effects, such as
hypoglycemia, need to be taken into account during
treatment with the hormone. It should also be noted
that the present experiments lasted for only 24 h and
the influence of long-term treatment with IGF-I on
burn-induced muscle catabolism remains to be deter-
mined.

Table 3. Plasma glucose levels in nonburned (sham)
and burned rats treated with saline or IGF-I

<table>
<thead>
<tr>
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<th>Sham</th>
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<tr>
<td>Saline</td>
<td>107 ± 3</td>
<td>128 ± 4*</td>
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<tr>
<td>IGF-I (3.5 mg·kg⁻¹·24 h⁻¹)</td>
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<td>107 ± 4†</td>
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<tr>
<td>Experiment 2</td>
<td></td>
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Results are means ± SE in milligrams per deciliter; n = 7 or 8 in
each group. *P < 0.05 vs. sham. †P < 0.05 vs. saline by ANOVA.

The rat has served as a useful model to study the
effects of IGF-I (11, 49). Because of the lack of commer-
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studies have often involved the administration of hu-
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infused with human IGF-I, determination of plasma
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IGF-I. The finding that total IGF-I levels were not
increased in rats that received 3.5 mg·kg⁻¹·24 h⁻¹ of
the hormone was surprising considering the fact that
muscle protein breakdown was reduced in these rats.
We cannot offer any explanation for this result at
present, but it may be speculated that some of the
human IGF-I was rapidly cleared from the plasma and
taken up by muscle tissue, where it exerted its biologi-
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probably reflected changes in protein metabolism in other tissues.

To our knowledge, this is the first study in which muscle protein turnover rates were measured after burn injury and treatment in vivo with IGF-I. Cioffi et al. (6) measured whole body protein kinetics after a 3-day infusion of IGF-I in patients with thermal injury and found that protein oxidation, estimated from the rate of lysine oxidation, was significantly reduced by the hormone. The IGF-I infusion also inhibited whole body protein degradation, assessed from the rate of appearance of endogenous lysine in plasma, and improved nitrogen balance, although these effects were not statistically significant. Because no measurements of protein turnover rates were performed in individual tissues, the influence of IGF-I on protein balance in skeletal muscle is not known from this study.

In addition to burn-induced catabolism, there is evidence that IGF-I may influence the response to other catabolic conditions as well, including fasting, cancer, sepsis, and treatment with glucocorticoids or tumor necrosis factor (13, 14, 28, 31, 36, 47). A recent study in which healthy human volunteers were infused with IGF-I suggests that the hormone may also exert anabolic effects under noncatabolic conditions (39). It should be noted that the beneficial effects of IGF-I may not be universal to all catabolic conditions. For example, in recent studies, IGF-I did not improve nitrogen balance or protein degradation, determined as 3-MH excretion, in postoperative patients after gastrectomy or colon resection (22, 34, 40). In recent studies, we found evidence that muscle protein breakdown becomes resistant to IGF-I during sepsis (28). Thus the influence of IGF-I on muscle protein turnover probably varies with the underlying cause of muscle catabolism. Alternatively, the apparently contradicting results in previous reports may reflect differences in dose and method of IGF-I administration. In the present study and in the report by Cioffi et al. (6), beneficial effects were noted following continuous infusion of IGF-I. In contrast, the hormone was administered as two daily subcutaneous injections in postoperative patients with no improvement of nitrogen balance (22, 34, 40). The total daily dose of IGF-I used in the study by Cioffi et al. (6) was ~34 mg, whereas in the postoperative patients the total dose was ~11 mg (22, 34, 40). Further studies are needed to determine the role of continuous infusion versus repeated injections and the influence of the dose of IGF-I to prevent the catabolic response in skeletal muscle. Results in the present study suggest that at least the effect of IGF-I on muscle protein synthesis following thermal injury is dose dependent.

In a recent study from our laboratory, treatment of incubated muscles from burned rats with IGF-I in vitro stimulated protein synthesis and inhibited protein breakdown in a dose-dependent fashion (20). Thus it is possible that the anabolic effects of IGF-I noted in the present study were caused by a direct effect of the hormone on muscle tissue. Potential indirect effects of IGF-I on muscle protein turnover need to be considered as well. For example, in previous studies, administration of IGF-I increased circulating levels of IGF-I binding proteins (8), and although the binding proteins typically limit the bioavailability of the hormone, the IGF-I binding protein 3 may potentiate the action of IGF-I under certain circumstances (48). Other potential indirect effects of IGF-I that may influence muscle protein balance include the interaction between IGF-I and insulin (24), growth hormone (32), and proinflammatory cytokines (17).

The finding that infusion of 7 mg·kg⁻¹·24 h⁻¹ of IGF-I did not completely normalize muscle protein synthesis rates in burned rats may reflect the need for an even higher dose of the hormone to fully reverse the catabolic response. We did not examine the effect of a higher dose of IGF-I than 7 mg·kg⁻¹·24 h⁻¹ because of the potential risk of hypoglycemia. Furthermore, the increase in muscle protein breakdown, which is the predominant component of burn-induced muscle catabolism (18), was completely blocked by both doses of the hormone tested in this study.

The mechanism(s) by which IGF-I inhibits burn-induced muscle protein breakdown are not known from the present study. In a recent report, IGF-I stimulated the degradation of a mRNA transcript encoding the 14-kDa ubiquitin-conjugating enzyme (46), suggesting that IGF-I may inhibit ubiquitin-dependent muscle proteolysis. This is particularly important considering the role of the ubiquitin-proteasome pathway in muscle protein breakdown after burn injury (21). The present finding of reduced ubiquitin mRNA levels in muscles from IGF-I-treated burned rats supports the concept that the hormone may inhibit ubiquitin-dependent muscle proteolysis. Further studies are needed to better define the cellular mechanisms of inhibited muscle protein breakdown by IGF-I following burn injury and to determine whether proteolytic pathways other than the ubiquitin pathway are influenced by the hormone.

The mechanisms of IGF-I-induced increase in muscle protein synthesis following burn injury remain to be determined as well. Potential mechanisms include stimulated glucose and amino acid uptake (10, 12, 16) and increased cellular glutamine levels, secondary to inhibited efflux of glutamine from skeletal muscle (37). In muscle from septic rats, IGF-I reduced the abundance of free 40S and 60S ribosomal subunits, suggesting that protein synthesis was increased through a stimulation of peptide-chain initiation (31). Previous studies suggest that the anabolic effects of IGF-I in hepatocytes may be mediated by cell swelling (26). Recent experiments in our laboratory, however, suggest that the anabolic effects of IGF-I in muscle are independent of cell swelling (19).

The finding that administration of IGF-I did not influence muscle protein metabolism in sham-burned rats suggests that IGF-I exerted its anabolic effect mainly by inhibiting elevated protein breakdown rates and by stimulating reduced protein synthesis rates under the present experimental conditions. The results should not be interpreted as indicating that IGF-I does not influence basal muscle protein turnover rates because several previous reports provided evidence that
the hormone stimulates protein synthesis and inhibits protein breakdown in muscle cells under basal conditions (38, 44). This was further supported by a recent study in which we found that treatment in vitro of incubated muscles from both burned and nonburned rats with IGF-I stimulated protein synthesis and inhibited protein breakdown (20).

Perspectives

The present report is the first to provide evidence that administration of IGF-I in vivo can inhibit the catabolic response in skeletal muscle following thermal injury. The results suggest that the anabolic effect of IGF-I reflects both inhibited muscle protein breakdown and stimulated protein synthesis, although inhibition of protein breakdown may be the most important mechanism, at least under the present experimental conditions. Considering the pronounced catabolic response in burned patients, the results in this study may have important clinical implications. It will be important in future studies to determine whether the hormone can improve muscle protein balance following burn injury in humans as well.

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