Published in final edited form as:

Ann Surg. 2012 February ; 255(2): 370–378. doi:10.1097/SLA.0b013e31823e76e7.

Severe Injury Is Associated With Insulin Resistance, Endoplasmic Reticulum Stress Response, and Unfolded Protein Response

Marc G. Jeschke, MD, PhD^{*}, Celeste C. Finnerty, PhD^{†,‡}, David N. Herndon, MD^{†,‡}, Juquan Song, MD^{†,‡}, Darren Boehning, PhD[§], Ronald G. Tompkins, MD[¶], Henry V. Baker, PhD^{||}, and Gerd G. Gauglitz, MD^{†,**}

^{*}Ross Tilley Burn Centre, Sunnybrook Health Science Centre, Department of Surgery, Sunnybrook Research Institute, University of Toronto, Ontario, Canada

[†]Shriners Hospitals for Children, Galveston, TX

[‡]Department of Surgery University of Texas Medical Branch, Galveston, TX

[§]Department of Neuroscience and Cell Biology, University of Texas Medical Branch, Galveston, TX

[¶]Department of Surgery, Massachusetts General Hospital, Shriners Hospital for Children, and Harvard Medical School, Harvard University, Boston, MA

^{||}Department of Molecular Genetics and Microbiology, University of Florida College of Medicine, Gainesville, FL

^{**}Department of Dermatology and Allergology, Ludwig-Maximilian-University Munich, Frauenlobstrasse, Munich, Germany

Abstract

Objective—We determined whether postburn hyperglycemia and insulin resistance are associated with endoplasmic reticulum (ER) stress/unfolded protein response (UPR) activation leading to impaired insulin receptor signaling.

Background—Inflammation and cellular stress, hallmarks of severely burned and critically ill patients, have been causally linked to insulin resistance in type 2 diabetes via induction of ER stress and the UPR.

Methods—Twenty severely burned pediatric patients were compared with 36 nonburned children. Clinical markers, protein, and GeneChip analysis were used to identify transcriptional changes in ER stress and UPR and insulin resistance–related signaling cascades in peripheral blood leukocytes, fat, and muscle at admission and up to 466 days postburn.

Results—Burn-induced inflammatory and stress responses are accompanied by profound insulin resistance and hyperglycemia. Genomic and protein analysis revealed that burn injury was

Copyright © 2012 by Lippincott Williams & Wilkins

Reprints: Marc G. Jeschke, MD, PhD, Ross Tilley Burn Centre, Department of Surgery, Division of Plastic Surgery, Sunnybrook Health Science Centre and University of Toronto, 2075 Bayview Avenue, Room D704, Toronto, ON M4N 3M5, Canada. Marc.Jeschke@sunnybrook.ca..

Drs. Jeschke and Finnerty contributed equally to this work.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.annalsofsurgery.com).

associated with alterations in the signaling pathways that affect insulin resistance, ER/sarcoplasmic reticulum stress, inflammation, and cell growth/apoptosis up to 466 days postburn.

Conclusion—Burn-induced insulin resistance is associated with persistent ER/sarcoplasmic reticulum stress/UPR and subsequent suppressed insulin receptor signaling over a prolonged period of time.

A burn injury represents one of the most severe forms of trauma and occurs in more than 2 million people in the United States per year.¹ A severe burn is a devastating injury, affecting nearly every organ system and leading to significant morbidity and mortality.^{2,3} Hyperglycemia and insulin resistance are common pathophysiologic phenomena in burn and critically ill patients and are hallmarks of burn-induced diabetes.³ During the early phases, postburn hyperglycemia is caused by an increase in the rate of glucose production coupled with impaired tissue extraction of glucose, ultimately resulting in increased levels of circulating glucose and lactate.^{4,5} Burn injury leads not only to inefficient insulin-mediated glucose⁶ and lipid⁷ metabolism, but also to impaired protein anabolism.⁸ The clinical relevance of hyperglycemia after a severe burn was shown in recent studies by our group. We assessed the relationship between hyperglycemia and adverse clinical outcomes after severe burn.^{9,10} Patients with poor glucose control had a significantly higher incidence of bacteremia/fungemia and mortality.^{9,10} In addition, we found that hyperglycemia exaggerated protein degradation, enhancing the catabolic response. Hemmila and colleagues¹¹ confirmed these results in their recent study indicating that hyperglycemia associated with insulin resistance represents a significant clinical problem in burn patients, as has been demonstrated in critically ill and trauma patients. Although the metabolic consequences of postburn hyperglycemia and insulin resistance have been delineated, the molecular mechanisms underlying stress-induced insulin resistance are essentially unknown.

It has become increasingly clear that a variety of cellular stress signaling and inflammatory pathways are activated as a consequence of burn injury.³ A critical player in the cellular stress response is the endoplasmic reticulum (ER) [the sarcoplasmic reticulum (SR) in muscle], a membranous organelle that functions in synthesizing and processing secretory and membrane proteins.^{12–14} Certain pathologic stress conditions disrupt ER homeostasis and lead to accumulation of unfolded or misfolded proteins in the ER lumen. To cope with this stress, a signal transduction pathway, called the unfolded protein response (UPR), is activated to limit the accumulation of unfolded polypeptides in the ER lumen. The UPR functions as a prosurvival response that reduces the unfolded protein burden and restores ER homeostasis.¹⁵ However, when protein misfolding is persistent and cellular stress cannot be resolved, UPR signaling switches from prosurvival to proapoptotic. The central role of the ER stress/UPR was recently shown in 2 studies in diabetic mice in which the authors suggested that ER stress and the UPR are of major importance in the development of insulin resistance.^{12,16} Conditions that trigger ER stress include the presence of hypermetabolism, specific proinflammatory cytokines [such as interleukin (IL)-6, monocyte chemoattractant protein-1 (MCP-1), or tumor necrosis factor- α (TNF- α)], stress hormones (cortisol and catecholamines), increased synthesis of secretory proteins, many of which are present after burn injury.^{3,12,14,17} The ER stress response and UPR thus seem to be central links between stress, inflammatory, and hypermetabolic responses postburn. Although we have recently demonstrated that burn injury induces ER stress and the UPR in mice and rats,^{18,19} neither the occurrence of these phenomena nor their association with hyperglycemia and insulin resistance have been reported in human patients with burns. In this study, we examined whether postburn insulin resistance and hyperglycemia is associated with ER/SR stress and the UPR for up to a year after the burn injury and compared the expression pattern of genes and proteins known to be involved in inflammation, ER/SR stress, and insulin resistance

signaling pathways in peripheral blood leukocytes, fat, and muscle from severely burned and nonburned patients.

PATIENTS AND METHODS

Twenty pediatric burn patients admitted to the Shriners Hospitals for Children, Galveston, TX, were selected from the patients enrolled in the Inflammation and the Host Response to Injury Collaborative Research Program. Patients were selected for this analysis if they were 0 to 18 years of age, admitted to our institute within 96 hours after the burn injury, had burns covering more than 40% of their total body surface area (TBSA), did not receive anticatabolic or anabolic agents, and had genomic data for at least 1 tissue available at 2 time points. The nonburned cohort (36 controls) included volunteers [plasma, peripheral blood leukocytes, and resting energy expenditure (REE)] and patients undergoing elective surgeries (plasma, REE, peripheral blood leukocytes, and tissue samples). Tissue samples were obtained during surgery from a location that allowed tissue collection. Skin, fat, or muscle were obtained and immediately snap frozen. These control samples were obtained only at one time point and not over a time course. However, because these patients are not burned, we hypothesized that a time course is not needed for comparison. The study was reviewed and approved by the institutional review board of the University Texas Medical Branch, Galveston, TX. Prior to the study, each subject, parent, or child's legal guardian had to sign a written informed consent form.

Admission Data

On admission, the extent and degree of burn was assessed and recorded on a standard Lund and Browder chart by the attending burns surgeon present. Information also recorded at the time of admission included burn-related (date and mechanism) and demographic data (age and sex), as presented in Table 1.

All patients were treated in our pediatric burns intensive care unit according to standardized protocols as previously published.²⁰ During acute hospitalization, all patients were fed enterally with Vivonex TEN at a rate of 1.4 times the predicted REE. None of the patients received total parenteral nutrition or any anabolic or anticatabolic agents. Once patients were discharged from the intensive care unit, food intake was monitored by our dietician and adjusted if needed. We have no information on feeding in the control group. We assume that these children eat regular meals, because none of the controls were diabetic at the time of surgery.

Time Points and Data Collection

Various parameters thought to be associated with stress-induced insulin resistance were measured at admission (0 to 10), 10 to 49, and 50 to 250 days postburn and compared with values from nonburned children. If any patient had more than 1 measurement performed during each time period, results were averaged to give a single mean result for each patient at each time period.

Urinary Catecholamine and Cortisol Measurements

Twenty-four-hour urine collections were taken regularly throughout acute hospital stay and during admissions for reconstructive operations and rehabilitation services. Samples were collected and chilled by the bedside prior to transport to our clinical laboratory for processing using high-performance liquid chromatography (HPLC) techniques. In brief, specimens were acidified to pH 2 with hydrochloric acid and 1 mL of acidified urine and were extracted on an HLB Oasis cartridge (Waters Corporation, Milford, MA) previously conditioned with 1 mL of methanol and 1 mL of deionized water. The cartridge was washed

with a solution of 25% methanol in water (pH 10.9) and eluted with 100% methanol (pH 10.9 with ammonium hydroxide). The eluent was evaporated under a gentle stream of air to dryness and reconstituted with 50% methanol in HPLC grade water (pH 2.9) and submitted to HPLC analysis. HPLC analysis was performed using a symmetry shield 3.5 μm , 4.6 $\times$ 150-mm column (Waters Corporation, Milford, MA), using a mobile phase consisting of 30% to 35% methanol in water with 0.1% trifluoroacetic acid, with UV detection at 245 nm. Urinary catecholamines were analyzed using HPLC techniques. Extraction of the catecholamines from acidified urine samples were performed using a Bio-Rad kit (Bio-Rad, Hercules, CA), according to the manufacturer's instructions.

Serum Cytokine and Hormone Measurements

Blood was collected from the burn patients within the earlier-mentioned time frame for serum cytokine analysis. Blood was drawn in a serum-separator collection tube and centrifuged for 10 minutes at 1320 rpm; the serum was removed and stored at -70°C until assayed. The Bio-Plex Human Cytokine 17-Plex panel was used with the Bio-Plex Suspension Array System (Bio-Rad) to profile expression of MCP-1 and IL-6. The assay was performed according to the manufacturer's instructions.

Indirect Calorimetry

As part of our routine clinical practice, all patients underwent REE measurements within the time period studied. Resting energy expenditure was measured using a Sensor-Medics Vmax 29 metabolic cart (Yorba Linda, CA) as previously published.^{3,21} REE was calculated from the oxygen consumption and carbon dioxide production by equations described before. For statistical comparison, measured energy expenditure was expressed as the percentage of the basal metabolic rate predicted compared with predicted norms based on the Harris-Benedict equation and with body mass index.

Fasting Serum Glucose and Insulin Measurements

Serum glucose concentrations were quantified using a hexokinase assay on a Dimension Instrument (Dade Behring/Siemens Healthcare Diagnostics, Dade Behring, Deerfield, IL). Serum insulin concentrations were determined by common enzyme-linked immunosorbent assay techniques (Diagnostic Systems Laboratories/Beckmann-Coulter, Webster, TX).

Tissue Sampling

For genomic analysis, muscle and fat biopsies were taken in the operating room, placed in RNA later for genomic analysis, and subsequently stored at -80°C according to the Glue Grant tissue collection protocol (www.gluegrant.org). For protein analysis, tissue biopsies were immediately snap frozen and stored at -80°C .

Sample Processing for Genomic Analysis

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

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis and Immunoblotting

To determine the effect of burn injury on insulin and ER-related signaling pathways in muscle, Western blot analysis was conducted. Each muscle biopsy was homogenized and solubilized in 150 mM NaCl, 50 mM Tris-HCl (pH 7.8), 1% (wt/vol) Triton X-100, 1 mM EDTA, 0.5 mM phenylmethanesulfonyl fluoride, and 13 complete protease inhibitor mixture

(Roche Molecular Biochemicals, Basel, Switzerland) for 5 minutes on ice and spun at 12,000g. The resultant supernatant (20 mg) was loaded and run on a 4% to 20% sodium dodecyl sulfate polyacrylamide gel and subsequently electrotransferred to nitrocellulose membranes (Bio-Rad Laboratories). Nitrocellulose sheets were then probed with primary antibody against inositol-requiring enzyme-1 (IRE-1), phosphorylated IRE-1 (Abcam Inc., Cambridge, MA), cleaved (p50ATF6), and precursor (p90ATF6). Activating transcription factor (ATF)-6, c-Jun N-terminal kinase (JNK), Thr^{183/185}-phosphorylated JNK, sarco-endoplasmic reticulum calcium Adenosine Triphosphatase (ATPase) (SERCA), insulin receptor substrate (IRS)-1, Ser⁶¹²-phosphorylated IRS-1, Tyr⁶¹²-phosphorylated IRS-1, phosphatidylinositol 3-kinase^{p85} (PI3K), phosphorylated PI3K^{p85}, protein kinase B (Akt), Thr³⁰⁸-phosphorylated AKT, B-cell lymphoma (BCL)-2, Cyclic Adenosine Monophosphate (cAMP)-dependent protein kinase, eukaryotic initiation factor (EIF)-2 α , signal transducers and activators of transcription (STAT)-1 and -3, extracellular signal-regulated kinases (ERK)-1/2, actin, and glyceraldehyde-3-phosphate-dehydrogenase (all from Cell Signaling Technology, Danvers, MA).

The signal was amplified using horseradish peroxidase-conjugated secondary antibody and developed with chemiluminescent substrates (Pierce Biotechnology, Rockford, IL). Results were quantified by using a densitometer to scan the blots (GENE GENIUS, Bio Imaging System; Syngene, Frederick, MD). For correcting the intensities of the phosphorylated protein bands, the respective phosphorylated form was divided by the total form of the respective protein or, in the case of nonphosphorylated proteins, by the intensity of the respective actin or glyceraldehyde-3-phosphate-dehydrogenase band. Quantitative values of the respective protein are expressed as mean $\pm$ SEM of at least 3 independent measurements per group and are presented as fold change to values of nonburned controls. In those cases where a single blot was probed sequentially with more than one antibody, the nitrocellulose was stripped at 60°C for 30 minutes in stripping buffer (2% sodium dodecyl sulfate, 100 mM β -mercaptoethanol, 62.5 mM Tris-HCl) before probing with the next antibody.

Statistics

Graphs were created using Origin Pro 8.0 (STATCON, Wittenhausen, Germany). Paired and unpaired Student *t* test, χ^2 analysis, and Mann-Whitney *U* tests were used where appropriate. Data are expressed as mean $\pm$ SD or SEM, where appropriate. Significance was accepted at *P* < 0.05.

Genomics Data Analysis

Genomics data from the Inflammation and the Host Response to Injury Collaborative Research Program trial database web site (<https://www.gluegrant.org/trdb>) were used to identify, in peripheral blood leukocytes, fat, and muscle, the signaling pathways impacted by a severe burn injury by comparing expression data to tissue from normal nonburned children. Forty-four microarrays corresponding to peripheral blood leukocyte samples from 13 patients, 44 microarrays corresponding to fat samples from 14 patients, and 60 microarrays corresponding to muscle samples from 18 patients, and harvested between 2 and 425 days postburn were selected for this analysis. Microarrays data from 36 nonburned children were included as well: 25 fat, 15 muscle, and 32 peripheral blood leukocyte. Normalized microarray expression data were downloaded for analysis. We analyzed 54,613 probe sets on the U133 plus 2.0 Affymetrix GeneChips. Significance Analysis for Microarrays²² was used to compare the expression values in burn patients and nonburn 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 nonburned patients (parameters were set broadly to facilitate discovery of pathways that are overrepresented.). The resulting probe sets (Table 2) were then uploaded into the Ingenuity Pathways

Knowledge Base to identify the genes and pathways that were significantly modulated in response to a severe burn injury. The canonical pathways in Supplemental Digital Content Figure 1 (<http://links.lww.com/SLA/A197>) were generated through the use of Ingenuity Pathways Analysis (Ingenuity Systems, Redwood City, CA, www.ingenuity.com). A literature search was used to identify genes associated with insulin resistance, ER stress, the UPR, inflammation, and diabetes; the genomic expression data sets were then interrogated to determine the involvement of these pathways after a severe burn injury. We then created a comprehensive interaction network comprised for each cell type of these genes and generated prototypical cells for peripheral blood leukocytes, fat, and muscle for each time point with the fold change in postburn versus nonburn gene expression values superimposed on this genomic network (Supplemental Digital Content Figs. 2–4, <http://links.lww.com/SLA/A197>). The identified pathways used to determine the protein signaling cascades to be elucidated via Western blotting.

RESULTS

Patient Demographics

Characteristics of burn patients and controls at the time of acute hospitalization are shown in Table 1. Patients were on average 8 years of age and suffered from severe burn injury of 64% TBSA burn and a third-degree burn of 54% TBSA. Nonburned control patients were similar in race and sex distribution but were significantly older, due to the nature of the surgical intervention conducted in these nonburned control patients. However, both groups were prepubertal, thus having similar hormonal profiles and, therefore, can be used for comparison, $P < 0.05$ (Table 1). None of the burn patients had sepsis or multiple organ failure, and only few patients had wound infections (Table 1).

Inflammatory and Stress Responses Postburn

We determined stress hormones in serum and urine and found that total urine cortisol levels initially increased 10- to 20-fold to $163 \pm 56 \mu\text{g}/24 \text{ hours}$ and remained significantly elevated compared with normal values ($5\text{--}21 \mu\text{g}/24$) for the entire length of the study, $P < 0.05$ (Fig. 1A). Epinephrine levels increased 4-fold ($P < 0.05$) after the burn injury, peaked approximately 1 week postburn and then gradually decreased for the remainder of the study; levels of epinephrine in burned children remained significantly higher than those in nonburned children, $P < 0.05$ (Fig. 1B). Norepinephrine levels followed a very similar pattern and were significantly increased throughout the entire study period, $P < 0.05$ (data not shown).

The 17 serum cytokines measured were significantly affected by the burn injury and increased between 2- and 1000-fold; here, we would like to focus on present expression data for 2 cytokines related to insulin resistance: MCP-1 and IL-6. Both cytokines were increased 100- to 1000-fold immediately postburn with levels of $1032 \pm 365 \text{ pg/mL}$ and $637 \pm 166 \text{ pg/mL}$, respectively, at the time of admission, $P < 0.05$ (Figs. 1C, D). Both cytokines remained significantly elevated throughout the entire study period.

Predicted REE demonstrated an immediate significant increase upon burn injury that was maintained for the remaining of the study indicating marked hypermetabolism, $P < 0.05$ (Fig. 1E). These data clearly indicate that burn induces vast inflammatory and stress responses.

Hyperglycemia and Insulin Resistance Postburn

Fasting serum glucose significantly increased postburn to $155 \pm 8 \text{ mg/dL}$ and then gradually decreased over time but remained significantly elevated for the entire study period, $P < 0.05$

(Fig. 1F). Fasting insulin levels were significantly increased with a peak around 40 to 60 days postburn, $P < 0.05$ (Fig. 1G). Increased fasting glucose levels along with increased fasting insulin levels are indicative of profound insulin resistance.

Genome-Wide Transcriptional Alterations in Peripheral Blood Leukocytes, Fat, and Muscle From Severely Burned Patients

Genomic expression data collected over 4 time periods (0–10, 11–49, 50–250, and 251 days postburn) in burned patients were compared with expression data from nonburned patients. We identified the following canonical signaling pathways in Ingenuity Knowledge Base that are associated with insulin resistance and ER/SR stress (Supplemental Digital Content, Fig. 1, <http://links.lww.com/SLA/A197>): ER/SR stress (A), insulin receptor (B), phosphoinositide-3-kinase (PI3K) (C), Extracellular regulated MAP kinase 1/2/mitogen-activated protein kinase 1 (ERK/MAPK) (D), c-Jun N-terminal kinase (SAPK/JNK) (E), acute phase response (F), calcium (G), and apoptosis (H). Superimposition of the expression data for the significantly altered genes (Table 2) onto the networks of known molecular interactions and canonical signaling pathways enabled identification of genes that may mediate the postburn insulin resistance response in peripheral blood leukocytes (Supplemental Digital Content, Table 1, <http://links.lww.com/SLA/A197>), fat (Supplemental Digital Content, Table 2, <http://links.lww.com/SLA/A197>), and muscle (Supplemental Digital Content, Table 3, <http://links.lww.com/SLA/A197>). Within the identified pathways, transcripts of 455 genes in peripheral blood leukocytes, 360 genes in fat, and 448 genes in muscle were significantly changed in a temporal manner after a severe burn injury. These genes were used to create prototypical blood, fat, and muscle cells (Supplemental Digital Content, Figs. 2–4, respectively, <http://links.lww.com/SLA/A197>), depicting the tissue-specific changes overtime at postburn days (a) 0 to 10, (b) 11 to 49, (c) 50 to 250, and (d) 251. Surprisingly, the majority of these genes did not return to normal expression levels by even 251 days after the burn injury, indicating pervasive and persistent ER/SR stress, UPR, and insulin resistance. Modulation of these molecules and their signaling pathways are predicted to alter a variety of cellular processes including protein synthesis, cell growth, cell survival, lipolysis, and cytokine expression. Thus, the dramatic changes in gene expression in various tissues may provide the molecular basis for clinical sequelae such as insulin resistance, muscle wasting, fat loss, and persistent hyperinflammation during the first year after a burn injury.

Genomic Response in Peripheral Blood Leukocytes

The genomic response in peripheral blood leukocytes (Supplemental Digital Content, Fig. 2, <http://links.lww.com/SLA/A197>) indicates long-term alteration of the genes involved in ER stress, UPR, insulin resistance, inflammation, the acute phase response, calcium signaling, and apoptosis. Transcriptional changes in *IRE-1*, *TAO kinase 3 (JIK)*, and *ATFs (ATF4 and ATF6)* demonstrate the involvement of ER stress and UPR. Insulin receptor mediated signaling in peripheral blood leukocytes may also be impacted via changes in expression of the insulin receptor and downstream mediators of PI3K and protein kinase B (*AKT*) signaling such as 3-phosphoinositide dependent protein kinase-1 (*PDPK1*), PI3K3C2A, PIK3C3, PIK3CD, PIK3R1, PIK3R2, PIK3R3, PIK3R5, AKT1, AKT2, AKT3, BCL2-associated agonist of cell death, phosphodiesterase 3B, Cyclic Guano-sine Monophosphate-inhibited (phosphodiesterase 3B), protein kinase A and B (*PRKAR1A*, *PRKAR1B*), glycogen synthase kinase 3 (*GSK3*), eukaryotic translation initiation factor 2 β (*eIF2B*), forkhead box O1, mechanistic target of rapamycin, eukaryotic translation initiation factor 4E (*eIF4E*), and signaling molecules within the ERK1/2 signaling pathways such as Src homology 2 domain containing transforming protein 1 (*SHC1*), growth factor receptor-bound protein-2 (GRB2), son of sevenless homologs (*SOS1*, *SOS2*), V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog, MAPK kinase 1/2 (*MAP2K*), *MAPK1*, cAMP responsive

element binding protein 1 (*CREB1*), and *STAT1* and *STAT3*. Changes in the genomic expression patterns of these molecules may potentially mediate insulin receptor signaling-related alterations in gene transcription, cell growth, apoptosis, protein synthesis, and insulin resistance. Associated changes in JNK expression, which may have a direct inhibitory effect on insulin receptor mediated signaling, may be involved with the development of insulin resistance along with changes in *MAPK* kinase kinase 2 (*MAP3K2*), *MAPK* kinase 7 (*MAP2K7*), cell division cycle 42 (*CDC42*), and *ATF2* expression. Modulation of genes downstream of the IL-1, IL-6, and TNF- α receptors up to and beyond 251 days postburn may indicate prolonged inflammation and acute phase signaling; these genes include albumin (*ALB*), apolipoproteins A1 and A2 (*APOA1*, *APOA2*), retinal binding protein 4 (*RBP4*), IL-6, nuclear factor of kappa light polypeptide gene enhancer in B-cells (*NF κ B2*), *NF κ B* inhibitors (*NF κ BIB*, *NF κ BIE*), glucocorticoid receptor (NR3C1), suppressors of cytokine signaling (*SOCS1*, *SOCS3*, *SOCS6*), amyloid p component serum (APCS), and serpin peptidase inhibitor clade A (*SERPINA1*). Concurrent modulation of key molecules that are anti- or proapoptotic, such as caspase (*CASP2*), B-cell CLL/lymphoma 2 (*BCL2*), *BCL2*-like 1 (*BCLXL*), Diablo, HtrA serine peptidase 2 (*HtrA2*), and *CASP3* and *CASP9* may indicate temporal changes in cell fate as a shift from proapoptotic to antiapoptotic signaling occurs. Broadscale alterations of key calcium signaling molecules including glutamate receptors, *ATPase*, Ca^{++} transporting, plasma membrane, *CREB1*, nuclear factor of activated T-cells, calcineurin-dependent 2 and 4, calcium/calmodulin-dependent protein kinases, sarco-ER calcium *ATPase*, calmodulin (*CALM3*), and inositol 1,4,5-triphosphate receptor (*ITPR1*) may also impact cell growth, survival, and apoptosis, potentially tying the changes induced by the postburn inflammatory response to the ER stress/UPR, and insulin resistance responses. Overall, the gene expression data from peripheral blood leukocytes indicate the reprioritization of cell function from initial proinflammatory and proapoptosis signaling to cell growth and survival signaling.

Genomic Expression in Fat

Following a severe burn injury, the genomic response in fat (Supplemental Digital Content, Fig. 3, <http://links.lww.com/SLA/A197>) also suggests wide-spread alterations of the pathways involved in postburn ER stress and the resultant insulin resistance. One of the main findings is the strong participation of molecules crucial to the ER stress response and activation of the UPR such as heat shock 70 kDa protein 5, *ATF4*, *DNAJ* (*HSP40*) homolog, *IRE-1*, eukaryotic translation initiation factor 2 α , X-box binding protein 1 (*XBPI*), *JNK1*, and *MAPK* kinase kinase 5 (*MAP5K5*). Concurrent alterations in insulin receptor signaling suggest potential functional alterations that may result in lipolysis, protein synthesis and transcription, cell growth signaling, and receptor internalization, via changes in expression of insulin receptor, members of the PI3K/AKT signal transduction pathway, *IRS-1*, phosphatase and tensin homolog (PTEN), *AKT1/2/3*, *eIF4E*, and forkhead box O1, and members of the *ERK1/2* signaling pathway such as *CREB3*, and peroxisome proliferator-activated receptor gamma (PPAR γ). Concurrent modulation of JNK-related signaling is believed to bridge the gap between ER stress and insulin resistance; we report alterations in *TNFRSF1A*-associated via death domain (TRADD), Fas-associated via death domain (FADD), *MAP3K1*, *MAP3K5*, *MAP3K3*, *MAP2K4*, *JNK3*, *JNK1*, and *ATF2*. The participation of the inflammatory and acute phase responses is also supported by the modulation of key players in the acute phase response *IL-6*, *IL-1*, *MAP3K1*, *MAPK14*, *SERPINF*, complement component 1 (C1), von Willebrand factor (vWF), *SOCS1*, *SOCS6*, and haptoglobin (HP) with downstream perturbations of inflammatory mediator signaling via receptors for TNF- α , *IL-1*, and *IL-6* continuing out to >251 days postburn. Participation of apoptotic signaling pathways downstream of the TNF- α and cytokine receptors is indicated as well, with modulation of *CASP6*, 8, 9, and 10, fodrin, acinus, *BCL-2*-associated X protein (BAX), *BCL2*-antagonist/killer 1 (BAK), *BCL2*, and *BCLXL*. Changes in calcium

signaling molecules such as calreticulin (*CALR*), *CALM3*, calpain 6 (*CAPN6*), *CREB3*, and 5-hydroxytryptamine (serotonin) receptor 3A (*5HT3R*), further relate the initial ER stress response with insulin resistance. The changes in the genomic expression patterns may provide molecular insight into postburn lipolysis and fat deposition in the liver.

Genomic Response in Muscle

Following a burn injury, the genomic expression profiles in muscle (Supplemental Digital Content, Fig. 4, <http://links.lww.com/SLA/A197>) also support activation of the SR stress response and insulin resistance pathways. Induction of SR stress and the UPR are indicated by altered genomic expression of eukaryotic translation initiation factor 2-alpha kinase 3 (*PERK*), *JIK*, *EIF-2α*, *ATF4*, and *ATF6*. Insulin receptor signaling is impacted at the transcript level and expression changes are seen for the insulin receptor and downstream signaling molecules associated with *PI3K/AKT* signaling such as *IRS-1*, *PI3K*, *PTEN*, pyruvate dehydrogenase kinase, isozyme 1 (*PDK1*), *AKT1/2/3*, *GSK3B*, *eIF2B*, *4E-BP1*, and FKHR and participants in *ERK* signaling such as *MRAS*, *RRAS*, *MAPK1*, *CREB1*, *CREB5*, *PPARγ*, *STAT1*, *STAT3*, and *ATF1*. *JNK* signaling is impacted in muscle as well, with alterations noted in genomic expression patterns of *ATF2*, *NFAT5*, *NFATC1*, *NFATC3*, *cJun*, and *TP53*. Systemic elevation of *TNF-α*, *IL-1*, and *IL-6* as part of the acute phase signaling response may be related to the alterations in the genomic expression levels of receptors for *TNF-α*, *IL-1*, and *IL-6* and downstream mediators of the inflammatory and acute phase responses including *TRADD*, *MAP2K4*, *JNK2*, *MAPK14*, *NFκB2*, *NFκB1B*, *STAT1*, *STAT3*, *SOCS1*, *SOCS2*, *SOCS3*, *SOCS4*, *SOCS5*, *SERPINE1*, *RBP4*, *RBP7*, *α2-macroglobulin*, and *SERPINA3*. As seen in both peripheral blood leukocytes and fat, burn alters apoptotic signaling pathways as seen by the modulation of *CASP2*, 6, and 7, fodrin, and acinus, and the calcium signaling molecules such as *CALM3*, *CREB1*, *CREB5*, *NFATC1*, *NFATC3*, *ITPR1*, *ITPR2*, *ITPR3*, and calcineurin (*PPP3CC*). The changes in these signaling pathways may provide mechanistic insight into documented postburn changes in muscle such as decreases in protein synthesis, cell proliferation, peripheral insulin resistance, and increases in protein catabolism and muscle loss.

Of the genes involved in the pathways related to postburn insulin resistance and ER stress, approximately one-third of the response is unique in each of the tissues examined (Supplemental Digital Content, Fig. 5, <http://links.lww.com/SLA/A197>). The expression of 301 genes (100 uniquely) changed in peripheral blood leukocytes, 209 genes (52 uniquely) in fat, and 299 genes (87 uniquely) in muscle. The differences in gene expression patterns may be related to the different postburn recovery responses that occur within each tissue.

Proteomic Alterations in Muscle

Burn injury was associated with marked SR stress and UPR in muscle as demonstrated by significant elevations of phosphorylated IRE-1 and accumulations of cleaved ATF6 for up to 250 days postburn when compared with nonburned controls, $P < 0.05$ (Figs. 2A–C). We found that phosphorylated JNK, a major mediator of insulin resistance signaling, was also significantly increased postburn. Phosphorylated JNK was markedly increased in muscle from burned patients for up to 50 days postburn when compared with normal controls, $p < 0.05$ (Figs. 2A, D). We further found that SERCA-2, a major mediator of SR calcium homeostasis in muscle, was significantly increased for 50 days postburn, possibly indicating a compensatory mechanism secondary to SR calcium depletion, $P < 0.05$ (Figs. 2A, E). These data confirm the genomic expression results and clearly indicate that burn causes ER/SR stress and UPR in muscle postburn.

To dissect how burn alters insulin receptor signaling, we measured insulin receptor subunits- α and $-\beta$, and phosphorylated and total IRS-1. We found that a burn causes marked

increases in insulin receptor subunits- α and - β in muscle (Figs. 2A, O, P). Similarly, both phosphorylated and total IRS-1 are markedly altered postburn (Figs. 2A, F, G). One of the most important findings, however, is that burn induces serine phosphorylation of the IRS-1 (Figs. 2A, F). Serine phosphorylation of IRS-1 reduces insulin receptor mediated signaling by blocking phosphorylation of IRS-1 at tyrosine 612 (Figs. 2A, G).

Levels of phosphorylated AKT were decreased throughout the whole study period in muscle, demonstrating further impairment of insulin signaling (Figs. 2A, H). Expression of EIF-2 α , an essential factor for protein synthesis, and the antiapoptotic BCL-2, both proteins acting downstream of AKT were not significantly affected by burn trauma (Figs. 2A, J, K). Protein kinase, a key regulator of glycogen, sugar, and lipid metabolism, was significantly upregulated in muscle tissue upon burn injury, $P < 0.05$, and remained increased for the whole time period studied (Figs. 2A, I). Protein analysis further revealed significantly increased accumulation of STAT-1 and -3 in muscle tissue at 0 to 10 and 0 to 49 days postburn, respectively, $P < 0.05$ (Figs. 2A, L, M). Both proteins regulate many aspects of cell growth, survival, and differentiation and may play an important role in the development and function of the immune system. Levels of ERK1/2, proteins downstream of the insulin receptor which regulate cell proliferation, were increased in muscle tissue throughout the entire length of the study with significantly elevated values at 11 to 50 days postburn, $P < 0.05$ (Figs. 2A, N).

DISCUSSION

Stress-induced diabetes, a hallmark of burn, trauma, and critical illness, worsens clinical outcomes by increasing morbidity and mortality.^{9–11} Despite insulin resistance and hyperglycemia being well-documented pathophysiologic responses in severely burned and critically ill patients, the underlying mechanisms are poorly understood. Our study is the first to identify cellular and molecular alterations in burned patients allowing development of novel treatments to attenuate hyperglycemia and insulin resistance.

Burn injury induces a vast inflammatory response, which leads to cellular stress responses in many tissues—even those not directly burned. Recently, ER stress and the UPR were identified as central intracellular stress signaling pathways associated with inflammation.^{13,15} More importantly, in a model of type 2 diabetes, ER stress and UPR have been shown to be causally linked with hyperglycemia and insulin resistance.^{12,16} It, therefore, seems that ER stress and UPR are central for the development of insulin resistance and hyperglycemia postburn. We have recently reported in animal models that burn induces marked ER stress and UPR leading to impaired insulin receptor (IR) signaling via activation of JNK resulting in insulin resistance and hyperglycemia.^{18,23} The aim of the present study was to determine whether ER stress and UPR are present in humans who suffered a severe burn injury. It is well documented that glucose kinetics are almost always abnormal in burn patients, with glucose utilization occurring predominantly via inefficient anaerobic mechanisms as characterized by increased lactate production, which accounts for increased glucose consumption.^{4,24–26} Plasma insulin levels are markedly increased over a prolonged period after a severe burn injury along with increased glucose levels.^{3,5,6} The fact that the rate of basal glucose production is elevated despite elevated plasma insulin levels indicates insulin resistance, because under normal conditions elevated serum insulin would lower the rate of glucose production.^{4,27,28} As mentioned previously, the importance of hyperglycemia and insulin resistance is that both worsen the outcomes of severely burned patients.^{9–11,29} It is well known that many stress hormones can increase glucose levels, including glucagon, glucocorticoid, adrenalin, and so on. All these hormones are significantly increased by severe burn injury. There is thus the possibility that insulin increased secondary to the

dramatically increased glucose and that the glucose level still remains high because of a relatively insufficient insulin concentration.

In this study, we showed for the first time that a trauma, severe burn in our case, induces systemic ER stress and UPR in humans. We determined the genomic changes in peripheral blood leukocytes, fat, and muscle postburn and compared these changes to the same tissues from normal, healthy, nonburned volunteers. Genomic analysis revealed that burn injury activated ER stress and UPR in blood, fat, and muscle for up to 1 year postburn. The genomic expression pattern was not identical in blood, fat, and muscle, indicating some tissue-specific responses, but in all 3 tissues, we found that major components of the ER stress and UPR were activated. These findings indicate that ER stress and UPR is not limited to one tissue type but is present in many, if not all, organs and tissues postburn.

The ER is recognized as the site of protein synthesis and folding of secreted and membrane-bound proteins.¹⁵ Protein folding is essential for cell function and cell survival, and the presence of an excess of misfolded proteins results in the activation of signaling pathways to restore homeostasis.¹⁵ Calcium depletion from ER stores leads to increased unfolded and/or misfolded proteins in the ER lumen. This results in the activation of the UPR.¹⁵ The accumulation of unfolded protein is detected by the cell *via* 3-key ER transmembrane receptors, protein kinase RNA (PKR)-like ER kinase (PERK), IRE-1, and ATF6. In resting cells, all 3 ER receptors are maintained in an inactive state. On accumulation of unfolded proteins, GRP78/BiP (an ER chaperone) dissociates from the receptors, which leads to their activation and triggers the UPR. The UPR is a prosurvival response that reduces the accumulation of unfolded proteins and restores normal ER function.¹⁵ However, if protein aggregation is persistent and the stress cannot be resolved, signaling switches from prosurvival to proapoptotic and protein synthesis decreases and catabolism occurs. In the present study, we were able to show that burn injury induces proapoptotic and calcium signaling pathways, indicating the enormous stress response postburn.¹⁸ The link between calcium signaling and ER stress seems important and will require further elucidation in future studies. We have shown that there appears an association between the two.

An intriguing result is the strong, coordinated downregulation of caspases in muscle. It does not seem to fit with the hypothesis of induced stress/apoptosis in burn patients. We hypothesize that this is a compensatory mechanisms to attenuate the vast muscle catabolism that occurs postburn and persists for 3 years. We hypothesize that the body adapts to the stress and tries to preserve as much muscle mass as possible, thus decreasing caspase activation.

It is currently unknown by which exact mechanisms ER stress and UPR are induced, but several studies suggest that proinflammatory cytokines (IL-6, IL-8, MCP-1, or TNF), metabolic stress (hypermetabolism), and stress hormones (cortisol, catecholamines) activate ER stress and UPR.¹⁴ In the present study, and in a large clinical study from our group, we showed that a burn injury is associated with dramatically increased proinflammatory cytokines, hypermetabolism, and release of stress hormones, all of which could activate ER stress and UPR postburn.³ The mechanisms by which ER stress and UPR induces insulin resistance was recently identified by Ozcan and colleagues.¹² They showed in a type 2 diabetic mouse model that ER stress leads to suppression of insulin receptor signaling through hyperactivation of JNK and subsequent serine phosphorylation of IRS-1.¹² The authors concluded that ER stress is a central feature of peripheral insulin resistance and type 2 diabetes at the molecular, cellular, and organismal levels.¹² Phosphorylation of JNK leads to serine phosphorylation of the insulin receptor inhibiting the essential tyrosine phosphorylation of the insulin receptor and PI3K signaling resulting not only in insulin resistance and hyperglycemia but also in increased cell stress and apoptosis.³⁰ In the present

study, we have shown on both a transcriptome and protein level that burn activates proinflammatory and metabolic mediators, key indicators of ER stress/UPR, and JNK, the interactions of which result in vast insulin resistance and hyperglycemia. We furthermore confirmed that burn decreases prosurvival PI3K/Akt signaling, and consequently alters apoptosis and proapoptotic calcium signaling pathways. These results confirm our recent study in burned rats demonstrating that a severe burn causes ER stress and UPR and gross alterations in ER calcium with increased cytosolic calcium concentrations.¹⁸ Increased cytosolic calcium induces mitochondrial dysfunction and damage, leading to apoptosis and tissue dysfunction. Here, we demonstrate the relationship between systemic postburn inflammation and stress-induced diabetes with alterations in tissue-specific molecular signaling pathways related to ER stress, UPR, insulin signaling, and insulin resistance.

Limitations of this study need to be mentioned and are severalfold. This study provides a link between ER stress and insulin resistance; however, we lack direct evidence. However, this study was setup to determine whether ER stress and UPR occurs in humans after burn injury, and we feel that we have shown that these responses do occur. This will allow further studies to determine cause-effect relationships and potential therapeutic targets for severely burned patients. In addition, we believe that this study may have implications for other forms of trauma patients as similar responses occur in these patients. Thus, this study showing the presence of ER stress and UPR for up to 1 year posttrauma has importance as it may open new therapeutic windows. New therapeutic options to be tested in in vitro and in vivo models may include established clinical perturbations or novel agents such as chemical chaperones. Another limitation is associated with the use of GeneChip analysis. We found 455 genes in peripheral blood leucocytes, 360 genes in fat, and 448 genes in muscle that were significantly changed. This is a high number of genes, because we used a lower cutoff and higher *P* value for discovery purposes to screen for novel genes. However, this discovery genomics is associated with false detection and may be responsible for an “artificial” enrichment of deregulated genes within the selected pathways.

In summary, we showed that a severe thermal injury induces vast insulin resistance and hyperglycemia, which our data indicate are associated with the systemic induction of ER/SR stress and the UPR. Furthermore, we demonstrated that ER stress and UPR occurs in peripheral blood leukocytes, fat, and muscle, and also that alterations of these pathways persist for up to 1 year postburn after a severe burn injury. ER stress and UPR alters JNK, PI3K/Akt, calcium, and apoptosis signaling pathways leading to insulin resistance and cellular dysfunction postburn. We suggest that ER stress and UPR is induced by inflammation, hypermetabolism, and release of stress hormones as reported in this study. This study is the first to describe these pathologic phenomena in severely traumatized humans.

Acknowledgments

The authors thank DingZhong Yang, Rong Chu, Gabriela Kulp, Maricela Pantoja, and Geoffrey Lerew for their technical assistance and Liz Montemayor, Ryan Griffin, Mary Kelly, and Karen Henderson for their assistance in collecting the samples. They also thank Steven Schuenke and Eileen Figueroa for their help and support.

This manuscript was prepared using a data set obtained from the Glue Grant program and does not necessarily reflect the opinions or views of the Inflammation and the Host Response to Injury Investigators or the NIGMS.

Disclosure: Supported by the American Surgical Association Foundation, Shriners Hospitals for Children grants (8507, 8660, 8640, 8740, and 9145), National Institute of General Medical Sciences (R01-GM56687, T32-GM008256, P50-GM60338, and U54 GM-62119-04), and National Institute on Disability and Rehabilitation Research (H133A020102). C.C.F. is supported in part by an Institute for Translational Sciences Career Development Fellowship and 1KL2RR029875. Supported by Inflammation and the Host Response to Injury Large-Scale Collaborative Project Award 2-U54-GM062119 from the National Institute of General Medical Sciences. The

Inflammation and the Host Response to Injury “Glue Grant” program is supported by the National Institute of General Medical Sciences.

REFERENCES

1. Bringham PA, McLoughlin E. Burn incidence and medical care use in the United States: estimates, trends and data sources. *J Burn Care Rehabil.* 1996; 17:95–107. [PubMed: 8675512]
2. Herndon DN, Tompkins RG. Support of the metabolic response to burn injury. *Lancet.* 2004; 363:1895–1902. [PubMed: 15183630]
3. Jeschke MG, Chinkes DL, Finnerty CC, et al. Pathophysiologic response to severe burn injury. *Ann Surg.* 2008; 248:387–401. [PubMed: 18791359]
4. Gore DC, Ferrando A, Barnett J, et al. Influence of glucose kinetics on plasma lactate concentration and energy expenditure in severely burned patients. *J Trauma.* 2000; 49:673–678. [PubMed: 11038085]
5. Wolfe RR, Miller HI, Spitzer JJ. Glucose and lactate kinetics in burn shock. *Am J Physiol.* 1977; 232:E415–E418. [PubMed: 15459]
6. Wolfe RR, Durkot MJ, Allsop JR, et al. Glucose metabolism in severely burned patients. *Metabolism.* 1979; 28:1031–1039. [PubMed: 491960]
7. Wolfe RR, Herndon DN, Peters EJ, et al. Regulation of lipolysis in severely burned children. *Ann Surg.* 1987; 206:214–221. [PubMed: 3606248]
8. Hart DW, Wolf SE, Mlcak R, et al. Persistence of muscle catabolism after severe burn. *Surgery.* 2000; 128:312–319. [PubMed: 10923010]
9. Gore DC, Chinkes D, Heggors J, et al. Association of hyperglycemia with increased mortality after severe burn injury. *J Trauma.* 2001; 51:540–544. [PubMed: 11535907]
10. Gore DC, Chinkes DL, Hart DW, et al. Hyperglycemia exacerbates muscle protein catabolism in burn-injured patients. *Crit Care Med.* 2002; 30:2438–2442. [PubMed: 12441751]
11. Hemmila MR, Taddonio MA, Arbabi S, et al. Intensive insulin therapy is associated with reduced infectious complications in burn patients. *Surgery.* 2008; 144:629–637. [PubMed: 18847648]
12. Ozcan U, Cao Q, Yilmaz E, et al. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science.* 2004; 306:457–461. [PubMed: 15486293]
13. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol.* 2007; 8:519–529. [PubMed: 17565364]
14. Ogawa W, Kasuga M. Cell signaling. Fat stress and liver resistance. *Science.* 2008; 322:1483–1484. [PubMed: 19056968]
15. Szegezdi E, Logue SE, Gorman AM, Samali A. Mediators of endoplasmic reticulum stress-induced apoptosis. *EMBO Rep.* 2006; 7:880–885. [PubMed: 16953201]
16. Ozcan U, Yilmaz E, Ozcan L, et al. Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science.* 2006; 313:1137–1140. [PubMed: 16931765]
17. Gauglitz GG, Song J, Herndon DN, et al. Characterization of the inflammatory response during acute and post-acute phases after severe burn. *Shock.* 2008; 30:503–507. [PubMed: 18391855]
18. Jeschke MG, Gauglitz GG, Song J, et al. Calcium and ER stress mediate hepatic apoptosis after burn injury. *J Cell Mol Med.* 2009; 13:1857–1865. [PubMed: 20141609]
19. Song J, Finnerty CC, Herndon DN, et al. Severe burn-induced endoplasmic reticulum stress and hepatic damage in mice. *Mol Med.* 2009; 15:316–320. [PubMed: 19603103]
20. Klein MB, Silver G, Gamelli RL, et al. Inflammation and the host response to injury: an overview of the multicenter study of the genomic and proteomic response to burn injury. *J Burn Care Res.* 2006; 27:448–451. [PubMed: 16819346]
21. Jeschke MG, Finnerty CC, Suman OE, et al. The effect of oxandrolone on the endocrinologic, inflammatory, and hypermetabolic responses during the acute phase postburn. *Ann Surg.* 2007; 246:351–362. [PubMed: 17717439]
22. Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci USA.* 2001; 98:5116–5121. [PubMed: 11309499]

23. Gauglitz GG, Halder S, Boehning DF, et al. Post-burn hepatic insulin resistance is associated with ER stress. *Shock*. 2010; 33:299–305. [PubMed: 22011639]
24. Wilmore DW. Hormonal responses and their effect on metabolism. *Surg Clin North Am*. 1976; 56:999–1018. [PubMed: 982246]
25. Herndon DN, Wilmore DW, Mason AD Jr. Development and analysis of a small animal model simulating the human postburn hypermetabolic response. *J Surg Res*. 1978; 25:394–403. [PubMed: 713539]
26. Wilmore DW, Long JM, Mason AD Jr, et al. Catecholamines: mediator of the hypermetabolic response to thermal injury. *Ann Surg*. 1974; 180:653–669. [PubMed: 4412350]
27. Wolfe RR, Shaw JH, Jahoor F, et al. Response to glucose infusion in humans: role of changes in insulin concentration. *Am J Physiol*. 1986; 250:E306–E311. [PubMed: 2869694]
28. Wolfe RR, Nadel ER, Shaw JH, et al. Role of changes in insulin and glucagon in glucose homeostasis in exercise. *J Clin Invest*. 1986; 77:900–907. [PubMed: 2869053]
29. Gore DC, Wolf SE, Herndon DN, et al. Metformin blunts stress-induced hyperglycemia after thermal injury. *J Trauma*. 2003; 54:555–561. [PubMed: 12634538]
30. Urano F, Wang X, Bertolotti A, et al. Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science*. 2000; 287:664–666. [PubMed: 10650002]

FIGURE 1.

Severe burn injury leads to vast inflammatory stress responses, hyperglycemia, and hyperinsulinemia. Levels of urinary cortisol (A), epinephrine (B), serum MCP-1 (C), serum IL-6 (D), and REE% predicted (E), fasting serum of glucose (F), and insulin (G) were determined at various time points postburn and compared with nonburned children. Bars represent mean; error bars correspond to SEM. *Statistical difference between burned children versus non-burned children, $P < 0.05$.

FIGURE 2.

Burn injury induces ER/SR stress and the UPR in muscle. Western blot analysis of muscle from normal, unburned patients, and burned patients taken at various time points postburn (0–10, 11–49, and 50–250 days postburn, respectively). Glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) and actin served as control loading proteins (A). Quantitative analysis of proteomic alterations in muscle. Expression of proteins involved in ER/SR stress, the UPR, inflammation, and insulin signaling-related pathways were measured at the respective time periods (B–P). Histograms depict intensities of the phosphorylated protein bands divided by the intensity of the total form of the respective protein band or in the case of nonphosphorylated proteins, by the intensity of the respective actin or glyceraldehyde-3-phosphate-dehydrogenase band. Results are presented as fold change from unburned controls and represent 6 to 8 different patients per group, as indicated in the main text. Bars represent mean; error bars correspond to SEM. *Statistical significance: $P < 0.05$ for every comparison between groups and compared with controls.

TABLE 1

Patient Demographics *

	Burned Patients	Nonburned Controls
N	20	36
Age, yr	8 ± 5	11 ± 2 [†]
Sex, Female/Male	8/12	16/20
Race, African American/American Indian/Asian/White/Other	1/4/0/2/13	1/4/1/29/1
Height, cm	127 ± 33	146 ± 14
Weight, kg	37 ± 25	42 ± 15
Time to admit, days	2 ± 1	
Length of stay, days	36 ± 21	
Burn mechanism, flame/scald/other	16/2/2	
TBSA total, %	64 ± 12	
TBSA 3rd, %	54 ± 22	
Surgical interventions	6.3 ± 0.2	1
Wound infection, n	1.4 ± 0.05	
Inhalation injury, Yes/No	11/9	
Ventilated, Yes/No	17/3	
Total number of operations, n	11 ± 5	
Number of pneumonias, n	0.5 ± 1.4	
APACHE score	11 ± 6	

APACHE indicates Acute Physiology and Chronic Health Evaluation.

* Data presented as mean ± SD.

[†]Significant difference between burn versus control patients, $P < 0.05$.

TABLE 2

Genetic Profile for Fat, Muscle, and Blood

Tissue	Days Postburn	No. of Burn Patient Arrays	No. of Nonburn Patient Arrays	FDR, %	No. of Significant Genes
Fat	0–10	20	25	5	3989
	11–49	21	25	4.60	4213
	50–250	12	25	5.80	529
Muscle	251	10	25	3.35	8352
	0–10	16	15	4.65	12039
	11–49	18	15	9	3078
	50–250	18	15	5.20	4326
	251	12	15	5.20	6381
Blood	0–10	13	32	0	3106
	11–49	13	32	0	3503
	50–250	9	32	1.20	9017
	251	9	32	0.20	3754

FDR indicates false detection rate.