Acute response of IGF-I and IGF binding proteins induced by thermal injury

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Lang, Charles H., Xiaoli Liu, Gerald J. Nystrom, and Robert A. Frost. Acute response of IGF-I and IGF binding proteins induced by thermal injury. Am J Physiol Endocrinol Metab 278: E1087–E1096, 2000.—Previous studies demonstrate that thermal injury decreases circulating levels of insulin growth factor I (IGF-I) and alters the plasma concentration of several IGF binding proteins (IGFBP), but the mechanisms for these alterations have not been elucidated. In the current study, a 30% total body surface area full-thickness scald burn was produced in anesthetized rats, and animals were studied 24 h later. The plasma concentration of both total and free IGF-I was decreased (38 and 65%, respectively) in burn rats compared with values from time-matched control animals. Thermal injury decreased the IGF-I peptide content in liver ~40%, as well as in fast-twitch skeletal muscle (56–69%) and heart (28%). In contrast, IGF-I content in kidney was elevated by 36% in burn rats. Northern blot analysis of liver indicated that burn decreased the expression of small (1.7- and 0.9- to 1.2-kb) IGF-I mRNA transcripts but increased the expression of the 7.5-kb transcript. In contrast, there was a coordinate decrease in all IGF-I mRNA transcripts in muscle and kidney of ~30%. For liver, muscle, and kidney, there was no significant difference in the expression of growth hormone receptor mRNA between control and burn rats. Thermal injury increased plasma IGFBP-1 levels, and this change was associated with increased IGFBP-1 mRNA in both liver and kidney. IGFBP-3 levels in plasma were concomitantly decreased by burn injury. This change was associated with a reduction in IGFBP-3 mRNA in liver but an increased expression of IGFBP-3 in kidney and muscle. Thermal injury also decreased the concentration of the acid-labile subunit (ALS) in plasma and ALS mRNA expression in liver. Finally, hepatic expression of IGFBP-related peptide-1 was increased twofold in liver but was unchanged in kidney or muscle of burn rats. These results characterize burn-induced changes in various components of the IGF system in select tissues and thereby provide potential mechanisms for alterations in the circulating IGF system and for changes in tissue metabolism.

insulin-like growth factor binding protein-1 and -3; mac25; acid-labile subunit; amino acids; rats

THERMAL INJURY PRODUCES A NUMBER of well-characterized hormonal and metabolic alterations. One such hormonal alteration is the pronounced decrease in the circulating concentration of insulin-like growth factor (IGF-I). A decrease in plasma IGF-I has been demonstrated by several investigators to occur early (within 24 h) after thermal injury and to remain reduced for several weeks thereafter (1, 16, 23, 32, 34). Furthermore, the burn-induced decrease in plasma IGF-I appears to be proportional to the severity of the insult (1, 32). The liver is the primary source of blood-borne IGF-I (24), and previous studies have demonstrated that hepatic synthesis and secretion of IGF-I are impaired in other catabolic-inflammatory conditions, such as sepsis and endotoxemia (8, 9, 22, 46). Hence, it is assumed, but not proven, that a similar defect in hepatic IGF-I synthesis occurs after thermal injury. Furthermore, a variety of extrahepatic tissues contain the peptide and mRNA for IGF-I (33, 46), suggesting that IGF-I functions as both a classical hormone and an autocrine-paracrine regulator. In this regard, tissuespecific changes in IGF-I peptide and mRNA have been previously reported in other catabolic conditions (8, 9, 38) and may impact upon metabolic pathways in those tissues.

IGF-I in the blood and various body fluid compartments is bound noncovalently to one of several IGF binding proteins (IGFBPs). The majority of the circulating IGF-I is bound to IGFBP-3 and the acid-labile subunit (ALS) to form a ternary complex (27, 37). Because of its large molecular weight, this complex is restricted to the vascular compartment and is believed to represent a storage reservoir for IGF-I (27, 37). In contrast, a relatively small amount of the total IGF-I in the blood is carried by IGFBP-1 (26). Because of the size of this binary complex, IGFBP-1 represents a potential mechanism for the translocation of IGF-I across the capillary endothelium (26). Although the exact functions of these binding proteins are not known, it is clear that they represent another mechanism by which the tissue availability of IGF-I can be modulated. In addition, several of the IGFBPs have IGF-independent effects (13, 21). To our knowledge, there are only three studies reporting IGFBP levels after thermal injury, and all have been performed in humans (16, 23, 34). All studies clearly demonstrate a decrease in the circulating concentration of IGFBP-3 at various times after burn. However, for IGFBP-1, two of the studies showed increased levels (23, 34), and the third reported no change (16). Burn-induced changes in tissue mRNA for these IGFBPs have not been reported.

The purpose of the present study was to characterize changes in IGF-I, IGFBP-3, and IGFBP-1 in the sys-
temic circulation and in liver and selected extrahepatic tissues after acute (24-h) thermal injury in the rat. Burn is associated with alterations in the release of numerous hormones that are capable of modulating various components of the IGF system. The stimulation of hepatic IGF-I, IGF-BP-3, and ALS synthesis is generally considered to be growth hormone (GH) dependent (7, 12, 35, 43), whereas insulinopenia has been reported to decrease IGF-I and IGF-BP-3 and to increase IGF-BP-1 levels (15, 23, 28). Elevated glucocorticoid levels also decrease IGF-I and increase IGF-BP-1 (28, 29, 41). Finally, alterations in the concentration of dietary protein and amino acids can differentially regulate the hepatic synthesis of IGF-I and IGF-BP-1 (20, 36). Therefore, we have also measured the plasma concentrations of these various hormones/substrates to begin to assess their potential role in regulating the IGF system in response to thermal injury.

METHODS AND MATERIALS

Animal preparation and experimental protocol. Adult specific pathogen-free male Sprague-Dawley rats (285–310 g; 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 ≥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 guidelines for the use of experimental animals.

On the two days before the study, each animal was placed in a metabolic cage, and urine volume was recorded for the next two 24-h periods. Thereafter, one-half of the rats were assigned to the sham-control group and the other one-half to the burn group. All rats were deeply anesthetized with an intraperitoneal injection of pentobarbital (Milenax GV, Millipore, Molsheim, France) to ensure death. The prefilled samples were then added to Amicon YMT 30 membranes and MRS-1 supporting devices (Amicon Division, Milford, MA) and centrifuged at 300 g (1,500 rpm) at 24°C for 1 h. The ultrafiltrate was collected from 40 to 100 min of centrifugation and used for the IGF-I RIA.

RNA extraction and Northern blotting. Total RNA was isolated with TRI reagent TR-118, as outlined by the manufacturer (Molecular Research Center, Cincinnati, OH). Twenty-one hundred-microgram samples of total RNA were run under denaturing conditions in 1% agarose-6% formaldehyde gels. The running buffer was 1× TBE. Northern blotting occurred via capillary transfer to Zeta-Probe GT blotting membranes (Bio-Rad Laboratories, Hercules, CA). An 800-bp probe from rat IGF-I (Peter Rotwein, St. Louis, MO), a 600-bp probe of the rat growth hormone receptor (GHR) comprising the extracellular domain, the putative transmembrane region, and a short section of the intracellular domain (Lawrence et al., Ann Arbor, MI), and a 407-bp probe from IGF-BP-3 (Millicourt, Kyoto, Japan) were labeled by use of a random primed DNA labeling kit (Roche Molecular Biochemicals, Indianapolis, IN). To probe for rat ALS, a 36-mer oligonucleotide was constructed. The complement of the second exon position 3843–3878 (5'-GAC GCT TCG GAG TGC GTT CCT GCT-3') was the sequence chosen for the oligonucleotide. A rat 18S oligonucleotide was used for normalization of RNA loading. Both oligonucleotides were synthesized by the Macromolecular Core Facility at the Pennsylvania State College of Medicine with a Perceptive Biosystems Expedite 8909 nucleic acid synthesizer. Each oligonucleotide was radiactively end-labeled with T4 polynucleotide kinase (Amersham Pharmacia Biotech, Piscataway, NJ). Membranes were prehybridized and hybridized at 42°C in 50% formamide-6× SSPE-5× Denhardt’s-1% SDS-10% dextran sulfate-herring testis DNA (100 µg/ml), with the exception that ULTRAhyb (Ambion, Austin, TX) was used as the hybridization solution for rat ALS and mac25. All membranes were washed at room temperature twice in 2× SSC-0.1% SDS for 5 min and once in 0.1× standard sodium citrate (SSC)-0.1% SDS for 15 min. Additionally, membranes hybridized with rat IGF-I, GHR, or mac25 were washed at 55°C in 0.1× SSC-0.1% SDS for 15–30 min. Finally, membranes were

Tissue samples were rinsed in ice-cold saline, blotted, and then frozen between aluminum blocks precooled to the temperature of liquid nitrogen. All tissue, plasma, and urine samples were stored at –70°C until analysis.

IGF-I determination. The concentration of total IGF-I in plasma was determined by a modified acid-ethanol (0.25 N HCl-87.5% ethanol) procedure with cryoprecipitation, and tissues were processed by use of acid homogenization and Sep-Pak (C-18) extraction (8, 9, 22, 46). Urine samples were concentrated tenfold by centrifugal concentration, and the dried sample was reconstituted with RIA buffer containing 0.25% BSA for IGF-I determination. IGF-I in plasma, urine, and tissues was determined by RIA. Recombinant human

[Thr59]IGF-I was used for iodination and standards (Gene-tech, South San Francisco, CA). The ED₅₀ for this assay is 0.03–0.08 ng/tube. The tissue protein concentration was determined by the biuret method, and tissue IGF-I content was expressed as nanograms of IGF-I per microgram of tissue protein.

The plasma concentration of free IGF-I was determined by centrifugal ultrafiltration (3). Briefly, the plasma samples were diluted 1:5 with Krebs-Ringer bicarbonate buffer (pH 7.4, with 5% BSA) and prefilled through a 0.22-µm filter (Millipore, Millpore, Molsheim, France) to remove debris. The prefilled samples were then added to Amicon YMT 30 membranes and MRS-1 supporting devices (Amicon Division, Milford, MA) and centrifuged at 300 g (1,500 rpm) at 24°C for 100 min. The ultrafiltrate was collected from 40 to 100 min of centrifugation and used for the IGF-I RIA.

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exposed to a phosphoimager screen and the resultant data quantitated by ImageQuant software (Molecular Dynamics, Sunnyvale, CA). Data for all probes were normalized to the level of ribosomal 18S RNA. Relative mRNA abundance was expressed as the ratio between the particular mRNA and 18S mRNA. This ratio was arbitrarily set at 1.0 for tissues from control animals.

Ligand blotting. IGFBP-3 in plasma was determined by Western ligand blot analysis (8, 9, 22, 46). Samples were subjected to SDS-PAGE without reduction of disulfide bonds. The electrophoresed proteins were transferred onto nitrocellulose in Tris-methanol-glycine buffer. Nitrocellulose sheets were washed and then incubated overnight with radiolabeled IGF-I. The nitrocellulose sheets were washed extensively in Tween 20, dried, and autoradiographed with X-ray film (Kodak X-Omat AR, Eastman Kodak, Rochester, NY) and intensifying screens (Du Pont, Wilmington, DE) at −70°C for 2–4 days.

Western immunoblotting. Plasma samples were separated on a 12.5% SDS-PAGE gel under nonreducing conditions, as previously described (46). Separated proteins were electrophoresed onto nitrocellulose and blocked for 2 h at room temperature with Tris-buffered saline containing 1% nonfat dry milk. The membranes were then incubated with a 1:2,000 dilution of antiserum against rat IGFBP-1 at room temperature for 2 h. Antigen-antibody complexes were identified with goat anti-rabbit IgG tagged with horseradish peroxidase (Sigma, St. Louis, MO) and exposed to the enhanced chemiluminescence detection system (Amersham, Arlington Heights, IL) for 1 min and to X-ray film for 10–30 s. Bands were scanned (Microtek ScanMaker IV) and quantitated with NIH Image 1.6 software. Representative samples from all experimental groups were electrophoresed on the same gel; data are expressed as a percentage of the control value.

IGFBP-3 proteolysis. Proteolysis was determined on plasma from control and burned rats (n = 5 for each group). Plasma samples were incubated either alone or mixed and incubated in 0.1 M Tris buffer (pH 7.4) at 37°C for 18 h. Samples were subsequently separated by SDS-PAGE, and IGFBPs were detected by ligand blotting, as described above. IGFBP-3 proteolysis was defined as a loss of IGFBP-3 by ligand blotting compared with control plasma. Plasma from a pediatric AIDS patient was used as a positive control for IGFBP-3 proteolysis.

Plasma glucose, hormone, and amino acid concentrations. The plasma glucose concentration was determined by means of a rapid analyzer (GL5, Analox Instruments, Lumenburg, MA). The plasma concentrations of rat insulin (Linco, St. Louis, MO), GH (Amersham), and corticosterone (Diagnostic Products, Los Angeles, CA) were determined by specific RIA.

Statistics. Data were obtained from two separate experimental series, each containing sham-control and burn rats (5–6 rats per group). Experimental values are presented as means ± SE. The numbers of rats per group are indicated in the figure legends. Data were analyzed with Student’s t-test to determine treatment effect. Statistical significance was set at P < 0.05.

RESULTS

Plasma IGF-I and IGFBPs, and IGF-I excretion. The concentration of total IGF-I in plasma was decreased 38% in rats 24 h after thermal injury, compared with time-matched control animals (Fig. 1A). Burn animals also showed a 65% decrease in the plasma concentration of free IGF-I (Fig. 1B). As a result of these changes, the relative amount of free IGF-I, compared with total IGF-I, decreased from 5.8 to 3.2% in burn rats (Fig. 1C).

Western blot analysis indicated a dramatic elevation in circulating IGFBP-1 after thermal injury (Fig. 2A). Ligand blot analysis also demonstrated a 45% reduction in plasma IGFBP-3 levels in burn rats (Fig. 2B). Furthermore, there was a burn-induced decrease in the circulating level of ALS (56%) compared with control values (Fig. 2C).

Tissue IGF-I peptide and mRNA. A concomitant reduction in IGF-I peptide content was also detected in liver (43%) from burn rats (Fig. 3). Thermal injury decreased IGF-I in muscles composed predominantly of mixed fast- and slow-twitch fibers. In this regard, IGF-I content was decreased by 56% in the gastrocnemius and 69% in the extensor digitorum longus (EDL). The response in muscles with a high proportion of slow-twitch fibers (heart and soleus) was less pronounced. In burn rats, IGF-I content of heart was decreased (28%), whereas there was no significant difference in IGF-I in the...
soleus from sham-control and burn rats (Fig. 3). In contrast to the above-mentioned changes, thermal injury increased the IGF-I content of kidney by 36%.

Northern blot analysis of liver indicated three major bands for IGF-I mRNA: one band at ~7.5 kilobases (kb), a second band at ~1.7 kb, and a third broad band between 0.9 and 1.2 kb (Fig. 4B). This banding pattern is comparable to that reported by others (42). In liver, the relative abundance of both the 1.7-kb and 0.9- to 1.2-kb IGF-I transcripts was significantly decreased (35–45%) in burn rats compared with controls (Fig. 4A). In contrast, there was a significant 53% increase in the 7.5-kb transcript after thermal injury. Collectively, the cumulative abundance of all three hepatic IGF-I transcripts tended to be reduced in burn rats (~25%; \( P < 0.08 \)). For kidney and muscle, there was a coordinate decrease in all three IGF-I transcripts in response to burn (data not shown). Quantitation of the 7.5-kb band showed that thermal injury decreased IGF-I mRNA expression by 31% in kidney and 38% in muscle. Similarly, quantitation of the two smaller IGF-I transcripts indicated a 30% decrease in both tissues in burn rats. As a result of these changes, the cumulative abundance of the three transcripts was significantly reduced by ~30% in both tissues (Fig. 4, C and D).

During the 24-h period before injury, the volume of urine excreted was not different between groups (control, 18.9 ± 2.6 ml/day vs. burn, 17.9 ± 2.4 ml/day). After burn, urinary volume was decreased ~50% compared with control values (control, 15.9 ± 3.5 ml/day vs. burn, 8.3 ± 0.9 ml/day; \( P < 0.05 \)). The urinary excretion of IGF-I (the product of urinary volume and urinary IGF-I concentration) was subsequently calculated. During the 24-h period before injury, urinary excretion of IGF-I was similar in both control (904 ± 203 ng/day) and burn (743 ± 76 ng/day) rats. Although IGF-I excretion tended to decrease after either sham or burn injury, these changes did not reach statistical significance. More importantly, there was no significant difference in the urinary excretion of IGF-I between control (746 ± 126 ng/day) and burn (678 ± 91 ng/day) rats during the 24-h period after burn injury.

Growth hormone receptor and GH binding protein mRNA. After hybridization, two bands were visualized: a 4.4-kb transcript encoding the GH receptor (GHR) and a 1.2-kb transcript encoding GH binding protein (GHBP) (data not shown). There was no significant difference between the expression of GHR mRNA in control and burned rats for either liver [control, 1.00 ± 0.6 vs. burn, 1.06 ± 0.18 arbitrary units (AU) of volume] or kidney (control, 1.00 ± 0.10 vs. burn, 1.24 ± 0.15 AU), as assessed by Northern blot analysis. There was a tendency for GHR mRNA in skeletal muscle to be decreased by thermal injury, but this change did not reach statistical significance (controls, 1.00 ± 0.09 vs. burn, 0.84 ± 0.05 AU). Likewise, there was no signifi-
cant difference in GHBP mRNA expression between control and burned rats for liver, kidney, or skeletal muscle (data not shown).

Tissue IGFBPs and ALS. IGFBP-1 mRNA expression was increased ~5-fold in liver and ~12-fold in kidney (Fig. 5) in response to thermal injury. IGFBP-1 mRNA expression could not be detected in skeletal muscle by Northern blot analysis (data not shown).

Tissue expression of IGFBP-3 was also determined in selected tissues from control and burn rats (Fig. 6). Thermal injury decreased IGFBP-3 mRNA by 31% in liver. In contrast, IGFBP-3 mRNA expression was increased by 63% in kidney and almost threefold in the gastrocnemius muscle after burn injury. Furthermore, there was no detectable increase in the extent of IGFBP-3 proteolysis in plasma from burn rats compared with control values (data not shown).

The expression of ALS mRNA was decreased 94% in liver from burn rats compared with time-matched control values (Fig. 7).

The expression of IGFBP-related protein-1 (IGFBP-rP1), also termed mac25 or IGFBP-7, was also determined. IGFBP-rP1 was increased more than twofold in liver in response to burn injury (control, 1.00 ± 0.11 vs. burn, 2.18 ± 0.18 AU; P < 0.05). In contrast, no burn-induced change was detected for IGFBP-rP1 in kidney or skeletal muscle (data not shown).

Glucose, hormone, and amino acid concentrations. The plasma concentrations of glucose and several hormones capable of influencing the IGF system were determined. Thermal injury increased the concentration of both insulin (196 ± 30 vs. 422 ± 54 pmol/l; P < 0.05) and corticosterone (198 ± 16 vs. 326 ± 29 ng/ml; P < 0.05) compared with control values. A single-point determination of plasma GH was not different between groups (control, 18 ± 2 vs. burn, 19 ± 2 ng/ml). There was also no significant difference in the plasma glucose concentration between sham-control and burn rats (9.1 ± 0.4 vs. 9.5 ± 0.7 mM, respectively) at the 24-h time point.

The effect of burn injury on the concentration of individual plasma amino acids is presented in Table 1. Overall, burn rats demonstrated an 8% decrease in the concentration of total amino acids, but this change was not statistically significant. However, several individual amino acids did show significant decreases in response to thermal injury; these included glutamine (~32%), arginine (~27%), proline (~20%), glycine (~31%), and lysine (~23%). In contrast, the concentrations of several amino acids were elevated in response to burn, including valine (55%), leucine (59%), isoleucine (40%), tyrosine (20%), phenylalanine (30%), histidine (60%), and 3-methylhistidine (34%).

DISCUSSION

In the present study, we used a well defined thermal injury model in rats to characterize selected aspects of the IGF system in plasma and tissues. The plasma concentration of total IGF-I was markedly reduced in burn rats. This finding is consistent with a number of animal and human studies of burn injury (1, 16, 23, 32, 34). Moreover, we have extended these original observations by determining that burn decreased the absolute concentration of free IGF-I, as well as the relative amount of free IGF-I compared with the total IGF-I concentration in the plasma. The mechanism for the burn-induced decrease in circulating IGF-I has not been previously elucidated, but it could result from a decreased rate of synthesis and/or increased rate of
removal from the blood. Our results confirm data from a previous report (19), which indicates that the content of hepatic IGF-I peptide is also reduced in burn rats. Burn also tended to decrease IGF-I mRNA expression in the liver, based on the combined abundance of the three major transcripts detected. However, whereas burn clearly decreased the smaller IGF-I transcripts (1.7 kb and 0.9–1.2 kb), the abundance of the 7.5-kb transcript was significantly increased. The overall decrease in hepatic total IGF-I mRNA was qualitatively similar to, albeit quantitatively smaller than, that observed in other catabolic conditions, including sepsis (22, 49), endotoxemia (29, 46), or cytokine-induced inflammation (8, 9). However, these latter insults produced a coordinated decrease in all detectable IGF-I transcripts. The reasons for this difference are unclear. It is also possible that an enhanced rate of IGF-I removal from the circulation contributes to the burn-induced decrease in plasma IGF-I. An enhanced rate of clearance may result from an increase in IGF-I binding by specific cells, such as leukocytes, enterocytes, and various renal cell types, which could be mediated by an upregulation of the IGF type I receptor and/or the amount of one or more of the IGF binding proteins. These possibilities were not examined in the present study; however, our data do indicate that the burn-induced decrease in plasma IGF-I is not a consequence of an increase in urinary IGF-I excretion.

GH is a potent stimulator of hepatic IGF-I synthesis and secretion. Therefore, burn-induced changes in plasma IGF-I may result because of a decrease in circulating levels of GH or an impaired responsiveness of tissues to GH. Although a single-point determination of plasma GH failed to detect any major difference between control and burn rats, we cannot exclude the possibility that pulsatile release of GH was impaired by thermal injury. Additionally, we were unable to detect significant alterations in GHR mRNA in any tissue examined. Therefore, it seems unlikely that the burn-induced changes in tissue IGF-I (or other GH-dependent processes described below) were mediated by the number of receptors for GH. Other studies have reported that the number of GHR in liver is either markedly decreased (5) or not altered (31) after the injection of endotoxin. The reason for the difference in GHR levels in these studies remains unclear but may be related to the severity of the insult and/or the time point at which samples were obtained. Regardless of the exact mechanism, both studies demonstrate the presence of endotoxin-induced hepatic GH resistance (5, 31), which has also been reported in response to thermal injury (19). Hence, the burn-induced decrease in plasma IGF-I and other GH-dependent processes appears most likely to result from a defect in the GH signaling pathway in liver. To our knowledge, there are no studies that examine the influence of catabolic stimuli on GH signal transduction in extrahepatic tissues.

IGF-I is an important anabolic agent in skeletal muscle, where it promotes protein synthesis and glucose uptake and inhibits protein degradation (2, 14). Burn rats showed a consistent decrease in IGF-I peptide content in several fast-twitch skeletal muscles (gastrocnemius and EDL). There was no decrease in IGF-I peptide content in the slow-twitch soleus muscle, and there was a relatively small burn-induced decrease in heart. This fiber type selectivity is similar to that observed by other investigators for burn-induced changes in protein metabolism (10). In the gastrocnemius muscle, which has a fiber type similar to that of the whole body musculature, we also demonstrated a burn-induced decrease in IGF-I mRNA. The decrease in both IGF-I peptide and mRNA in skeletal muscle is similar to the response observed in other catabolic conditions (8, 9, 22, 46) and has been suggested to be at least partially responsible for the impairment in muscle protein balance and the negative nitrogen balance (22). After thermal injury, there was an increase in IGF-I peptide but a decrease in IGF-I mRNA in kidney. This response suggests that burn injury increases the movement of IGF-I peptide from the circulation to the kidney rather than increasing renal IGF-I synthesis. These changes are similar to those reported in rats made...
acutely acidotic (4), but previous work by Horton et al. (18) indicates that rats are not acidotic or hypoxic at this time point after burn injury. However, these changes differ from those observed in other catabolic conditions that show an increase in both renal IGF-I peptide and mRNA levels (9, 29, 46). The burn-induced increase in renal IGF-I did not result in a concomitant increase in urinary IGF-I excretion. This suggests that IGF-I present in the kidney was largely sequestered or trapped in this tissue and that the reduction in plasma IGF-I did not result from an enhanced rate of excretion. Other catabolic conditions are characterized by an increase in IGFBP-1 content in the kidney (8, 9, 22, 46), and indeed, renal synthesis of IGFBP-1, as evidenced by an increase in IGFBP-1 mRNA, was increased in burn rats. Therefore, it seems likely that the renal sequestration of IGF-I occurred secondarily to the increase in IGFBP-1. We speculate that this burn-induced increase in renal IGF-I represents a beneficial response that...
may aid in maintaining renal perfusion and glomerular filtration (30).

The large majority of IGF-I in blood is carried bound to IGFBP-3 (37). Previous studies in humans report that thermal injury produces a rapid and sustained decrease in circulating IGFBP-3 (16, 23, 34). The current investigation demonstrates that a qualitatively similar IGFBP-3 response can be produced in rats. This decrease could result from an increased rate of clearance and/or a decreased rate of synthesis. In other catabolic conditions, some of the decrease in plasma IGFBP-3 may result from an enhanced rate of proteolysis (6). In the present study, however, we failed to detect a burn-induced increase in IGFBP-3 proteolysis. The decreased plasma concentration of IGFBP-3 in burn rats was associated with a comparable reduction in IGFBP-3 mRNA in liver. Previous studies indicate that exogenous administration of IGF-I increases hepatic IGFBP-3 mRNA in hypophysectomized rats (12); therefore, it is possible that the burn-induced decrease in hepatic IGFBP-3 mRNA results from the reduction in hepatic and/or circulating levels of IGF-I. Alternatively, we cannot exclude the possibility that a decrease in GH levels or the presence of hepatic GH resistance is responsible for the burn-induced decrease in hepatic IGFBP-3 mRNA. In contrast to liver, IGFBP-3 mRNA was increased in both kidney and muscle by burn injury. The threefold elevation in skeletal muscle IGFBP-3 mRNA expression was particularly dramatic. Based on the assumption there is a corresponding increase in IGFBP-3 protein in the muscle and surrounding interstitial fluid of burn rats and that elevations in IGFBP-3 appear to largely inhibit IGF-I mediated processes (37), it is possible that this local change is partially responsible for the metabolic disturbances seen in muscle. The mechanism for the increase in IGFBP-3 in muscle and kidney is not known.

A moderate decrease in the circulating concentration of ALS, the third component of the ternary complex, was also observed in burn rats. This decrease faithfully mimics the response seen in human subjects after burn (23). In rats, however, the burn-induced decrease in hepatic ALS mRNA was far more dramatic, with expression being reduced to <10% of control levels. The greater reduction in hepatic ALS mRNA, compared with plasma ALS protein, may be a consequence of its large molecular weight and corresponding relatively long half-life in the blood. The amount of ALS in the circulation is determined primarily by GH-dependent transcriptional activation of the ALS gene within the liver (35). The suppression of hepatic ALS mRNA in burn rats is comparable to the decrease observed in hypophysectomized rats completely lacking GH (12). Although our data suggest that such a severe reduction in plasma GH is not present in burn rats, the same response could be observed in animals with a defect in hepatic GH signal transduction. Thermal injury increases the hepatic expression or the systemic concentration of various proinflammatory cytokines (44). Recently, Delhanty (7) has reported that relatively low doses of interleukin-1β can inhibit GH-induced increases in ALS in primary hepatocytes; hence, the overexpression of one or more cytokines remains a possible mediator for the burn-induced decrease in ALS. Elevated cytokine levels also inhibit IGF-I synthesis in isolated hepatocytes (43), which is consistent with the reduction in abundance of the smaller IGF-I mRNA transcripts observed in liver from burn rats. An elevation in plasma IGFBP-1 is commonly observed in a variety of catabolic conditions (8, 9, 22, 46) and has been reported in some (23, 34), but not all (16), of the studies on burn trauma. In the current study, plasma IGFBP-1 was markedly and consistently elevated 24 h after thermal injury. We have extended these observations to reveal that IGFBP-1 mRNA expression was also elevated 5- to 10-fold in both liver and kidney. Known regulators for the elevations in IGFBP-1 include decreases in insulin (28), as well as increases in glucocorticoids and various cytokines (25, 28, 29). Because mild hyperinsulinemia was present in our burn rats, this hormone appears to be an unlikely candidate. Thus the latter group of agents remain as potential mediators for the elevation in blood and tissue IGFBP-1.

In addition to the six well-characterized high-affinity IGFBPs, there are several new members of the IGFBP superfamily that have a relatively low sequence homology and IGF binding affinity. The impact of thermal injury on one of these peptides, IGFBP-rP1 (mac25 or IGFBP-7), was determined in the present study. We examined IGFBP-rP1 because of its reported ability to bind insulin and impair insulin-stimulated phosphorylation (45). It has been suggested that the overproduction of IGFBP-rP1 might play an important role in the pathogenesis of the insulin resistance that accompanies certain catabolic conditions (45). In this regard, thermal injury is known to produce a marked impairment of insulin action in muscle (39). Our data demonstrate that IGFBP-rP1 mRNA expression was elevated in liver, but not in muscle or kidney, in response to burn. Therefore, if IGFBP-rP1 is a mediator of the burn-induced muscle insulin resistance, it must be functioning via an elevation in the plasma concentration, as opposed to having an autocrine-paracrine effect.

The plasma concentrations of numerous individual amino acids were altered in response to burn. In general, the increased concentration of the branched-chain amino acids, as well as the elevation in tyrosine, phenylalanine, and 3-methylhistidine, strongly suggests a breakdown of myofibrillar protein (40). Similar changes have been reported previously in burn rats (11). However, the concentrations of other amino acids, such as glutamine, arginine, proline, glycine, and lysine, were decreased by burn. Hence, there was no significant change in the plasma concentration of total amino acids in burn rats. Therefore, it seems unlikely that burn-induced changes in total amino acid availability mediate the observed changes in the IGF system. Furthermore, IGFBP-1 secretion by HepG2 cells has been shown to be increased under conditions where the individual concentration of valine, leucine, isoleucine, phenylalanine, or histidine in the medium is decreased.
(20). However, the plasma concentration of each of these amino acids was increased in burn rats. Therefore, changes in individual amino acids do not appear to be important regulators of IGFBP-1 synthesis in this particular stress condition.

Based on the above-mentioned data, we speculate that the burn-induced decrease in plasma IGF-I occurs in the following manner. Hepatic expression of ALS is dramatically downregulated, resulting in a progressive decrease in plasma ALS. In addition, there is a concomitant impairment in hepatic synthesis and secretion of IGFBP-3, albeit less severe than that observed for ALS. The loss of ALS from the circulation prevents the formation of the ternary complex. Initially, there is still adequate IGFBP-3 available to bind IGF-I and form a binary complex. However, because of its smaller molecular weight, the IGF-I-IGFBP-3 complex is rapidly removed from the circulation, leading to a decline in plasma IGF-I. In addition, because of the upregulation of IGFBP-1, the relative percentage of IGF-I bound to low-molecular-weight, the IGF-I-IGFBP-3 complex is rapidly removed from the circulation, leading to a decline in plasma IGF-I. In addition, because of the upregulation of IGFBP-1, the relative percentage of IGF-I bound to this low-molecular-weight binding protein is expected to increase, thereby decreasing the circulating concentration of free IGF-I. Collectively, these changes would be expected to exacerbate the catabolism of muscle protein.

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