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Thermal injury activates the eEF2K-dependent eEF2 pathway in pediatric patients

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Abstract

Background—Burn induces a hypermetabolic state characterized by alterations in protein metabolism which is associated with increased morbidity and mortality. Eukaryotic elongation factor 2 (eEF2) plays a crucial role in regulating protein synthesis in many diseases, but whether it participates in burn-induced hypermetabolism is unclear. The aim of this study was to determine the expression of eEF2 and the upstream eEF2-inactivating kinase, eEF2K, in severely burned pediatric patients.

Methods—Eight pediatric patients (> 40% TBSA) and three non-burned pediatric volunteers were enrolled in this study. Muscle and skin biopsies were collected at early (0–10 days post burn [dpb]), middle (11–49dpb), and late (50–365dpb) time points. Resting energy expenditure (REE), body composition, and muscle protein synthesis rate (FSR) were measured. Proteins were extracted and analyzed by Western blotting. To further investigate the protein synthesis pathway, microarray data from muscle and skin were examined from 22 non-burned and 20 burned children.

Results—Burn patients exhibited a profound hypermetabolic response, as seen by a significant increased in REE ($p < 0.05$) and a loss of lean body mass (LBM) without altered muscle FSR, indicating a shift to catabolism after thermal injury. In muscle, the phosphorylation of eEF2K-dependent eEF2 was down-regulated early and middle post-burn. Similar changes in eEF2K and

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Clinical Relevancy Statement: This project provides novel data on how stress in human causes dramatic catabolic and hypermetabolic responses and discusses the profound impairment in protein synthesis.

eEF2 levels occurred in skin at the early time point. Total amounts of eEF2 and eEF2K were not altered.

Conclusions—Burn induces prolonged activation of eEF2K and eEF2. Alterations in these mediators may contribute to profound hypermetabolism seen in severely burned patients.

Keywords

burn; muscle; skin; protein synthesis; hypermetabolism

INTRODUCTION

Severe burn injury is associated with broad hypermetabolic alterations, including hyperglycemia, lipolysis, and protein catabolism.^{1, 2} Without proper treatment, multiorgan failure (MOF) and sepsis may occur, leading to significant morbidity and mortality.^{3, 4} One of the major causes of MOF, sepsis, and mortality after burn injury is increased protein catabolism. Marked alterations in protein metabolism persist for a prolonged period after burn injury and have been shown to influence burn patient survival and recovery.^{4, 5}

Protein metabolism relies on the equilibrium between protein synthesis and protein breakdown. Fang and his colleagues⁶ showed that thermal injury is associated with profound catabolism by the increased muscle protein breakdown and reduced protein synthesis in the animal model. Understanding the mechanism and regulation of post-burn protein metabolism can be of further benefit when developing approaches to reduce protein breakdown as well as increase protein synthesis.⁷ By reducing the net protein loss in severe burned patients, we expect to see dramatic improvements in patient survival and outcomes.

Protein synthesis includes initiation, elongation and termination steps during the translation of messenger ribonucleic acid (mRNA) into an amino acid chain that becomes the protein.⁸ Many protein molecules participate in regulation of protein translation at each single step. Protein biosynthesis is mainly regulated by mammalian target of rapamycin (mTOR) interacting with eIF4E-binding proteins-1 (4E-BP-1), eukaryotic initiation factor 2 α (eIF2 α), and ribosome p70 S6 kinase at the beginning point of protein translation.⁹ However, other mechanisms may contribute toward protein synthesis control as well.

Eukaryotic elongation factor 2 (eEF2) is a key factor in the elongation step of protein synthesis.¹⁰ It functions to translocate nascent protein chains from the A site to the P site of ribosomes in a guanosine-5'-triphosphate (GTP)-dependent manner. Activation of eEF2 is regulated by a highly specific calcium/calmodulin-dependent kinase known as eEF2 kinase (eEF2K). eEF2K phosphorylates and inactivates eEF2, thereby inhibiting protein synthesis.

Several studies have endorsed the importance of the eEF2K/eEF2 pathway in protein translation control.^{11, 12} Huichalaf et al¹¹ isolated myoblasts in patients with myotonic dystrophy 2 disease and found that ZNF9 gene regulates protein synthesis by binding to the 5' UTRs of key regulators of protein translation such as eEF2. In 2005, Li X et al¹² reported that mTOR-eEF2k-eEF2 translational control pathway is required to regulate tau protein accumulation in human Alzheimer's disease. As a key factor in regulating protein biosynthesis, eEF2K dependent eEF2 showed its important role in clinical diseases. However, there is lack of information about the eEF2K/eEF2 pathway in response to hypermetabolic status after severe burn.

An *in vitro* study recently revealed that fluctuation in calcium levels and endoplasmic reticulum (ER) stress can induce eEF2K-dependent eEF2 phosphorylation to promote protein translational arrest.¹³ The ER is the essential compartment for the synthesis and

folding of secreted proteins with post-translational modifications. ER stress occurs in response to many external factors that stimulate events accompanied by disruption of ER function. We have recently reported that severe burn injury is associated with depletion of hepatic ER calcium stores and induction of the ER stress response.¹⁴ We have also shown that in pediatric patients, severe burn injury induces ER stress that persists for up to one year in peripheral blood leukocytes, muscle, and fat up to one year post-burn.¹⁵ These findings have led us to hypothesize that severe burn injury modulates the interaction of eEF2K with eEF2 through alterations in intracellular calcium and induction of ER stress, further reducing protein synthesis after burn injury. Here, we investigated changes in expression of eEF2K/eEF2 in muscle and skin from pediatric burn patients.

PATIENTS AND METHODS

Eight severely burned pediatric patients were enrolled in this study within 96 hours of burn injury and followed for up to one year after injury at the Shriners Burns Hospital-Galveston. Patients had a minimum burn size of 40% of total body surface area (TBSA). The study was approved by the Institutional Review Board of the University of Texas Medical Branch, Galveston, Texas. Informed consent was obtained from the patient and/or the legal guardian prior to initiation of the studies. All patients were treated with the following standardized protocol: immediately following admission, resuscitation was guided by the Galveston formula of 5000 ml/m² TBSA burned + 2000 ml/m² TBSA lactated Ringer's solution given in increments over the first 24 hours. Within 48 hours of admission, all patients underwent total burn wound excision and the wounds were closed with available autograft skin and allograft on the remaining open areas. Patients returned to the operating room as soon as the donor sites healed and were available for reharvest. Sequential staged surgical procedures for repeated excision and grafting continued until the wounds were 95% healed.

Patients underwent the same nutritional treatment according to a standardized protocol. The intake was calculated as 1500 kcal/m² body surface + 1500 kcal/m² area burned, or the resting energy expenditure (REE) and multiplied by 1.4, with weekly adjustments as previously published.¹⁶ Briefly, patients received enteral nutrition via nasoduodenal tubes with Vivonex TEN (Sandoz Nutritional Corp., Minneapolis, MN). The composition of Vivonex is 82% carbohydrate, 15% protein, and 3% fat. This feeding regimen was started at admission and continued until the wounds were healed.

Muscle and skin biopsies were obtained in the operating room at early (0–10 days post burn [dpb]), middle (11–49 dpb), and late (50–365 dpb) time points. The final time point was particularly broad as it encompassed when the patients returned to the operating room for clinical needs. The time grouping for analysis was determined by the activation of intracellular ER stress signals in response to post-burn hypermetabolic status in a previous study.¹⁵ Three non-burned pediatric patients undergoing elective surgeries were enrolled as non-burned controls to provide normal, non-burned protein expression levels. None of the control patients had underlying diseases (including muscle wasting or paralysis), and all patients underwent the same biopsy procedures including anesthesia and specimen excision.

Protein metabolism after burn injury was assessed by measuring REE, body lean mass (LBM), and muscle protein fractional synthesis rate (FSR). All of these methods have been published previously,¹⁷ and briefly described as following.

Indirect calorimetry

Resting energy expenditure (REE) was measured using a SensorMedics 2900 metabolic cart (SensorMedics, Yorba Linda, Calif). All indirect calorimetry measurements were made at 30°C, which is the standard environmental setting while the subject was asleep between

midnight and 5 A.M. Composition of inspired and expired gases were sampled and analyzed at 60-second intervals. Values of V_{CO_2} , $\dot{V}O_2$, and REE were accepted when they were at a steady state for 5 minutes. The average REE was calculated from these steady-state measurements. For statistical comparison, energy expenditure was expressed both as absolute REE and as the percentage of the basal metabolic rate predicted by the Harris-Benedict equation. Estimated basal energy expenditure, based on age, sex, height, and weight, was calculated using the Harris-Benedict equation to normalize the measured REE values to a percentage of predicted expenditure.

Body composition

Total body lean mass (LBM), fat mass, and bone mineral content (BMC) were measured by dual-energy x-ray absorptiometry. A Hologic model QDR-4500W dual-energy x-ray absorptiometer (Hologic Inc, Waltham, Mass) was used to measure body composition. To minimize systematic deviations, the Hologic system was calibrated daily against a spinal phantom in the anteroposterior, lateral, and single-beam modes. Individual pixels were calibrated against a tissue bar phantom to determine whether the pixel was reading bone, fat, lean tissue, or air.

Muscle protein synthesis

Because phenylalanine is neither synthesized nor degraded in the peripheral tissues (it is metabolized only in the liver), a stable isotope tracer incorporation technique with a primed-constant infusion of L-[ring- 2H_5]-phenylalanine was applied to measure skeletal muscle protein synthesis rate. Blood samples were taken simultaneously from an ipsilateral femoral artery and vein for this determination. Indocyanine green was used to determine leg blood flow. The blood concentration of unlabeled phenylalanine was determined by gas chromatography-mass spectrometry using the internal standard approach and the tert-butyltrimethylsilyl esters, and muscle protein synthesis rates were calculated as previously published.¹⁸

Antibodies and reagents

Antibodies to phosphorylated eEF2K (Ser366), eEF2K, phosphorylated eEF2 (Thr56), and eEF2 were purchased from Cell Signaling Tech, Inc. (Danvers, MA). SuperSignal West Pico Chemiluminescent Substrate was purchased from Thermo Scientific Inc, (Rockford, IL).

Western blotting

Protein was extracted from the tissue samples using our standard lab protocol. Briefly, approximately 50 mg of frozen tissue was homogenized in 150 mM NaCl, 50 mM Tris-HCl, pH 7.8, 1% (w/v) Triton X-100, 1 mM EDTA, 0.5 mM phenylmethanesulfonyl fluoride, 1X Complete protease inhibitor mixture (Roche Molecular Biochemicals, Indianapolis, IN) and a phosphatase inhibitor cocktail (Sigma-Aldrich, St. Louis, MO). The homogenate was centrifuged at $20,000 \times g$ for 30 minutes at 4°C and the pellet discarded. Thirty micrograms of each protein sample was subsequently analyzed by SDS-PAGE and Western blotting. Band intensities were quantified using the GeneSnap/GeneTools software (Syngene, Frederick, MD). Activation of eEF2K and eEF2 was determined by normalizing the band intensities for phosphorylated eEF2K and eEF2 to intensities for the total forms of these proteins.

Patients for genomic analysis

Twenty pediatric burn patients, admitted to the Shriners Hospitals for Children, Galveston, Texas, were selected from the patients enrolled in the Inflammation and the Host Response to Injury Collaborative Research Program.¹⁵ Patients were selected for this analysis if they

were 0 to 18 years of age, admitted to our institute within 96 hours following the burn injury, had burns covering more than 40% of their TBSA, did not receive anti-catabolic or anabolic agents, and had genomic data for at least one tissue available at two time points. The non-burned cohort (22 controls) included patients undergoing elective surgeries (tissue samples). The study was reviewed and approved by the Institutional Review Board of the University Texas Medical Branch, Galveston, Texas. Prior to the study, each subject, parent or child's legal guardian had to sign a written informed consent form.

Muscle and skin biopsies were taken at similar time points in the operating room, placed in RNAlater, and subsequently stored at -80°C according to the Glue Grant tissue collection protocol (www.gluegrant.org). Tissue biopsies from both burned patients and non-burned patients were obtained and stored in identical manner.

Sample processing for genomic analysis

Commercial kits from Qiagen (Valencia, CA) were used to extract total cellular RNA from skin and muscle (RNeasy Fibrous Tissue Midi Kit). RNA purity was assessed by capillary electrophoresis (Agilent 2100 Bioanalyser, Agilent Inc, Santa Clara, CA). cRNA was synthesized and hybridized onto Affymetrix U311 Plus 2.0 arrays and processed according to the protocol developed by Affymetrix.

Genomics data analysis

Genomics data from the Inflammation and the Host Response to Injury Collaborative Research Program TRDB web site (<https://www.gluegrant.org/trdb>) were utilized to identify, in skin and muscle, the signaling pathways impacted by a severe burn injury by comparing expression data to tissue from normal non-burned children. Fifty-five microarrays corresponding to skin samples from 13 patients and 60 microarrays corresponding to muscle samples from 18 patients, and harvested between 2 and 425 days post-burn were selected for this analysis. Microarrays data from 22 non-burned children were included as well: 19 skin and 15 muscle. Normalized microarray expression data were downloaded for analysis. We analyzed 54,613 probesets on the U133 plus 2.0 Affymetrix GeneChips. Significance Analysis for Microarrays (SAM)¹⁹ was used to compare the expression values in burn patients and non-burn patients for each tissue at each time point. An estimated False Discovery Rate of less than 10% was used to identify probe sets that were different in burned versus non-burned patients (parameters were set broadly in order to facilitate discovery of pathways that are over-represented). The resulting probe sets were then uploaded into the Ingenuity Pathways Knowledge Base to identify molecules in the eEF2 pathway that were significantly modulated in response to a severe burn injury. The canonical pathways in Supplemental Figures 1 and 2 were generated through the use of Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com).

Statistical analysis

Statistical analysis was performed by one-way ANOVA with Tukey's test. Data are reported as the mean $\pm$ SEM. Significance was accepted at $p < 0.05$.

RESULTS

Eight severely burned pediatric patients had an average burn size of 66% TBSA (Table 1). No significant differences between the non-burned or burn injury groups were found for age, gender, height or body weight. Demographics data for patients included in the genomics studies are presented in Table 2.

The percentage of predicted REE at the early ($132.3 \pm 3.9\%$), middle ($142.5 \pm 6.3\%$), and late ($128.5 \pm 6.3\%$) time points after burn injury were significantly greater than published pediatric control values ($103.6 \pm 5\%$) ($P < 0.05$).²⁰ This confirms that the hypermetabolic response persists for a prolonged period after burn in pediatric patients.

Dual energy X-ray absorptiometry (DEXA) revealed that the proportion of total LBM to body weight significantly decreased during the time course after burn injury (early time point $80.25\% \pm 1.72\%$ vs. middle time point $71.25\% \pm 2.69\%$ and late time point $74.21\% \pm 1.51\%$ respectively, $P < 0.05$), while the proportion of total fat to body weight increased (early time point $21.58\% \pm 2.17\%$ vs. middle time point $27.03\% \pm 1.96\%$ and late time point $23.30\% \pm 1.45\%$, $P < 0.05$).

Compared to published non-burned FSR values ($0.0857 \pm 0.0119\%/h$),²¹ the muscle FSR in pediatric burn patients did not differ significantly at the early ($0.1020 \pm 0.0079\%/h$), middle ($0.1250 \pm 0.0408\%/h$) or late ($0.0859 \pm 0.0220\%/h$) time points following the burn injury.

Post-burn eEF2K/eEF2 alteration in muscle tissue

Expression of total eEF2K and total eEF2 remained constant across all time points, indicating that changes in these signaling molecules would be at the post-translational level (Figure 1). Genomic expression data from muscle taken at the early, middle, and late time points confirm that regulation of eEF2 and eEF2K does not occur at the transcript level (Supplemental Figure 1). Western blot analysis revealed that the ratio of phosphorylated eEF2K to total eEF2K significantly increased at early and middle time points after burn injury ($p < 0.05$) (Figure 1), indicating that eEF2K activation persisted for up to 49 days after burn injury. Surprisingly, the ratio of phospho-eEF2 to total eEF2 was significantly decreased at early and middle time point post-burn ($P < 0.05$) (Figure 1), indicating that eEF2 exhibited the same prolonged pattern of activation as eEF2K.

Post-burn eEF2K/eEF2 alteration in skin tissue

Expression of total eEF2K and total eEF2 remained constant across all time points (Figure 2). Analysis of the post-burn skin genome confirmed that the messages for eEF2 and eEF2K, demonstrating that the alterations in these molecules were not at the transcript level (Supplemental Figure 2). The ratio of phosphorylated eEF2K to total eEF2K significantly increased at early time point after burn injury in skin tissue ($P < 0.05$). However, no alterations in eEF2K were detected thereafter. The ratio of phosphorylated eEF2 to total eEF2 significantly decreased at early time point post-burn (Figure 2) with no significant differences occurring at any of the later time points.

DISCUSSION

In the current study, we found that severe burn injury induces activation of the eEF2K dependent eEF2 pathway both in muscle and skin in pediatric patients. Patients presented a hypermetabolic status of increased REE and decreased LBM in addition to activation to eEF2K dependent eEF2 pathway, but we were unable to conclusively demonstrate a reduction in muscle protein synthesis.

Recent studies have shown that severe burn injuries induce ER stress²² and that ER stress inhibits protein synthesis via eEF2K/eEF2 pathway.²³ We hypothesized that eEF2K phosphorylates and inhibits eEF2, leading to reduced protein synthesis in response to severe thermal injury. However, we found that eEF2K activation was associated with decreased eEF2 phosphorylation in both muscle and skin in burn pediatric patients. Furthermore, the muscle FSR in burned patients demonstrated that no augmentation of protein synthesis occurred alongside eEF2 activation.

Muscle protein synthesis after thermal injury varies depending on species, injury severity, and muscle type. Fang and his colleagues⁶ showed that thermal injury inhibited protein synthesis and increased protein breakdown, leading to a pronounced catabolic response in a burned rat model. Shangraw, et al²⁴ reported that protein synthesis by burned-limb soleus and plantaris muscles was significantly elevated by 114% and 67%, respectively. In a previous clinical study, our group reported that, after burn injury, significant protein breakdown occurred in the absence of any alteration in protein synthesis, resulting in a negative net balance in muscle protein.²⁵ The patients included in the current study exhibited the same pattern: there were no alterations in muscle protein fractional synthesis rate (FSR). Furthermore, the proportion of LBM to body weight significantly decreased after a severe burn confirming that protein catabolism is a dominant response to burn injury.

In the current study, muscle protein synthesis was not altered despite the activation of the eEF2K/eEF2 pathway, which may suggest a non-canonical role for eEF2 in the skeletal muscle protein synthetic pathway after thermal injury. Lang, et al⁹ found that protein synthetic rates in rat heart, liver, gastrocnemius and kidney were different in response to burn; the protein synthesis in rat skeletal muscle was unaltered after burn which is compatible of our data. They further concluded that the initiation step, not the elongation step, is the main contributor toward protein synthesis in myocardium (only ventricle from rat heart). However, they did not exam eEF2k/eEF2 in skeletal muscle, so that we are unable to observe the role of eEF2k/eEF2 in skeletal muscle protein synthesis after burn. A practical assumption of the unexpected response in the current study is that the muscle fiber types were not recorded during sample collection. Rose and his colleagues²⁶ showed that eEF2 phosphorylation is decreased by half in type I fibers compared to type II fibers. Thus, the study of protein synthetic signaling pathways in the context of specific muscle fiber types should be further discerned.

Intracellular molecules including AMP-activated protein kinase (AMPK), mTOR, and MAPK, mediate the eEF2K/eEF2 pathway. Knebel et al²⁷ demonstrated that eEF2K is phosphorylated and inhibited by MAPK. Proud and colleagues²⁸ showed that eEF2K is inactivated by insulin via mTOR. In response to burn injury, tissue adenosine triphosphate (ATP) levels gradually fall, and increasing levels of adenosine monophosphate (AMP) lead to the increased ratio of AMP to ATP.²⁹ As a result, AMPK is activated, phosphorylating eEF2 to inhibit protein synthesis.³⁰

In the current study, we found that eEF2 phosphorylation was down-regulated following burn injury. In an *in vitro* model, deprivation of leucine led to eEF2 phosphorylation and inhibition of protein synthesis in myoblasts, revealing the important role of essential amino acids in regulating global protein synthesis via the eEF2 pathway.³¹ The early stage of burn care, especially enteral nutrition and burn wound excision have already been shown to reduce the severe burn patients' morbidity and mortality.³² Early enteral nutrition has been shown to improve gut function, decreases bacterial translocation, and therefore, ameliorates post-burn hypermetabolism. In animal models, early nutrition significantly improved liver function with reduction of plasma tumor necrosis factor (TNF);³³ Chang and his colleagues³⁴ showed that early stage of escharectomy decreased pro-inflammation after burn. Though the molecular mechanism of its effect is not clear, we speculate that the early stage of clinical treatment and appropriate nutrition support could alleviate hypermetabolic status of severe burn patients by influencing intracellular energy consumption of ATP/AMP, therefore, modulating signaling pathway in protein synthesis.

In the current study, we found that the time course of eEF2K and eEF2 activation in severely burned pediatric patients was tissue-specific, with activation occurring early in both skin and muscle, but persisting longer in muscle (10 days in skin vs. 49 days in muscle). Patterson

and colleagues³⁵ found that protein synthesis rates in response to a high-protein diet differed between skin and muscle in burn patients. They concluded that high-protein intake improves wound healing but has little benefit of muscle protein synthesis rates. These data suggest that protein synthesis responses to severe burn injury are tissue-specific and show that improving protein synthesis rates after burn injury will require tissue-specific interventions (e.g. diet or drugs). We and others^{36, 37} showed that either nutrition supplementation, anabolic agents, or anti-catabolic agents such as insulin, oxandrolone and propranolol, may attenuate protein catabolism in burn patients. We also showed a different time response for activation of the eEF2K/eEF2 pathway between muscle and skin in response to thermal injury. We recommend that investigators remain cognizant of probable tissue-specific protein synthesis regulation after burn injury.

In the current study, we collected tissue samples in 3 non-burned subjects as controls for Western blotting analysis. The size of group is small as it is difficult to obtain skin samples from non-burned patients, which is a potential weakness of this study. However, the controls had similar data for protein expression. In the larger cohort used for the genomics studies, the 20 non-burned patient samples yielded similar results, giving us increased confidence in our findings.

In the summary, we have demonstrated that prolonged, tissue-specific eEF2K and eEF2 activation occurs in both muscle and skin tissue in pediatric burn patients. Skeletal muscle protein synthesis may not be related to eEF2K/eEF2 activation in response to thermal injury; further studies using specific muscle fibers are necessary for confirmation of this. We suggest that further investigation into other related protein synthesis and proteolysis signaling pathways is necessary to understand the entirety of post-burn hypermetabolic alteration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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GLOSSARY

Genomics

To study the whole set of DNA sequences of a cell or tissue, and determine fine-scale genetic mapping. By using a gene chip microarray

Protein translational arrest

technique, we analyze RNA sample to understand the complex of pathway signals in response to pathophysiological changes.

Transfer RNA (tRNA) translated to polypeptide on a ribosome through activation, initiation, elongation and termination steps. Alteration of any activators in regulating these steps may interfere with protein polypeptide synthesis, even cause protein translational arrest and inhibit protein synthesis.

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Figure 1. Alteration of eEF2K and eEF2 in muscle after burn injury

A) Western blot data showing expression of eEF2K and eEF2 proteins and their phosphoprotein levels, with GAPDH as a loading control. **B)** The ratio of phospho-eEF2K to total eEF2K significantly increased at early and middle time point post-burn. **C)** The ratio of phospho-eEF2 to total eEF2 correspondingly decreased in muscle samples isolated from three non-burned controls and eight burned patients. Data are presented as means $\pm$ SEM. *P < 0.05, significant difference between burned and non-burned.

Figure 2. Alteration of eEF2K and eEF2 in skin after burn injury

A) Western blot data showing protein expression of eEF2K and eEF2 proteins and their phosphoprotein levels in skin samples with GAPDH as a loading control. **B)** The ratio of phospho-eEF2K to total eEF2K and **C)** the ratio of phospho-eEF2 to total eEF2 in skin tissue changed only at the early time point post burn, as seen by protein band intensity quantification from the same study population above. Data are presented as means $\pm$ SEM. * $P < 0.05$, significant difference between burned and non-burned.

TABLE 1

Patient Demographics

Patient Demographics	Non-Burned	Burned
n	3	8
Age (year)	11 $\pm$ 3	8 $\pm$ 2
Gender (M/F)	2/1	6/2
Body weight (kg)	53 $\pm$ 14	32 $\pm$ 8
Height (cm)	142 $\pm$ 18	125 $\pm$ 11
LOS ICU (day)	N/A	35 $\pm$ 7
% TBSA burn	N/A	66 $\pm$ 4
% TBSA third degree	N/A	55 $\pm$ 10

Data are presented as means $\pm$ SEM.

TABLE 2

Patient Demographics for Genomic Studies

Patient Demographics	Non-Burned	Burned
n	22	20
Age (year)	11 $\pm$ 2	8 $\pm$ 1
Gender (M/F)	13/9	12/8
LOS ICU (day)	N/A	39 $\pm$ 5
% TBSA burn	N/A	64 $\pm$ 3

Data are presented as means $\pm$ SEM.