Burn-induced increase in atrogin-1 and MuRF-1 in skeletal muscle is glucocorticoid independent but downregulated by IGF-I

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Lang CH, Huber D, Frost RA. Burn-induced increase in atrogin-1 and MuRF-1 in skeletal muscle is glucocorticoid independent but downregulated by IGF-I. Am J Physiol Regul Integr Comp Physiol 292: R328–R336, 2007. First published August 31, 2006; doi:10.1152/ajpregu.00561.2006.—The present study determined whether thermal injury increases the expression of the ubiquitin (Ub) E3 ligases referred to as muscle ring finger (MuRF)-1 and muscle atrophy F-box (MAFbx; aka atrogin-1), which are muscle specific and responsible for the increased protein breakdown observed in other catabolic conditions. After 48 h of burn injury (40% total body surface area full-thickness scald burn) gastrocnemius weight was reduced, and this change was associated with an increased mRNA abundance for atrogin-1 and MuRF-1 (3.1- to 8-fold, respectively). Similarly, burn increased polyUb mRNA content in the gastrocnemius twofold. In contrast, there was no burn-induced atrophy of the soleus and no significant change in atrogin-1, MuRF-1, or polyUb mRNA. Burns also did not alter E3 ligase expression in heart. Four hours after administration of the anabolic agent insulin-like growth factor (IGF)-I to burned rats, the mRNA content of atrogin-1 and polyUb in gastrocnemius had returned to control values and the elevation in MuRF-1 was reduced 50%. In contrast, leucine did not alter E3 ligase expression. In a separate study, in vivo administration of the proteasome inhibitor Velcade prevented burn-induced loss of muscle mass determined at 48 h. Finally, administration of the glucocorticoid receptor antagonist RU-486 did not prevent burn-induced atrophy of the gastrocnemius or the associated elevation in atrogin-1, MuRF-1, or polyUb mRNA. Burns increased polyUb mRNA content in the gastrocnemius twofold. In summary, acute muscle wasting accompanying thermal injury is associated with a glucocorticoid-independent increase in the expression of several Ub E3 ligases that can be downregulated by IGF-I.

muscle atrophy F-box; muscle wasting; leucine

EXTENSIVE THERMAL INJURY [e.g., >40% total body surface area (TBSA)] is a potent catabolic insult leading to a sustained hypermetabolic condition characterized by the erosion of lean body mass (61). When protracted, the depletion of both myofibrillar and sarcoplasmic protein leads to muscle weakness (14). Importantly, although this protein imbalance is observed acutely after injury it may persist for up to a year after the original burn insult (25), thereby impairing recovery. This wasting is associated with a greater risk for septic complications, longer hospital stays, and delayed rehabilitation (31, 59, 60). In general, the morbidity in this patient population is proportional to the magnitude of the catabolic response (25) and is considered a determinant of long-term patient outcomes (23). Tissue protein content in general and skeletal muscle protein in particular are maintained by the dynamic balance between rates of protein synthesis and degradation (36). Several catabolic insults, including sepsis, endotoxin, and excess inflammatory cytokines and glucocorticoids, alter both protein synthesis and degradation (16, 26, 34, 38, 39). In contrast, several laboratories have reported that burn-induced atrophy appears to result primarily from an elevated rate of muscle proteolysis (4, 11, 16, 30, 32, 39, 53, 59, 63). The etiology of this accelerated rate of protein degradation leading to negative nitrogen balance in burn injury is not completely understood.

Skeletal muscle contains multiple distinct pathways [e.g., lysosomal, calcium dependent, and ubiquitin (Ub)-proteasome dependent] responsible for regulating proteolysis (45), and, under certain experimental conditions, all of these pathways have been reported to be upregulated by thermal injury (11, 16, 17, 20). However, burn- and stress-induced changes in the Ub-proteasome proteolytic pathway are generally regarded as relatively more important in mediating the muscle cachexia observed after burn (27). In this regard, thermal injury to rats concomitantly increased muscle proteasome activity determined in vitro as well as the mRNA content of the 20 S proteasome α-subunits RC2 and RC3, the β-subunit RC7 (8, 17), and Ub (18, 19). Furthermore, the gene expression of the 14-kDa Ub-conjugating enzyme E214K is also increased in muscle from burned rats (8, 21). Finally, in vitro treatment of incubated muscles from burned rats with proteasome inhibitors reverses the increased rate of protein degradation (17); however, comparable studies have not yet been reported in which the proteasome inhibitor has been administered in vivo in an attempt to ameliorate the burn-induced muscle wasting.

Until recently the Ub-conjugating enzyme E2 and the Ub ligase E3α were considered the primary enzymes regulating muscle proteolysis in wasting states (28). However, a host of diverse conditions manifesting muscle atrophy, such as immobilization, denervation, and dexamethasone or inflammatory cytokine treatment, have all been reported to increase the gene expression of two additional E3 ligases—muscle ring finger (MuRF)-1 and muscle atrophy F-box (MAFbx; aka atrogin-1)—that are muscle specific (6). Furthermore, transgenic mice with null alleles for each gene individually display a robust resistance to denervation-induced atrophy (6), establishing an unequivocal in vivo role for MuRF-1 and atrogin-1 in promoting skeletal muscle degradation. Subsequent studies showed that diabetes, renal failure, as well as more traditional inflammatory conditions including cancer cachexia, peritonitis, and endotoxemia also increased expression of these “atrogenes”

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(13, 22, 44, 62). However, the response of atrogin-1 and MuRF-1 to burn injury has not been reported. Therefore, the purpose of the present study was to determine whether skeletal muscle atrogin-1 and MuRF-1 mRNA content is increased after burn and whether the resultant wasting observed can be ameliorated in vivo by treatment with the proteasome inhibitor Velcade (1, 43). Moreover, we examined the ability of the anabolic agents insulin-like growth factor (IGF)-I and leucine to acutely regulate mRNA expression of these genes as well as examining whether any burn-induced changes were glucocorticoid dependent.

**MATERIALS AND METHODS**

Animal preparation and experimental protocol. Adult specific pathogen-free male Sprague-Dawley rats (275–300 g for study 1 and 300–325 g for all other studies; Charles River Breeding Laboratories, Cambridge, MA) were housed at a constant temperature, exposed to a 12:12-h light-dark cycle, and maintained on standard rodent chow and water ad libitum for at least 1 wk before experiments were performed. All experiments were approved by the Animal Care and Use Committee at the Pennsylvania State University College of Medicine and adhered to the National Institutes of Health (NIH) guidelines for the use of experimental animals.

Rats in all studies were deeply anesthetized with an intraperitoneal injection of pentobarbital sodium (60–75 mg/kg body wt). The hair on the dorsal and ventral surfaces of the animal was closely clipped, and animals were secured in an insulated template that exposed only the area of skin to be injured, as originally reported by Walker and Mason (58) and described and characterized by our laboratory (39–42). For the burn group, the surface of the skin exposed through the aperture in the template was immersed in 100°C water for 12 s on the dorsal surface and 7 s on the ventral surface. The template limits the burn area to a predetermined 40% TBSA, and the technique produces a full-thickness scald injury with complete destruction of the underlying neural tissue. Rats were immediately dried and resuscitated by the subcutaneous injection of 0.9% saline (10 ml). Sham burn control rats were treated identically to those in the burn group, except they were immersed in 25°C water. All rats were injected subcutaneously with the analgesic buprenorphine (0.2 mg/kg) immediately after sham or burn injury. Rats in all studies were deeply anesthetized with an intraperitoneal injection of pentobarbital sodium (60–75 mg/kg body wt). The hair on the dorsal and ventral surfaces of the animal was closely clipped, and animals were secured in an insulated template that exposed only the area of skin to be injured, as originally reported by Walker and Mason (58) and described and characterized by our laboratory (39–42). For the burn group, the surface of the skin exposed through the aperture in the template was immersed in 100°C water for 12 s on the dorsal surface and 7 s on the ventral surface. The template limits the burn area to a predetermined 40% TBSA, and the technique produces a full-thickness scald injury with complete destruction of the underlying neural tissue. Rats were immediately dried and resuscitated by the subcutaneous injection of 0.9% saline (10 ml). Sham burn control rats were treated identically to those in the burn group, except they were immersed in 25°C water. All rats were injected subcutaneously with the analgesic buprenorphine (0.2 mg/kg) immediately after sham or burn injury and again 24 h later. Rats were returned to individual cages for the remainder of the experimental protocol. Burn rats displayed no discomfort or pain and moved freely within their cage. As described below, the majority of the experiments were performed ~48 h after the burn because previous studies indicated that severe alterations in skeletal muscle protein balance are observed at this time point (42) and because of the results from the first experimental series. In this study and unless otherwise indicated, burn rats were permitted to consume food (Harlan No. 2380, 18% protein rodent diet; Madison, WI) ad libitum and control rats were pair fed to match the food consumption of animals in the burn group. Previous studies indicated that during the first 24-h period after burn injury rats consumed 72% ± 5% of normal food consumption and thereafter food consumption was not significantly different from basal control values (data not shown). Therefore, any burn-induced changes in the expression of atrogin-1 and MuRF-1 cannot be attributed to differences in the caloric intake between control and burn rats.

In the first study, a limited time-course study was performed to determine the temporal progression of burn-induced changes in atrogin-1 and MuRF-1 mRNA in gastrocnemius. Therefore, rats were anesthetized and muscle was sampled at various time points from 12 h to 5 days after burn injury.

In the second study, animals in both the control (e.g., nonburned) and burn groups were then administered either saline (0.155 mol/l) or 200 μg/kg body wt IGF-1 subcutaneously. Comparable doses have been shown to increase muscle protein synthesis (5). Rats were returned to individual cages and anesthetized 4 h later by an intraperitoneal injection of pentobarbital sodium (75 mg/kg body wt). Thereafter, a blood sample (1 ml) was collected from the abdominal aorta, and the gastrocnemius, soleus, and heart (e.g., ventricle only) were excised. This time point was selected based on studies indicating that IGF-1 maximally decreased atrogin-1 mRNA content in C57Bl/6 mice within this time frame (56).

In a third group of burn and control rats we investigated the ability of the branched-chain amino acid leucine to alter E3 ligase expression. Leucine is a potent nutrient signal reported to both increase protein synthesis and decrease protein degradation (3, 7, 24, 51). For this study, rats were administered either saline (0.155 mol/l) or 1.35 g/kg body wt leucine (prepared as 54.0 g/l of l-amino acid in distilled water) by oral gavage. This dose of leucine was selected because it is equivalent to the amount of leucine consumed in a 24-h period when rats of this age and strain are provided free access to food and it simulates muscle protein synthesis (3). As for the studies using IGF-1 described above, tissues were sampled 4 h after leucine administration.

In the fourth experimental series, burn rats were treated 2 h before burn injury with the proteasome inhibitor Velcade (0.3 mg/kg body wt administered ip; Millennium Pharmaceuticals, Cambridge, MA) or an equal volume (1.5 ml) of vehicle (saline). Comparable doses were previously shown to produce moderate inflammation induced by the injection of streptococcal cell wall (54) and atrophy induced by hindlimb immobilization (33) as well as to inhibit proteasome function (43). This time point was selected to accentuate the burn-induced decrease in muscle protein content and to optimize detection of a Velcade effect. All rats were pair fed to match food consumption by the burn + Velcade group.

In the final study, control and burn rats were injected subcutaneously with the type II glucocorticoid receptor antagonist RU-486 (Mifepristone, 20 mg/kg body wt; Sigma, St. Louis, MO) 2 h before burn or sham injury and again 24 h later; time-matched control animals were injected with an equal volume (0.3 ml) of vehicle [ethanol-saline:1:1 (vol/vol)]. RU-486 is an antiprogestin with anti-glucocorticoid properties (51). The dose of RU-486 used in the present study attenuates glucocorticoid-induced increases in catabolism and ameliorates endotoxin- or cytokine-induced changes in the IGF system (15, 65).

**PolyUb immunoblot.** Gastrocnemius was homogenized in 4 volumes of ice-cold buffer containing (in mM) 20 HEPES, pH 7.4, 2 EGTA, 50 NaF, 100 KCl, 0.2 EDTA, 50 β-glycerophosphate, 10 N-ethylmaleimide, 0.1 PMSF, 1 benzamidine, and 0.5 sodium vanadate. Homogenates were centrifuged and Western blot analysis performed exactly as previously described (33).

**Northern blot analysis of Ub ligases.** Total RNA was isolated from rat tissues with TRI Reagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer’s protocol. Samples (25 μg) of total RNA were run under denaturing conditions in 1.1% agarose-6% formaldehyde gels using 1× HEPES running buffer. Northern blotting occurred via capillary transfer to Nytran SuPerCharge membranes (Schleicher & Schuell, Keene, NH). Rat ubiquitin C (Ub) mRNAs were isolated from total rat liver RNA using the PolyATtract mRNA isolation system (Promega, Madison, WI). For normalization of RNA loading, an 18S oligonucleotide was labeled by the same method. Northern blots were hybridized with ULTRAhyb (Ambion, Austin, TX). All membranes were initially washed twice in 2× saline sodium citrate (SSC)-0.1% SDS for 5 min at 42°C and once in 0.2× SSC-0.1% SDS for 15 min at 42°C. All probes except 18S were additionally washed with 0.2× SSC-0.1% SDS for 10 min at 48°C. Finally, membranes were exposed to PhosphorImager screen (Mo-
lecular Dynamics, Sunnyvale, CA), visualized, and quantified with Molecular Dynamics ImageQuant software (version 5.2).

A portion of powdered muscle was homogenized in saline, and the protein concentration was determined by the Biuret method, using crystalline bovine serum albumin as a standard. Muscle protein content was calculated as the product of the muscle weight (g) and the muscle protein concentration (mg protein/wet wt of muscle). We previously reported (42) that the wet weight-to-dry weight ratio for gastrocnemius is not altered by burn.

Statistical analysis. Experimental data for each condition are summarized as means ± SE, and the sample size is indicated in Figs. 1–4, 6, and 7 and Table 1. Statistical evaluation of the data from studies with four groups was performed with ANOVA followed by Student-Neuman-Keuls test to determine treatment effect (Instat, San Diego, CA). Differences between the groups were considered significant when P < 0.05.

RESULTS Temporal changes in gastrocnemius atrogin-1 and MuRF-1 mRNA after burn. In the first study, a limited time-course study was performed to evaluate the temporal progression of burn-induced changes in atrogin-1 and MuRF-1 mRNA in gastrocnemius. Figure 1 illustrates that 40% TBSA burn increased atrogin-1 expression by 12 h, and this increase was maintained for at least 5 days, compared with time-matched control rats. In contrast, the burn-induced increase in MuRF-1 mRNA was more transient and had returned to control values between 3 and 5 days after injury.

Muscle weight and protein content. Forty-eight hours after thermal injury the wet weight of the gastrocnemius was significantly reduced by 9% (Table 1). The protein content (mg protein/g muscle) also tended to be reduced, but this change did not achieve statistical significance (e.g., P < 0.08). As a result of these changes, the total protein per muscle was reduced by 20% (Table 1). We previously determined (42) that the wet weight-to-dry weight ratio is not altered by burn injury at this time point.

Ub ligases and polyUb: effect of IGF-I and leucine. Constitutive expression of atrogin-1 mRNA was observed in both skeletal and cardiac muscle of control rats (Fig. 2). Thermal injury increased atrogin-1 mRNA content of gastrocnemius muscle by 3.1-fold 48 h after injury (Fig. 2). In contrast, atrogin-1 mRNA content was reciprocally downregulated in gastrocnemius from both control and burn rats in response to exogenous IGF-I administration. IGF-I decreased atrogin-1 mRNA by ~50–60% in both groups. However, atrogin-1 expression in the burn + IGF-I group was reduced only back to basal control values. The regulation of atrogin-1 mRNA content differed between cardiac muscle and gastrocnemius in two regards: 1) there was no burn-induced change in atrogin-1 expression in heart under basal conditions, and 2) although IGF-I decreased atrogin-1 in hearts from control rats, no such decline was observed in hearts from burn rats.

Figure 3 illustrates that thermal injury selectively increased MuRF-1 mRNA in gastrocnemius (~8-fold) but not heart. Because of the relatively low constitutive expression of MuRF-1 in skeletal muscle under basal conditions, we were unable to detect a significant change in MuRF-1 expression in gastrocnemius from control rats in response to IGF-I. In contrast, IGF-I treatment decreased MuRF-1 mRNA by 40% in skeletal muscle from burn rats, but the mRNA expression of this E3 ligase remained higher than control values. Neither burn nor IGF-I produced a detectable change in MuRF-1 in cardiac tissue.

Overall, the change in polyUb mRNA produced by burn or IGF-I was similar to that described above for MuRF-1. For example, burn increased polyUb mRNA in skeletal muscle by twofold. Although this increase in burn muscle was reversed by IGF-I, there was no detectable acute effect of IGF-I on

Table 1. Effect of burn on net protein balance in gastrocnemius

<table>
<thead>
<tr>
<th></th>
<th>Muscle Weight, g</th>
<th>Protein Content, mg/g</th>
<th>Total Protein/Muscle, mg</th>
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<tbody>
<tr>
<td>Control</td>
<td>1.316±0.019</td>
<td>85.5±2.7</td>
<td>113.3±5.2</td>
</tr>
<tr>
<td>Burn</td>
<td>1.187±0.023*</td>
<td>75.9±3.1</td>
<td>90.1±5.4*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 13 and 18 rats in control and burn groups, respectively. There was no statistical difference between values from vehicle- and insulin-like growth factor (IGF)-I-treated rats within either the control or the burn group because of the acute nature of the IGF-I challenge (e.g., 4 h). Therefore, values from the control + vehicle and control + IGF-I groups were combined, as were the values from the burn + vehicle and burn + IGF-I groups. *P < 0.05.
polyUb mRNA in gastrocnemius from control rats (Fig. 4). Again, neither burn nor IGF-I altered polyUb mRNA expression in cardiac muscle at the time points examined.

Ub antibody immunoreactivity was increased in gastrocnemius from burn rats compared with basal control values (Fig. 5). Exogenous IGF-I treatment did not appear to consistently alter the content of ubiquitinated protein in skeletal muscle from either control or burn rats. In contrast to the ability of IGF-I to acutely reduce the mRNA content of atrogin-1, MuRF-1, and polyUb in gastrocnemius from burn rats, the expression of these transcripts was not significantly altered by oral administration of leucine in muscle from either control or burned rats (data not shown).

Differential effect of burn on soleus muscle. In contrast to the burn-induced atrophy observed in gastrocnemius, there was no detectable decrease in the wet weight of the soleus muscle between control and burn rats at either 1 day (127 ± 19 vs. 123 ± 15 mg) or 2 days (129 ± 13 vs. 125 ± 14 mg) after injury. Furthermore, there was no significant change in the mRNA content for atrogin-1 or MuRF-1 in soleus from control and burn rats (data not shown). Because of the lack of change at 24–48 h after burn, we did not determine whether burn altered atrogin-1 and MuRF-1 expression at additional time points.

Proteasome-dependent changes in burn-induced muscle atrophy. In the second experimental protocol the proteasome inhibitor Velcade was administered before burn and the extent of muscle atrophy was studied 48 h later. At this time, the wet weight of the gastrocnemius was significantly reduced by 14% (Fig. 6, top). Velcade pretreatment in vivo completely prevented the burn-induced muscle atrophy. Velcade also pre-

Fig. 2. Acute effect of insulin-like growth factor (IGF)-I on atrogin-1 mRNA in gastrocnemius (middle) and heart (bottom) from control and burn rats. Tissues were collected 48 h after thermal injury and 4 h after IGF-I administration. Atrogin-1 mRNA abundance is expressed in AUs determined by densitometry and normalized to 18S mRNA. Values are means ± SE; n = 8–10 per group. Groups with different letters are significantly different from each other (P < 0.05). Top: representative Northern blots for atrogin-1 in gastrocnemius and heart from the four treatment groups. 18S RNA is also shown for each group as a loading control. SAL, saline.

Fig. 3. Acute effect of IGF-I on MuRF-1 mRNA in gastrocnemius (middle) and heart (bottom) from control and burn rats. Tissues were collected 48 h after thermal injury and 4 h after IGF-I administration. MuRF-1 mRNA abundance is expressed in AUs determined by densitometry and normalized to 18S mRNA. Values are means ± SE; n = 8–10 per group. Groups with different letters are significantly different from each other (P < 0.05). Top: representative Northern blots for MuRF-1 in gastrocnemius and heart from the four treatment groups. 18S RNA is also shown for each group as a loading control.
vented the burn-induced decrease in total muscle protein content (data not shown). In a previous study, Velcade was shown not to influence muscle weight in control animals (33), and therefore this drug control was not included in the current study. However, it is important to note that there was a 50% lethality in Velcade-treated burn rats, compared with 0% lethality in saline-treated burn animals. Our previous study (33) indicated no lethality in control rats treated with Velcade.

Northern blot analysis of gastrocnemius from these three experimental groups revealed that Velcade also prevented the large majority of the burn-induced increase in atrogin-1 and Murf-1 mRNA (Fig. 6, middle and bottom). In this regard, although the atrogene expression in muscle from the burn + Velcade group was significantly greater than untreated control values, Velcade prevented ~50% of the burn-induced increase in atrogin-1 and 85% of the increase in Murf-1.

Role of endogenous glucocorticoids in regulating burn-induced E3 ligases. In the third experimental protocol rats were administered the glucocorticoid receptor antagonist RU-486 to assess the impact of elevated endogenous corticosterone on muscle atrogin-1 and Murf-1. Figure 7 illustrates that pretreatment of burned rats with RU-486 did not prevent the burn-induced decrease in muscle protein content or the elevated expression of either atrogin-1 or Murf-1 mRNA in gastrocnemius.

DISCUSSION

The present study provides evidence that mRNA expression of atrogin-1 and Murf-1 is coordinately upregulated in gastrocnemius between 12 h and 3 days after injury. However, at a later time point these atrogenes are differentially regulated. That is, atrogin-1 mRNA content was still increased 5 days...
after burn, whereas MuRF-1 mRNA had returned to control values by this time point. Hence, although both atrogin-1 and MuRF-1 might mediate the increased muscle proteolysis seen early after burn, any continued acceleration of protein breakdown at later times appears independent of MuRF-1. When examined 48 h after burn, the upregulation of both atrogin-1 and MuRF-1 occurred in the gastrocnemius, a muscle with a predominance of fast-twitch type II fibers, but not the soleus, a muscle composed primarily of slow-twitch type I fibers. Similarly, we confirmed that burn increased polyUb gene expression in the gastrocnemius (8, 18) and extend these observations by demonstrating no such change in soleus. Importantly, changes in E3 ligases and polyUb were closely associated with burn-induced muscle atrophy that was only detected in the gastrocnemius, but not the soleus. These results are consistent with previous studies reporting that the burn-induced stimulation of protein degradation is primarily or exclusively detected in fast-twitch skeletal muscle (8, 17–19). Moreover, the expression of the E3 ligases and polyUb was not increased in heart in response to burn and therefore is an unlikely mediator of any burn-induced protein imbalance in this tissue.

The above-mentioned data indicate a tissue-specific regulation of atrogin-1, MuRF-1, and polyUb after burn and suggest an upregulation of Ub-proteasome-dependent protein degradation in gastrocnemius. This conclusion is supported by two additional lines of evidence. First, there was a corresponding burn-induced increase in Ub immunoreactivity in homogenates of gastrocnemius. Such data corroborate the increased ubiquitinylation of muscle proteins reported in other catabolic conditions (57). Second, pretreatment of animals with the proteasome inhibitor Velcade prevented a significant loss of gastrocnemius weight and protein 2 days after burn injury. These data extend previous studies in which the short-term in
vitro incubation of muscles with different proteasome inhibitors attenuated the accelerated proteolysis produced by burn or sepsis (17, 29). Velcade also largely prevented the expression of atrogin-1 and MuRF-1 mRNA; however, the mechanism for this change was not investigated. Finally, although rates of muscle proteolysis were not directly assessed, our findings provide physiological evidence for the relative importance of increased proteasome-dependent proteolysis in mediating the burn-induced atrophy.

The anabolic hormone IGF-I is important for the normal accretion of muscle protein (36), but the circulating and tissue concentration of IGF-I is decreased by burn and other catabolic insults (34, 37, 38, 40). A reduction in bioavailable endogenous IGF-I would be anticipated to increase the rate of muscle proteolysis, whereas exogenous administration should decrease proteolysis (5, 18, 20). Specifically, IGF-I inhibits the breakdown of myofibrillar proteins under both in vitro (8, 18) and in vivo (19) conditions, and this attenuation is associated with a reduction in Ub mRNA content. Our present data indicate that in vivo injection of IGF-I acutely (e.g., within 4 h) reduced the burn-induced elevation in polyUb, atrogin-1, and MuRF-1 in gastrocnemius, and this response appears to be due primarily to a decreased rate of atrogene transcription (56). Collectively, these data are consistent with the ability of IGF-I to decrease whole body and muscle protein breakdown (10, 18) as well as inhibit both lysosomal and Ub-proteasome-dependent protein degradation after burn injury (20). In contrast to the decrement seen in burn rats, IGF-I only decreased atrogin-1 (not MuRF-1 or polyUb) mRNA content in gastrocnemius from control rats. Similar to the gastrocnemius, IGF-I also acutely downregulated atrogin-1 expression in heart from control rats at 4 h. However, a comparable IGF-I-induced decrease in atrogin-1 mRNA was not seen in hearts from burn rats. Collectively, these data suggest that the therapeutic efficacy of IGF-I in skeletal muscle is maintained in adult thermally injured rats.

In contrast to the IGF-I effect, the central nutritional regulator leucine had no detectable effect on atrogene expression in muscle. This was unexpected because IGF-I and leucine mediate their metabolic effects by sharing many of the same distal signaling elements (2). Branched-chain amino acids in general and leucine in particular decrease muscle proteolysis under both in vivo and in vitro conditions (7, 51). Moreover, leucine also dose-dependently inhibits chymotrypsin-like activity of the proteasome (24). Therefore, we anticipated that leucine, similar to IGF-I, would downregulate the expression of atrogin-1 and possibly MuRF-1. However, when the plasma leucine concentration was acutely elevated we were unable to detect a consistent leucine-induced decrease in either constitutive or burn-induced atrogene expression. Hence, the ability of leucine to inhibit muscle proteolysis in rats is not mediated by a concomitant downregulation of atrogene expression. These data further suggest that the mechanism by which IGF-I decreases atrogin-1 mRNA is mediated proximal to phosphatidylinositol 3-kinase (PI3-kinase) and PKB, which are both activated by IGF-I but not by leucine under in vivo conditions (2). This conclusion is consistent with findings from in vitro studies showing that inhibition of PI3-kinase prevents the ability of IGF-I to decrease atrogin-1 (56) and that adenoviral infection of myocytes with a dominant-negative PI3-kinase suppresses the inhibitory effect of insulin on protein degradation (46). Because of the acute nature of the present experimental protocol, we cannot exclude the possibility that a more chronic elevation in leucine would downregulate atrogene expression.

Burn injury produces a rapid and sustained elevation in the circulating concentration of glucocorticoids (16, 35, 41). Moreover, the catabolic effect of exogenously administered glucocorticoids, primarily dexamethasone, is well established in skeletal muscle and consistently characterized by an increased rate of degradation. Although dexamethasone dramatically increases proteolysis by stimulating various proteolytic pathways (9, 47, 57), this stimulation is associated with increased expression of atrogin-1 and MuRF-1 and can be largely inhibited by pretreatment with proteasome inhibitors (6, 56, 57). These data imply that dexamethasone-induced muscle wasting is primarily mediated by the Ub-proteasome pathway. Moreover, administration of the glucocorticoid receptor antagonist RU-486 prevents the decrement in muscle proteolysis seen in response to sepsis or burn (16, 57). The results of the present study, however, fail to confirm that the burn-induced increase in muscle proteolysis is mediated by endogenous glucocorticoids. Instead, our data on muscle weight as well as the mRNA content for atrogin-1, MuRF-1, and polyUb indicate that the increased rate of muscle proteolysis is glucocorticoid independent. These data corroborate an early study by Clark et al. (11) in which the burn-induced increase in muscle proteolysis was not ameliorated by either adrenalectomy or inhibition of glucocorticoid synthesis with metyrapone. Although the exact reason for this apparent discrepancy is not known, studies showing glucocorticoid dependence used smaller immature rats (40–60 g) compared with those studies purporting a glucocorticoid-independent effect (>250 g). In this regard, the rate of myofibrillar breakdown has been reported to be threefold greater in muscles from immature rats compared with rates in mature animals (64). Alternatively, it has been posited that a second signal, such as low insulin, might be required for glucocorticoids to activate muscle proteolysis (45). However, this permissive effect of insulinopenia would not be expected because the circulating insulin concentration is well preserved after burn in adult rats (39, 35). Finally, a glucocorticoid-independent activation of the Ub system has also been observed in cancer cachexia (48).

It is noteworthy that despite the ability of Velcade to prevent the atrophic response of fast-twitch skeletal muscle to burn injury, this proteasome inhibitor markedly increased mortality in burned rats. Previous studies in our lab (33) using the same Velcade dose and weight and strain of rats did not indicate any lethality in control animals. We have no direct evidence related to the cause of this increased mortality; however, a more thorough investigation of this adverse effect is certainly warranted.

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