

# Pathophysiologic Response to Severe Burn Injury

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**Objective:** To improve clinical outcome and to determine new treatment options, we studied the pathophysiologic response postburn in a large prospective, single center, clinical trial.

**Summary Background Data:** A severe burn injury leads to marked hypermetabolism and catabolism, which are associated with morbidity and mortality. The underlying pathophysiology and the correlations between humoral changes and organ function have not been well delineated.

**Methods:** Two hundred forty-two severely burned pediatric patients [ $>30\%$  total body surface area (TBSA)], who received no anabolic drugs, were enrolled in this study. Demographics, clinical data, serum hormones, serum cytokine expression profile, organ function, hypermetabolism, muscle protein synthesis, incidence of wound infection sepsis, and body composition were obtained throughout acute hospital course.

**Results:** Average age was  $8 \pm 0.2$  years, and average burn size was  $56 \pm 1\%$  TBSA with  $43 \pm 1\%$  third-degree TBSA. All patients were markedly hypermetabolic throughout acute hospital stay and had significant muscle protein loss as demonstrated by a negative muscle protein net balance ( $-0.05\% \pm 0.007$  nmol/100 mL leg/min) and loss of lean body mass (LBM) ( $-4.1\% \pm 1.9\%$ );  $P < 0.05$ . Patients lost  $3\% \pm 1\%$  of their bone mineral content (BMC) and  $2 \pm 1\%$  of their bone mineral density (BMD). Serum proteome analysis demonstrated profound alterations immediately postburn, which remained abnormal throughout acute hospital stay;  $P < 0.05$ . Cardiac function was compromised immediately after burn and remained abnormal up to discharge;  $P < 0.05$ . Insulin resistance appeared during the first week postburn and persisted until discharge. Patients were hyperinflammatory with marked changes in IL-8, MCP-1, and IL-6, which were associated with  $2.5 \pm 0.2$  infections and 17% sepsis.

**Conclusions:** In this large prospective clinical trial, we delineated the complexity of the postburn pathophysiologic response and conclude that the postburn response is profound, occurring in a timely

manner, with derangements that are greater and more protracted than previously thought.

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Despite improvements in mortality over the last decade, postburn morbidity is tremendous and remains a challenge for clinicians. We and others have shown that after a severe thermal injury, patients are hypermetabolic, disabled, and debilitated over a period of at least 24 months.<sup>1,2</sup> There is growing evidence that pathophysiologic responses that occur immediately or early after burn will affect long-term outcome of severely burned patients.<sup>1,3</sup> The inflammatory response starts immediately after burn and persists for up to several months.<sup>3</sup> The hypermetabolic response after major burn is characterized by a hyperdynamic response with increased body temperature, oxygen and glucose consumption, CO<sub>2</sub> production, glycogenolysis, proteolysis, lipolysis, and futile substrate cycling.<sup>4–7</sup> This hypermetabolic response begins on the fifth day postinjury and continues up to 24 months postburn causing loss of lean body mass (LBM), loss of bone density, muscle weakness, and poor wound healing.<sup>1,4,8</sup> The hypermetabolic response is associated with a marked acute phase response that persists for 8 to 12 weeks after the initial insult.<sup>9,10</sup>

Hypermetabolism is further associated with alterations in the endocrinologic response. The hypothalamic-pituitary-organ-hormonal axis acts as a regulator of many organ and cellular functions; however, it has been suggested that a hormonal dysbalance is present after a burn injury.<sup>11</sup> In critically ill patients, a biphasic endocrine response is present, which encompasses an acute and a long-term phase.<sup>12</sup> The acute phase is characterized by low effector hormones due to target-organ resistance.<sup>12</sup> The long-term phase encompasses the hypothalamic suppression of the endocrine axes, which contributes to the low serum levels of the subsequent target organ hormones.<sup>12</sup> Several clinical trials with the goal to correct hormonal dysbalances in critically ill patients have been ineffective or even harmful because of a lack of understanding of the pathophysiologic mechanisms.

Despite the identification and delineation of fragments of the postburn responses, no large prospective clinical trial examining the major responses during the postburn acute phase has been conducted. The purpose of the present study

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was to characterize the pathophysiologic responses postburn in terms of hypermetabolism, inflammation, hormonal and body composition changes, organ function, and muscle protein synthesis in a large prospective clinical trial to understand pathophysiologic mechanisms and develop new specific treatment options to improve outcome of severely burned patients.

## PATIENTS AND METHODS

All thermally injured children with burns over 30% of their total body surface area (TBSA) consented to an IRB-approved experimental protocol between 1998 and 2007, and were admitted to our burn unit and required at least 1 surgical intervention were included in this study. If needed, patients were resuscitated according to the Galveston formula with 5000 mL/m<sup>2</sup> TBSA burned + 2000 mL/m<sup>2</sup> TBSA lactated Ringer 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 covered with autograft. Any remaining open areas were covered with homograft. After the first operative procedure, patients were taken back to the operation theater when donor sites were healed. This procedure was repeated until all open wound areas were covered with autologous skin.

All patients underwent the same nutritional treatment according to a standardized protocol. The intake was calculated as 1500 kcal/m<sup>2</sup> body surface + 1500 kcal/m<sup>2</sup> area burn as previously published.<sup>13–15</sup> The nutritional route of choice in our patient population was enteral nutrition via a duodenal (Dobhoff) or nasogastric tube. Parenteral nutrition was only given in rare instances if the patient could not tolerate tube feeds.

Patient demographics (age, date of burn and admission, sex, burn size, and depth of burn) and concomitant injuries such as inhalation injury, sepsis, morbidity, and mortality were recorded. Sepsis was defined as a positive blood culture or pathologic tissue identifying the pathogen during hospitalization or at autopsy, in combination with at least 3 of the following: leucocytosis or leucopenia (>12,000 or <4000), hyperthermia or hypothermia (>38.5°C or <36.5°C), tachycardia (>150 BPM in children), refractory hypotension (systolic BP <90 mm Hg), thrombocytopenia (platelets <50,000/mm<sup>3</sup>), hyperglycemia (serum glucose >240 mg/dL), and enteral feeding intolerance (residuals >200 mL/h or diarrhea >1 L/d) as previously published.<sup>13,14,16</sup> We further determined time between operations as a measure for wound healing/re-epithelialization. We propose that the time between operations was indicative when donor sites were healed and thereby allowed determination of wound healing.

## Indirect Calorimetry

As part of our routine clinical practice, all patients underwent resting energy expenditure (REE) measurements within 1 week after hospital admission and weekly thereafter during their acute hospitalization. All REE measurements were performed between midnight and 5 AM while the patients were asleep and receiving continuous feeding. Resting energy expenditure was measured using a Sensor-Medics Vmax 29 metabolic cart (Yorba Linda, CA) as previously

published.<sup>15</sup> The REE was calculated from the oxygen consumption and carbon dioxide production by equations described by Weir et al.<sup>15</sup> Measured values were compared with predicted norms based upon the Harris-Benedict equation and to body mass index (BMI).<sup>15</sup> 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.

## Muscle Protein Synthesis

The degree of peripheral muscle protein net balance, taking into account synthesis and breakdown, was quantified using stable isotope tracers. Protein net balance was measured in a subset of 60 severely burned pediatric patients. Protein kinetic studies were performed between 5:00 and 7:00 AM, on postoperative day 5 after the first excision and grafting procedure. Because phenylalanine is neither synthesized nor degraded in the peripheral tissues (it is metabolized only in the liver), measurement across the leg reflects the net balance of protein synthesis and breakdown. 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 (GCMS) using the internal standard approach and the tert-butyldimethylsilyl esters, as previously described.<sup>17</sup> Indocyanine green concentrations were determined spectrophotometrically at  $\lambda = 805$  nm on a Spectronic 1001 (Bausch and Lomb, Rochester, NY). As phenylalanine is neither synthesized nor degraded in the periphery, the difference in concentration of this substrate in the femoral arterial and venous plasma pools reflects the net balance of leg skeletal muscle protein synthesis and breakdown. The net balance (NB) was calculated and standardized for leg volume by the following equation: NB = (C<sub>A</sub> – C<sub>V</sub>) × BF, where C<sub>A</sub> and C<sub>V</sub> are the blood-free amino acid concentrations of the femoral artery and vein, and BF is leg blood flow in mL/min/100 mL leg. Muscle protein synthesis and breakdown rates were measured and calculated as previously published.<sup>17</sup> Leg blood flow was determined from a modification of Fick equation. BF was normalized for each patient by leg volume. Subject weight, leg circumference at prescribed points relative to anatomic landmarks, and the distances between these points were used to mathematically model leg volume.<sup>17</sup>

## Body Composition

Height and body weight were determined clinically 5 days after admission and at discharge. Total LBM, fat, bone mineral density (BMD), and bone mineral content (BMC) were measured by dual energy x-ray absorptiometry (DEXA). A holologic model QDR-4500W DEXA (Hologic Inc., Waltham, MA) was used to determine body composition as previously published.<sup>1,2,18,19</sup>

## Hormones, Proteins, and Cytokines

Blood and/or urine was collected from burn patients at admission, preoperatively, and 5 days postoperatively for 4 weeks for serum hormone, protein, cytokine, and urine hormone analysis. Blood was drawn in a serum-separator col-

lection tube and centrifuged for 10 minutes at 1320 rpm; the serum was removed and stored at  $-70^{\circ}\text{C}$  until assayed.

Serum hormones and acute phase proteins were determined using HPLC, nephelometry (BNII; Plasma Protein Analyzer Dade Behring, MD), and ELISA techniques. The Bio-Plex Human Cytokine 17-Plex panel was used with the Bio-Plex Suspension Array System (Bio-Rad, Hercules, CA) to profile expression of seventeen inflammatory mediators interleukin IL-1 $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-17, granulocyte colony-stimulating factor (GCSF), granulocyte macrophage colony-stimulating factor (GMCSF), interferon-gamma (IFN- $\gamma$ ), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 beta (MIP-1 $\beta$ ), and tumor necrosis factor (TNF). The assay was performed according to the manufacturer's instructions. Briefly, serum samples were thawed and then centrifuged at 4500 rpm for 3 minutes at  $4^{\circ}\text{C}$ . Serum samples were then incubated with microbeads labeled with specific antibodies to one of the aforementioned cytokines for 30 minutes. After a wash step, the beads were incubated with the detection antibody cocktail, each antibody specific to a single cytokine. After another wash step, the beads were incubated with streptavidin-phycerythrin for 10 minutes, washed, and the concentrations of each cytokine were determined using the array reader.<sup>3,18–20</sup>

Urine creatinine, creatinine clearance, and cortisol were determined by standard laboratory techniques.

## Liver and Cardiac Changes

Liver ultrasound measurements in this study were made with the HP Sonos 100 CF echocardiogram (Hewlett Packard Imaging Systems, Andover, MA). The liver was scanned using an Eskoline B-scanner and liver size/volume was calculated using a formula as previously published.<sup>10,18,19,21</sup> Actual size was then compared with predicted size.

M-mode echocardiograms were completed as follows: at the time of the study, none of the patients presented with or previously suffered from other concomitant diseases affecting cardiac function, such as diabetes mellitus, coronary artery disease, long-standing hypertension, or hyperthyroidism. Study variables included: resting cardiac output (CO), cardiac index (CI), stroke volume (SV), resting heart rate (HR), and left ventricular ejection fraction (LVEF). Stroke volume and cardiac output were adjusted for body surface area and expressed as indexes. All cardiac ultrasound measurements were made with the Sonosite Titan echocardiogram with a 3.5-MHz transducer. Recordings were performed with the subjects in a supine position and breathing freely. M-mode tracings were obtained at the level of the tips of the mitral leaflets in the parasternal long axis position and measurements were performed according to the American Society of Echocardiography recommendations. Left ventricular volumes determined at end diastole and end systole were used to calculate EF, SV, CO and CI. Three measurements were performed and averaged for data analysis.<sup>18,19</sup>

## Physical Function Testing

In a subgroup analysis, we assessed physical function, which involved muscle strength and cardiopulmonary func-

tion. Studies were conducted in a timeframe ranging from immediately after ICU discharge up to 6 months postburn. Physical function was assessed in 46 burned patients (age:  $13 \pm 0.5$  years; height:  $152 \pm 2$  cm; weight:  $48 \pm 3$  kg). The results were compared with 46 age-matched nonburned control children (age:  $12 \pm 0.4$  years; height:  $154 \pm 3$  cm; weight:  $59 \pm 3$  kg). Age and height were similar in both groups; however, body weight was statistically significantly less in the burn group when compared with the control group,  $P < 0.05$ . Before muscle strength testing, the patient was familiarized with the exercise equipment and instructed on proper weight lifting techniques. Strength testing was conducted using a Biomed System-3 dynamometer (Shirley, NY) as previously published.<sup>22–24</sup> Values of peak torque were calculated by the Biomed software system. The highest peak torque measurement between the 2 trials was selected. Peak torque was corrected for gravitational moments of the lower leg and the lower arm.<sup>22–24</sup>

To determine peak oxygen consumption, patients underwent a standardized treadmill exercise test on day 2 using the modified Bruce protocol as part of their standard clinical outpatient evaluation. Heart rate and oxygen consumption were measured and analyzed using methods previously described.<sup>22–24</sup>

## Ethics and Statistics

The study was reviewed and approved by the Institutional Review Board of the University Texas Medical Branch, Galveston, TX. Before the study, each subject, parent or child's legal guardian had to sign a written informed consent form. Analysis of variance (ANOVA) with posthoc Bonferroni correction, paired and unpaired Student *t* test,  $\chi^2$  analysis, and Mann-Whitney tests were used where appropriate. Data are expressed as means  $\pm$  SD or SEM, where appropriate. Significance was accepted at  $P < 0.05$ .

## RESULTS

### Demographics

Two hundred forty-two severely burned children were included in the present study. Patients' demographics are shown in Table 1. Patients were, on average, 8 years of age, 41% were females and 59% were males. Patients suffered from a severe burn injury with 56% TBSA and a third-degree burn of 43% TBSA. Length of hospital/ICU stay was 31 days, which results in 0.55 days per percent TBSA burn. Patients were taken back to the OR every 8th day and required on average 4 operations. Minor infections occurred in 44% of the patients, whereas sepsis occurred in 18%, multiorgan failure in 21%, and 8% of our severely burned patients died (Table 1).

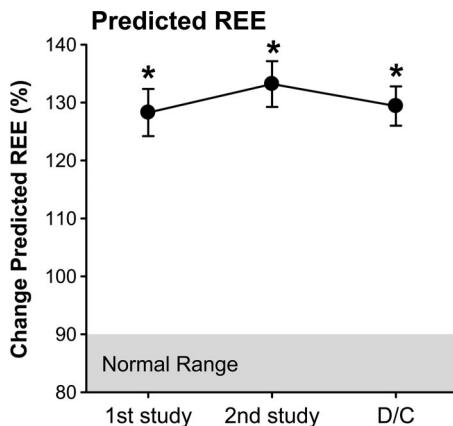
### Indirect Calorimetry

Percent predicted REE increased immediately postburn and peaked at 2 weeks postburn. Predicted REE decreased over time but remained elevated at discharge indicating marked hypermetabolism (Fig. 1; n = 212).

**TABLE 1.** Patient Demographics

	>30% TBSA (N = 242)
Age (yrs)	8.0 ± 0.2
Gender (F/M)	97/145
Time to admission (d)	6.7 ± 0.7
LOS (d)	31 ± 0.7
TBSA (%)	56 ± 0.3
3rd degree (%)	43 ± 0.3
Los/% TBSA (d/%)	0.55 ± 0.2
OR's (n)	4.3 ± 0.12
Time between operations (d)	8 ± 0.3
Inhalation injury (%)	32
Wound infections (n)	2.5 ± 0.2
Sepsis (%)	18
Multiorgan failure (%)	21
Mortality (%)	8

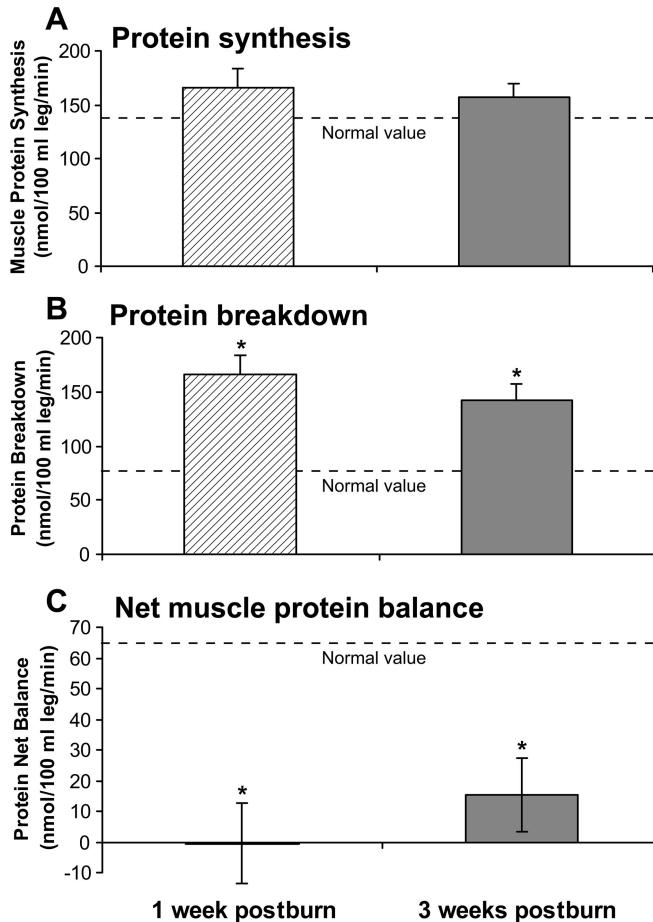
Data presented as means ± SEM or percentages.



**FIGURE 1.** Percent predicted REE. Predicted REE increased immediately postburn, peaked at 2 weeks postburn, and remained significantly elevated at hospital discharge indicating marked hypermetabolism. \*Significant difference between burned children versus normal range;  $P < 0.05$ .

### Peripheral Skeletal Muscle Net Balance

Stable isotope infusions were used to measure muscle protein synthesis and breakdown to determine net protein balance in severely burned patients ( $n = 59$ ) and unburned young adults ( $n = 5$ ) (Figs. 2A–C). These patients were selected as they had a complete set of stable isotope studies during week 1 and 3 postburn. There was no selection bias in choosing these patients, and their demographics are similar to the entire cohort. Peripheral muscle protein synthesis was not different at week 1 and week 3 postburn when compared with unburned young adults (Fig. 2A). Ninety-five percent confidence interval for protein breakdown is 130 to 202 nmol/100 mL leg/min at 1 week and 112 to 172 nmol/100 mL leg/min at 3 weeks postburn, which are both significantly above the normal value, indicating that muscle protein catabolism is twice the normal value (Fig. 2B). Likewise, the 95% confi-



**FIGURE 2.** Stable isotope infusions were used to determine muscle protein net balance in a subgroup of 59 burned patients and 5 unburned young adults. Peripheral muscle protein synthesis was not altered at week 1 and week 3 when compared with unburned young adults (A). Protein breakdown however was increased 3- to 4-fold at 1 and 3 weeks postburn (B) leading to a negative protein net balance (C).

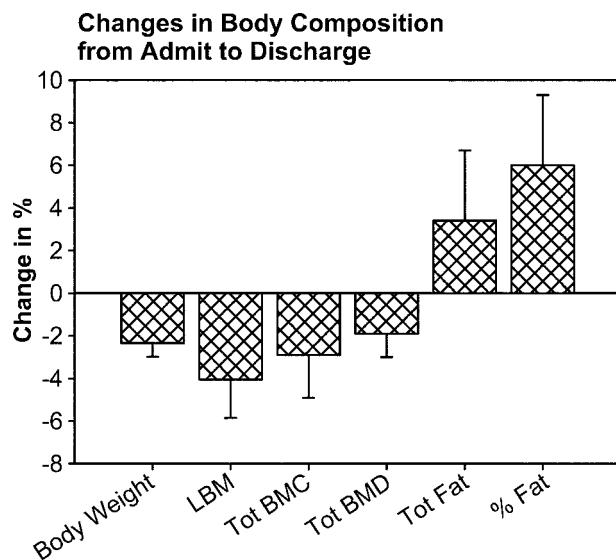
dence interval for protein net balance is -27 to +26 nmol/100 mL leg/min at 1 week and -9 to +39 nmol/100 mL leg/min at 3 weeks postburn, which is significantly worse than normal values in fed state (Fig. 2C).

### Body Composition

Severe burn causes marked changes in body composition during acute hospitalization. Severely burned children lost about 2% of their body weight (-5% LBM, -3% BMC, and -2% BMD) from admission to discharge. Total fat and percent fat increased from admission to discharge by 3% and 7%, respectively (Fig. 3;  $n = 105$ ).

### Hormones, Proteins, and Cytokines

Serum acute phase proteins were markedly increased postburn ( $n = 125$ –200 per time point). Serum complement C3 and  $\alpha_2$ -macroglobulin demonstrated a slow but significant increase over time (Figs. 4A–B). Serum haptoglobin,  $\alpha_1$ -acid glycoprotein, and CRP demonstrated a 4- to 10-fold



**FIGURE 3.** Severe burn causes marked changes in body composition during acute hospitalization ( $n = 105$ ). Severely burned children lost about 2% of their body weight, which is 5% lean body mass, 3% bone mineral content, 2% bone mineral density from admission to discharge. Total fat and percent fat increased from admission to discharge by 3% and 7%, respectively.

increase almost immediately postburn and remained significantly elevated through acute hospital stay (Figs. 4C–E). Serum constitutive hepatic proteins prealbumin, transferrin, and retinol-binding protein markedly decreased 4- to 8-fold almost immediately postburn and levels remained low up to 60 days postburn (Figs. 4F–H). Serum apolipoprotein A1 significantly decreased postburn, whereas apolipoprotein B showed an increase after an initial decrease (Figs. 4I–J). Serum-free fatty acids and triglycerides significantly increased 2- to 4-fold postburn (Figs. 4K–L).

Nonfasted serum glucose increased markedly during the acute phase postburn to 170 to 180 mg/dL along with increased levels of insulin implying that insulin resistance is present causing increased insulin associated with hyperglycemia (Fig. 5;  $n = 180$ –212).

Serum hormone levels showed a marked alteration postburn ( $n = 100$ –175). Serum insulin-like growth factor-I (IGF-I) decreased markedly immediately after burn and remained significantly decreased throughout acute hospitalization (Fig. 6A). Similar to IGF-I, serum insulin-like growth factor binding protein-3 (IGFBP-3) decreased almost immediately postburn and remained low through acute hospitalization (Fig. 6B). Serum GH did not decrease immediately postburn-like IGF-I and IGFBP-3. Serum GH started to decrease 8 to 10 days postburn and showed a steady decline through acute hospitalization (Fig. 6C).

Serum T4 decreased 2- to 3-fold immediately postburn (Fig. 6D). Serum T4, however, increased through acute hospitalization and approached normal levels at discharge (Fig. 6D). Free thyroid index (FTI) showed a significant decrease for 14 days postburn and then returned to normal levels (Fig. 6E).

Serum cortisol significantly increased immediately postburn and remained elevated for 3 weeks returning to normal levels (Fig. 6F). Urine-free cortisol increased 5- to 7-fold during the acute stay but decreased over time (Fig. 6G). Serum osteocalcin and iPTH were drastically decreased (5–7-fold) immediately after burn and showed almost no increase over time (Figs. 6H, I). Serum  $\beta$ -estradiol, testosterone, and progesterone showed very different patterns postburn. Although  $\beta$ -estradiol decreased immediately postburn with an increase over time (Fig. 6J), testosterone was normal during the early postburn phase but showed marked decreases beginning 4 weeks postburn (Fig. 6K). Progesterone demonstrated a very different pattern. Progesterone was increased at various time points when compared with normal levels (Fig. 6L).

Serum creatinine and creatinine clearance were not significantly different during the acute postburn response when compared with normal values (data not shown).

We found that all of the 17 serum cytokines measured are significantly altered, and these perturbations are possibly clinically relevant ( $n = 100$ –150). Dramatic changes were observed for serum G-CSF, IL-6, IL-8, MCP-1, and MIP-1 $\beta$  (Fig. 7A). These cytokines demonstrated up to a 100- to 200-fold increase when compared with normal levels. All 17 cytokines are depicted over time but also in a heat map, which illustrates the marked changes in the serum (Fig. 7B).

### Cardiac and Liver Changes

Analysis of cardiac output, predicted cardiac output, heart rate, predicted heart rate, cardiac index, and central venous pressure (CVP) showed postburn alterations. Cardiac output and predicted cardiac output postburn were increased immediately (up to 160% of predicted) and significantly decreased until discharge (Fig. 8;  $n = 212$ ). Heart rate and predicted heart rate were also significantly increased (up to 160% predicted) postburn and remained elevated at discharge (Fig. 8;  $n = 212$ ). Central venous pressure was not significantly altered postburn. Cardiac index was increased immediately postburn and significantly decreased from admit to discharge (Fig. 8;  $n = 212$ ).

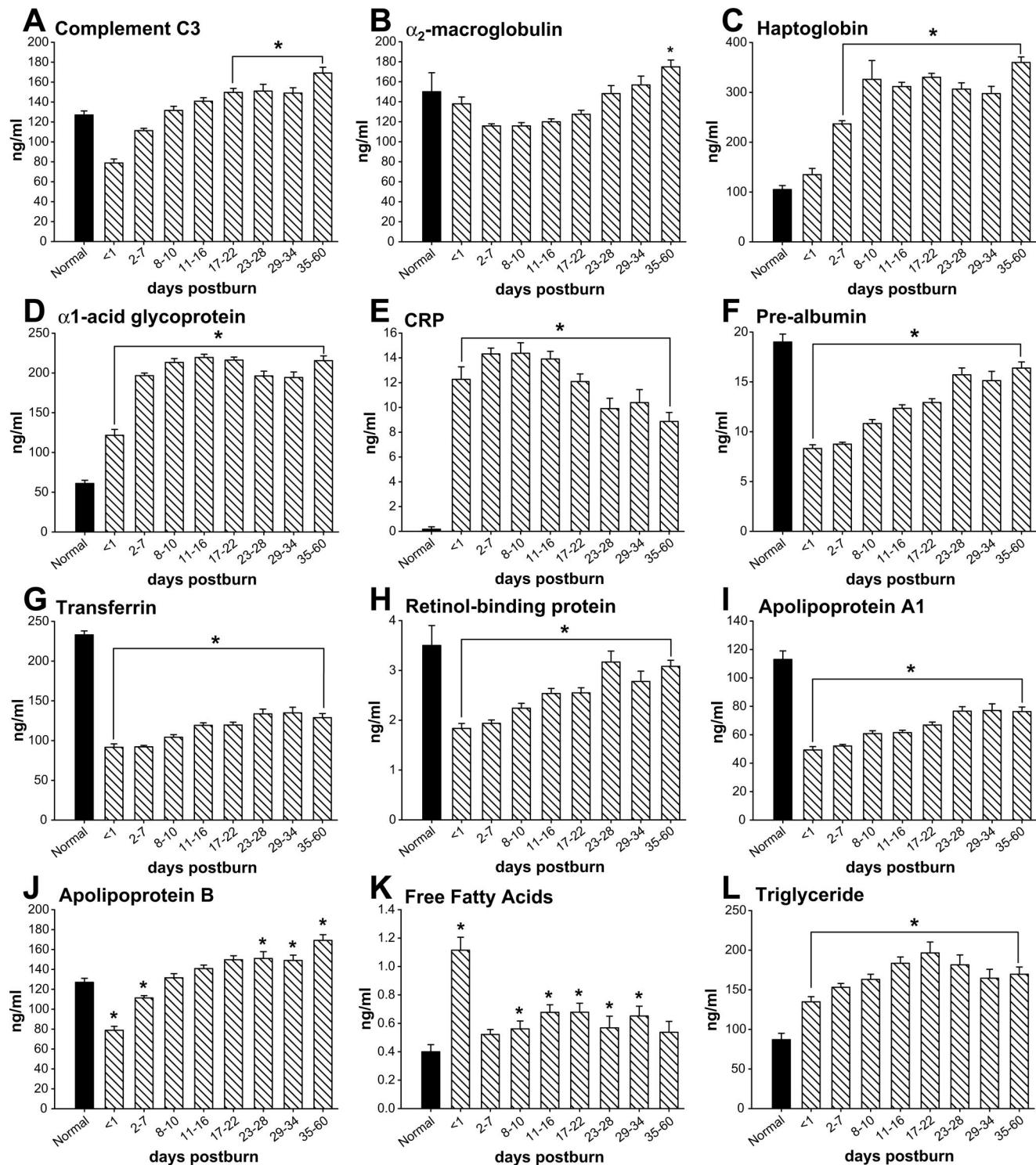
Immediately after burn, liver size markedly increased and remained elevated when patients were discharged from the hospital/ICU (Fig. 9;  $n = 178$ ).

### Physical Function

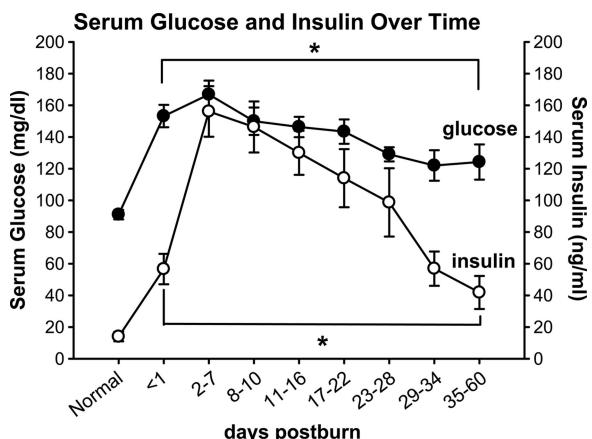
Both groups, burned and nonburned children, were similar in age and gender distribution. Peak torque (PT) was significantly lower in burned children ( $48 \pm 36$  Nm) when compared with nonburned control children ( $91 \pm 40$  Nm);  $P < 0.05$ . Similarly, peak cardiopulmonary capacity was significantly lower in burned children ( $27.0 \pm 6.8$  mL oxygen/kg/min) when compared with nonburned control children ( $34.9 \pm 8.4$  mL oxygen/kg/min);  $P < 0.05$ .

### DISCUSSION

Despite advances made in burn care over the last decade, burn injury remains a major clinical challenge and is associated with severe disabilities and impairment of the burn victim.<sup>4</sup> Burn over 30% to 40% of the body induces an



**FIGURE 4.** Serum complement C3 (A),  $\alpha_2$ -macroglobulin (B), haptoglobin (C),  $\alpha_1$ -acidglycoprotein (D), and CRP (E) were significantly increased postburn. Serum constitutive hepatic proteins prealbumin (F), transferrin (G), retinol binding protein (H) markedly decreased immediately postburn and levels remained low up to 60 days postburn. Serum apolipoprotein A1 (I) significantly decreased postburn, whereas apolipoprotein B (J) showed an increase. Serum-free fatty acids (K) and triglycerides (L) significantly increased postburn. \*Significant difference between burn versus normal ranges;  $P < 0.05$ .



**FIGURE 5.** Serum glucose increased during the acute phase postburn along with increased levels of endogenous insulin implying the presence of insulin resistance. \*Significant difference between burned children versus normal range;  $P < 0.05$ .

inflammatory and hypermetabolic response that persists for 2 years after the initial insult.<sup>1,2</sup> The metabolic demands and energy requirements are immense and are met by the mobilization of proteins and amino acids.<sup>25</sup> Increased protein turnover, degradation, and negative nitrogen balance are characteristics of this severe critical illness.<sup>25</sup> As a consequence, the structure and function of essential organs such as skeletal muscle, skin, immune system, and cellular membrane transport functions are compromised.<sup>26,27</sup> Chang et al<sup>28</sup> delineated in their study that a 10% loss of LBM leads to an impairment of immune function, 20% loss of LBM decreased wound healing with 30% mortality, 30% loss of LBM to pneumonia, and pressure sores with 50% mortality, and if 40% of LBM is lost, death will occur in 100%. Despite the identification and delineation of parts of the postburn response, no prospective large clinical study has ever fully characterized the major components during the acute phase postburn. The purpose of the present study was to determine the pathophysiologic response postburn in terms of hypermetabolism, inflammation, hormonal and body composition changes, organ function, and muscle protein synthesis in a large prospective clinical trial to enable developments of future interventions and treatment options.

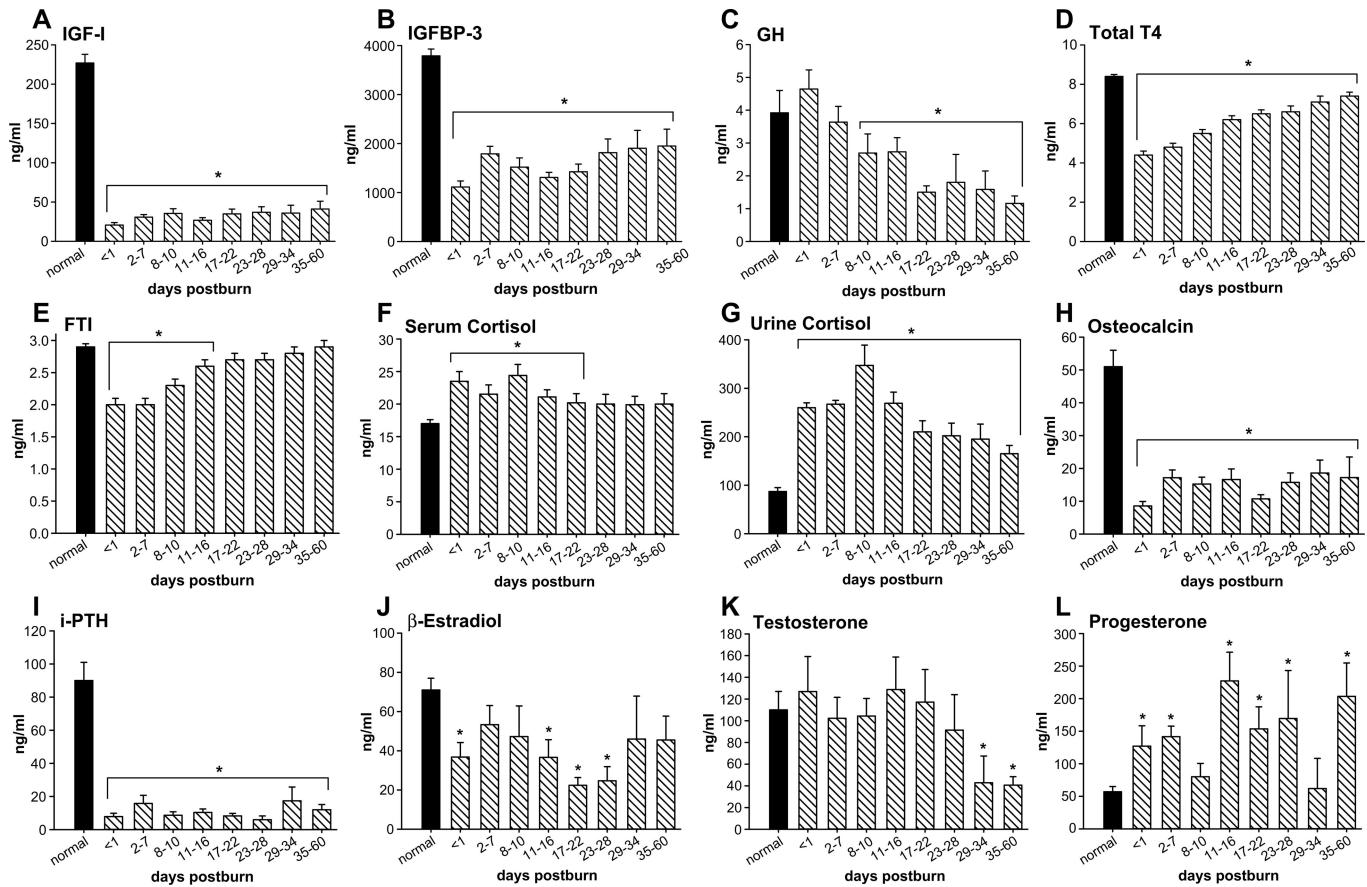
The patients in this study were severely burned children with an average age of 8 years and two thirds were males. The mortality was low (8%), indicating that mortality in severely burned children has, in fact, drastically decreased over the last decades and does not represent a valid outcome determinant for clinical studies in this patient population.<sup>29</sup> More striking was the effect of severe burn on metabolic and physiologic markers. Hypermetabolism measured by resting energy expenditure was markedly elevated to 130% to 140% predicted and remained elevated for the entire study period. The physiologic consequences of hypermetabolism are protein catabolism, loss of body weight, LBM, BMC, and BMD. This tremendous loss in essential body structures is not limited to acute hospitalization. We have shown that loss of proteins, muscle, and bone persists up to 2 years postburn

affecting patients' lives remarkably.<sup>1,2</sup> We, therefore, suggest that it is beneficial to attenuate the hypermetabolic response immediately postburn and subsequently preserve protein and amino acid stores. Agents known to affect postburn hypermetabolism and catabolism are insulin, IGF-I, oxandrolone, GH, and propranolol.<sup>4,18,30–33</sup> As GH increases mortality in critically ill adults<sup>34</sup> and IGF-I is at the moment limited in its availability, we recommend the use of insulin, oxandrolone, or propranolol to attenuate hypermetabolism and catabolism.

In this large prospective trial, we found that a severe burn affects expression of acute phase proteins. Serum complement C3 decreased initially but then increased over time, as did  $\alpha_2$ -macroglobulin. These 2 proteins seem to act as slow-acting acute phase proteins, whereas haptoglobin,  $\alpha_1$ -acid glycoprotein, and CRP act as more rapid acute phase proteins. Constitutive hepatic proteins, on the other hand, are significantly decreased throughout hospital stay. This decrease could be due to decreased production, increased consumption, or increased loss due to capillary leakage. These proteins are markers for general homeostasis indicating the severity and intensity of the postburn dysbalance. We found that apolipoprotein A1 is significantly decreased, whereas apolipoprotein B is initially decreased but increases at later time points. The exact role of these 2 proteins during the postburn response needs to be determined to evaluate their potential as targets for therapeutic interventions.

Of greater interest is the change in serum triglycerides and free fatty acids, both of which are significantly increased through almost the entire acute hospital stay. Fat transporter proteins are decreased postburn, whereas triglycerides and free fatty acids are increased, which could explain the fatty infiltration of the liver and other organs postburn. We have shown that hepatomegaly with fatty infiltration is associated with increased incidence of sepsis and mortality implying the importance of organ integrity and function.<sup>35</sup> A therapeutic approach to decrease lipolysis and fatty infiltration and reverse the acute phase response may thus improve morbidity and mortality.<sup>36</sup> We have recently shown that propranolol administration attenuates lipolysis and the hepatic acute phase response. Beta blockade decreases urinary nitrogen loss, peripheral lipolysis, whole-body urea production,<sup>37</sup> and resting energy expenditure.<sup>31</sup> Propranolol also decreases hepatic fat storage by limiting fatty acid delivery in severely burned pediatric patients.<sup>36</sup> In addition, we showed that propranolol decreased peripheral lipolysis and improved insulin responsiveness.<sup>38</sup> Recently, we further showed that propranolol has a profound effect on fat infiltration of the liver by reversing hepatomegaly.<sup>36</sup>

A striking finding of the current study was the change in the hormonal axis. In general, critical illness is characterized by marked alterations in the hypothalamic-anterior-pituitary-peripheral-hormone axes, the severity of which is associated with a high risk of morbidity and mortality.<sup>12</sup> We looked at several hormonal axes, such as the GH-IGF-I-IGFBP-3-axis, FTI-T4-axis, cortisone-cortisol-axis, insulin-glucose-axis, PTH-Osteocalcin axis, and sex hormones (testosterone,  $\beta$ -estradiol, progesterone). One of the most important endocrine axis after a severe injury and in the

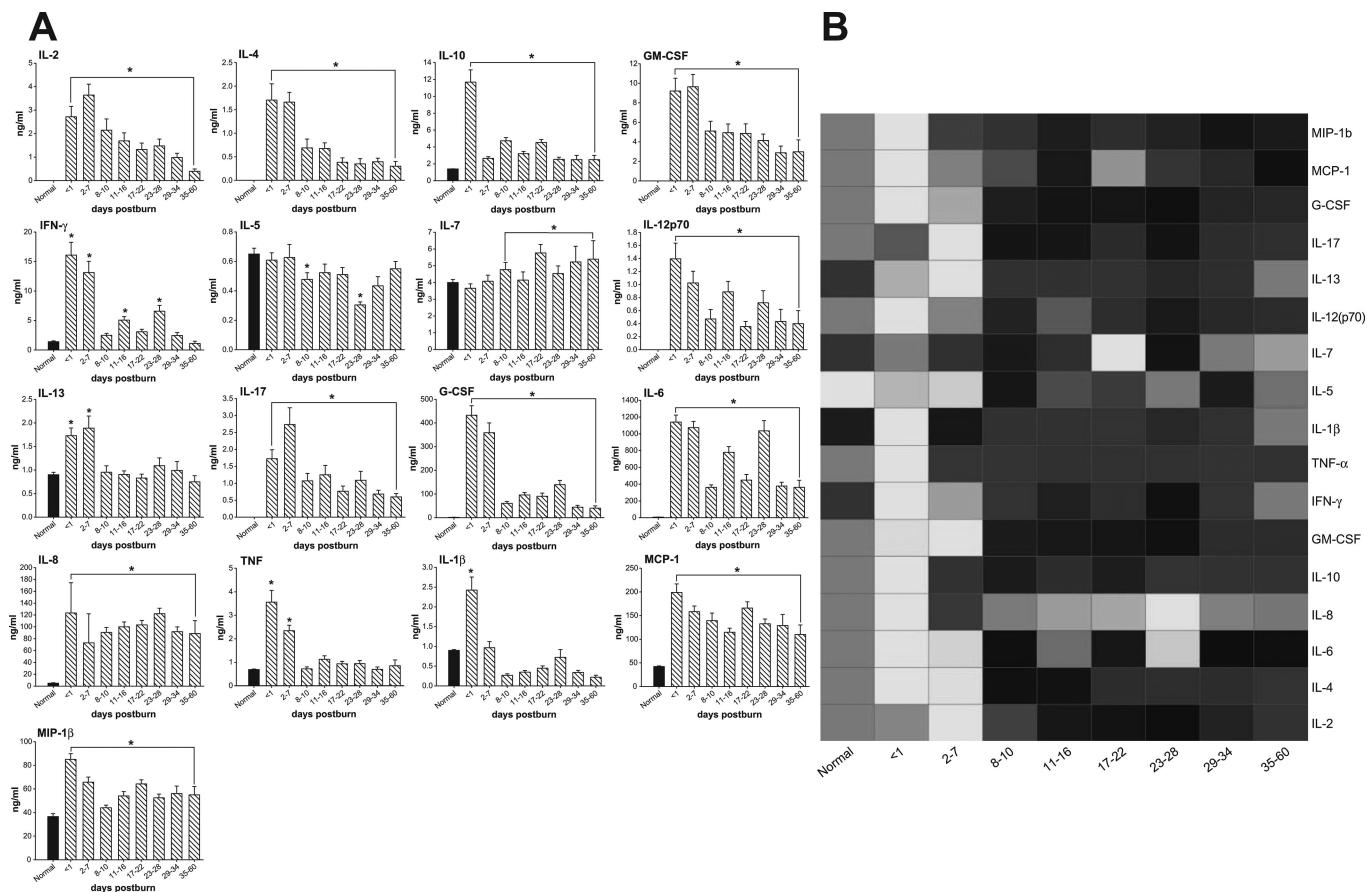


**FIGURE 6.** Serum hormone levels. Serum IGF-I and IGFBP-3 (A, B) decreased markedly immediately after burn and remained significantly decreased throughout acute hospitalization. Serum GH (C) started to decrease 8 to 10 days postburn and showed a steady decline through acute hospitalization. Serum T4 (D) decreased immediately postburn but increased through acute hospitalization. Free thyroid index (FTI) (E) showed a significant decrease for 14 days postburn and then returned to normal levels with no apparent difference. Serum cortisol (F) significantly increased immediately postburn and remained elevated for 3 weeks returning to normal levels. Urine cortisol (G) increased 5- to 7-folds during the acute stay but decreased over time. Serum osteocalcin (H) and iPTH (I) were drastically decreased (5–7-folds) immediately after burn and showed almost no increase over time. Serum  $\beta$ -estradiol (J) decreased immediately postburn but increased over time; testosterone (K) was normal during the early postburn phase but showed marked decreases beginning 4 weeks postburn. Progesterone (L) was increased at various time points when compared with normal. \*Significant difference between normal versus burn;  $P < 0.05$ .

critically ill is the GH-IGF-I axis. Recombinant human growth hormone (*rhGH*) has been shown to enhance immune function,<sup>39,40</sup> wound healing,<sup>41</sup> and to diminish the hypermetabolic response after major surgery, trauma, sepsis, or a thermal injury.<sup>42–44</sup> *rhGH* stimulates protein synthesis and attenuates the nitrogen loss after injury and improves clinical outcomes.<sup>45</sup> As animal and in vitro studies have shown, *rhGH* modulates the hepatic acute phase response by increasing constitutive hepatic proteins, decreasing acute phase proteins, modulating cytokine expression, and increasing IGF-I concentrations.<sup>46,47</sup> However, in a prospective, randomized, double-blind study in European ICU's, it has been demonstrated that *rhGH* treatment increased mortality among adult trauma patients when compared with placebo (42% vs. 18%).<sup>34</sup> Thus, GH administration is restricted and the indication for its administration is limited. In addition, by analyzing our data, it seems that GH administration may not be the best therapeutic agent for critically ill or burned patients. In the present

study, we showed that IGF-I and IGFBP-3 are much more affected when compared with GH.<sup>9,10,48,49</sup> As the clinical use of GH is restricted, it seems that IGF-I may be a better drug compared with GH to effectively attenuate the postburn. In fact, we conducted an animal and a clinical study in which we showed that IGF-I, in combination with its principle binding protein, improved muscle protein synthesis, hepatic acute phase and inflammatory response, and immune system.<sup>30,32,50</sup> However, we suggest that it would be necessary to test IGF-I in a large, multicenter trial to determine whether IGF-I would be a beneficial treatment option in severely burned patients.

The other hormonal axis that may play an important role is the thyroid hormone axis. Van den Berghe and co-workers<sup>51</sup> showed in patients who died after intensive care, not only did the hypothalamus-pituitary-thyroid axis undergo marked changes, but that also tissue-specific mechanisms are involved in the reduced supply of bioactive thyroid hormone in critical illness. We showed in the present study that T4



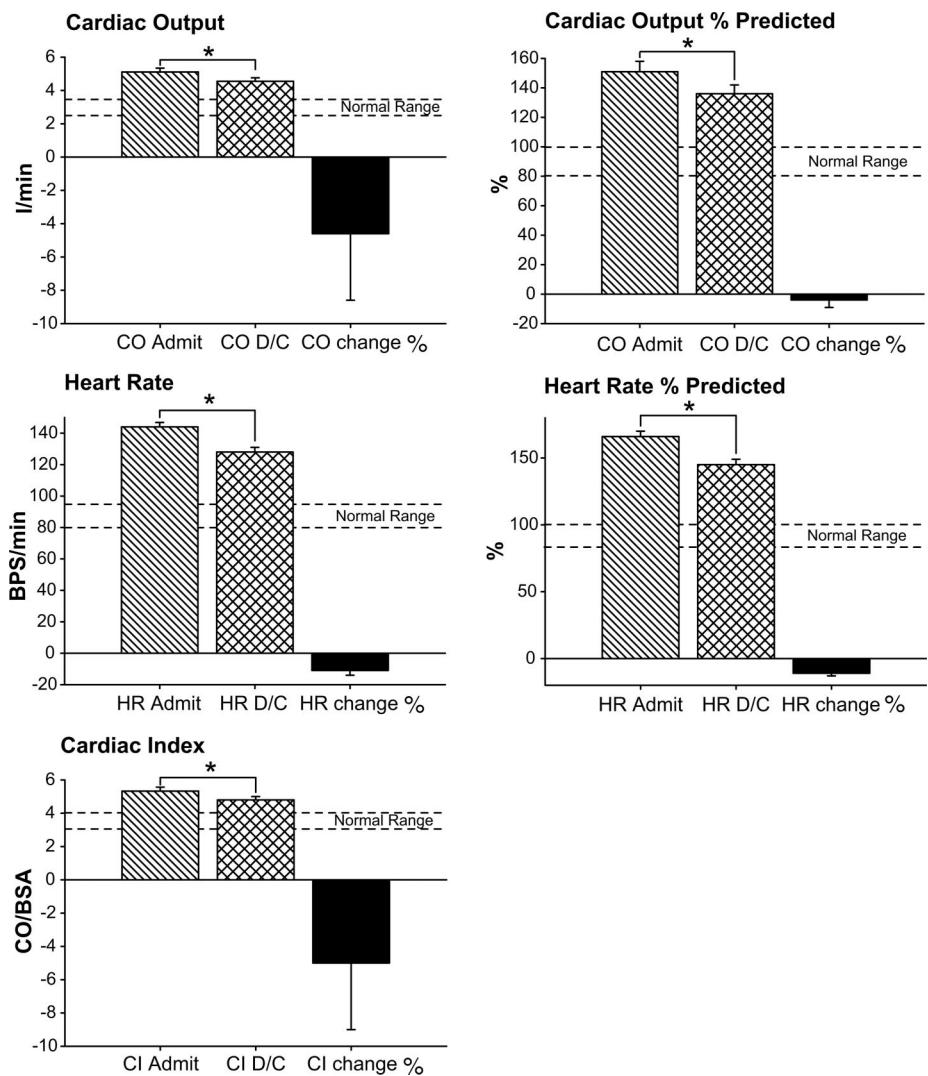
**FIGURE 7.** All of the 17 serum cytokines measured were significantly altered (A) and these perturbations are possibly clinically relevant. Dramatic changes were observed for serum G-CSF, IL-6, IL-8, MCP-1, and MIP-1 $\beta$ . \*Significant difference between burn versus normal ranges,  $P < 0.05$ . Heat map (B) comparing normal (noninjured, nonburned children), and burned children controls at each time point <1 day postburn, (2–7 days postburn, 8–10 days postburn, 11–16 days postburn, 17–22 days postburn, 23–28 days postburn, 29–34 days postburn, and 35–60 days postburn). Values are log<sub>10</sub> (average cytokine concentration, pg/mL); the color range for each cytokine is based on the detected values with blue indicating lower levels, yellow indicating highest levels, and black in the middle. Gray squares indicate that no expression was detected.

significantly decreased by 2-folds immediately postburn. Free thyroxine index also significantly decreased but reached normal levels 2 weeks postburn. The questions whether thyroid hormones should be replaced or not is difficult to answer and is controversially discussed.<sup>52–54</sup> As T4 and FTI levels did not change in the magnitude as other hormones measured, we suggest that a replacement with thyroid hormones is not warranted at this time and that T4 and FTI should be considered as a marker for the systemic homeostasis post stress.

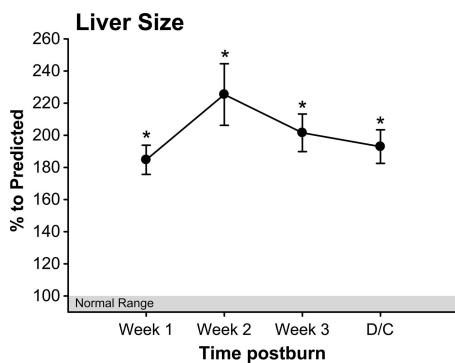
Catecholamines and stress hormones such as cortisol drive the hypermetabolic response to burn injury.<sup>5,7,55,56</sup> In the present study, we found that a severe burn injury increases serum and urine cortisol. Urine cortisol increased 5- to 8-fold and remained elevated throughout the entire acute hospital stay. Stress hormones such as glucocorticoids have been described as one of the major hormones responsible for proteolysis and catabolism.<sup>57–60</sup> Glucocorticoid levels are markedly increased postburn, and therefore, a hypothetical approach to attenuate protein breakdown and hypermetabolism would be to block cortisol production. We are currently

conducting a prospective randomized controlled trial to block cortisol production using ketoconazole.

Glucose kinetics in severely burned patients is almost always abnormal. Glucose utilization in burned patients is through inefficient anaerobic mechanisms as characterized by increased lactate production accounting for increased glucose consumption.<sup>5,7,61,62</sup> Glucose production, particularly from alanine, is elevated in almost all patients with severe burn.<sup>25</sup> The increased gluconeogenesis from amino acids renders these amino acids unavailable for reincorporation into body protein. Nitrogen is excreted, primarily in urea, thus contributing to the progressive depletion of body protein stores. In the present study, we showed that plasma insulin levels are significantly increased over 4 to 5 weeks postburn. This confirms data from other groups who showed that a severe burn causes increased insulin levels.<sup>63,64</sup> Increased insulin levels do not result in decreased glucose levels. In contrast, we showed that serum glucose is significantly increased for 4 to 5 weeks postburn. The fact that the basal rate of glucose production is elevated despite elevated plasma insulin levels



**FIGURE 8.** Cardiac output and predicted cardiac output postburn were increased immediately (up to 160% of predicted) and significantly decreased until discharge. Heart rate and predicted heart rate were also significantly increased postburn and remained elevated at discharge. Cardiac index was increased immediately postburn and significantly decreased from admission to discharge. Black bar depicts change from Admit to Discharge in %. \*Significant difference between admission and discharge;  $P < 0.05$ .



**FIGURE 9.** Immediately after burn, liver size doubled and at week 2, postburn was 220% of predicted liver size. Liver size remained elevated when patients were discharged from the ICU. \*Significant difference between burn versus normal range;  $P < 0.05$ .

indicates hepatic insulin resistance, since under normal conditions elevated serum insulin would lower the rate of glucose production.<sup>61,65,66</sup> We would like to point out that our values are not fasted values as most of our patients receive continuous feeding via a duodenal tube and we almost never stop feedings. Hyperglycemia is associated with increased mortality in critically ill patients<sup>67,68</sup> and worsens the outcome in severely burned patients.<sup>69–71</sup> In a recent study, we determined serum insulin and glucose levels in severely burned patients with different burn sizes. We found that insulin levels were significantly increased in the >80% TBSA burn group and lower in the smaller burn groups. This indicates that with the increased severity of the burn injury, insulin resistance increases and more insulin needs to be synthesized to maintain normoglycemia. It further indicates the necessity to attenuate hyperglycemia to improve outcome and survival. Agents to decrease glucose and improve insulin sensitivity encompass insulin, metformin, and fenofibrate.<sup>63,69,72–75</sup>

Another striking finding was that osteocalcin and parathyroid hormone were drastically decreased immediately after burn and remained decreased during the acute phase

postburn. Klein et al<sup>76–82</sup> published extensively on bone metabolism postburn. They showed that burned children have decreased BMC and BMD. Using labeled tetracycline, they determined bone turnover rates and found that burned patients have almost no bone formation and synthesis.<sup>76–82</sup> We did not determine bone turnover rates in this study; however, the biochemical markers that we determined indicate that bone metabolism is dysfunctional very early postburn indicating another target for therapeutic intervention. In a recent study, pamidronate<sup>81</sup> was shown to improve bone metabolism during the acute phase and long-term phase postburn. Another possible treatment approach would be sex hormone substitution. Estrogens have been shown to improve bone mineralization and metabolism.<sup>83</sup> Choudhry and coworkers have found that estrogens have a positive effect on inflammation and hypermetabolism and improve survival in a trauma hemorrhage model.<sup>84–87</sup> In the present study, we found that estrogen was significantly decreased immediately after the injury but increased during hospital stay, whereas testosterone decreased and progesterone increased over time. Therefore, it would be interesting to investigate the effects of estrogen on the postburn response. Although the effect of estrogens after burn has not been investigated, the effects of testosterone on postburn muscle metabolism were studied.<sup>88</sup> Testosterone improved muscle catabolism but is associated with side-effects.<sup>89,90</sup> Therefore, we and others determined the effects of oxandrolone, a synthetic testosterone analogue on the postburn response, and found that oxandrolone increased LBM and shortened acute hospitalization.<sup>18,91,92</sup>

We suggest that changes in protein expression are driven by the inflammatory response.<sup>3,20</sup> Cytokines and pro-inflammatory mediators are known via cellular mediators to block and therefore decrease endogenous anabolic agents.<sup>93,94</sup> Immediately postburn, there are marked changes in the cytokine expression profile. Of 17 cytokines, 16 drastically increased, most significantly IL-6, IL-8, MCP-1, MIP-1 $\beta$ , and G-CSF. Even so called anti-inflammatory cytokines increased significantly postburn. Only IL-5 was in the normal range postburn and decreased over time. IL-5 is a TH-2 cytokine produced by T helper-2 cells and mast cells. It stimulates B cell growth and increases immunoglobulin secretion and is also a key mediator in eosinophil activation. Unlike other members of this cytokine family (IL-3 and GM-CSF), this glycoprotein is a homodimer and is also expressed by eosinophils. Postburn immune exhaustion and compromise are present, and therefore, decreased IL-5 may be an important factor for this exhaustion.

Other intriguing findings of this study were changes in cardiac function. Burn patients with burns over 40% of their TBSA demonstrated an increased cardiac output and cardiac index accompanied by a massive tachycardia with 160% to 170% predicted heart rate. CO, CI, and heart rate remained high at ICU discharge at around 130% to 150% predicted. We now have evidence that the heart rate remains elevated up to 2 years postburn. Increased cardiac stress postburn is associated with myocardial depression.<sup>95–97</sup> The hypothesis that cardiac stress and myocardial dysfunction may be one of the main contributors to mortality in large burns was confirmed

in a recent retrospective autopsy study,<sup>98</sup> and clinical study,<sup>19</sup> implying the therapeutic need to improve cardiac stress and function. That this finding is not specific to our center is shown by the WHO report, in which the WHO delineates highest mortality rates in children <4 years and adults >65 years.<sup>99</sup> Propranolol decreases cardiac work and improves oxygen delivery to the heart representing a therapeutic approach to improve cardiac morbidity.<sup>31,100</sup>

That all these biomedical markers are related to real physical changes is demonstrated by the markedly compromised physical function in regards to muscle strength and cardiopulmonary capacity. An increase in both would go a long way in improving a burned child's capability to return to normal activities of daily living, in addition to improving quality of life. In fact, our group has an exercise program implemented at discharge as part of the outpatient rehabilitation and its effects are currently being evaluated. However, we have reported that an exercise program implemented at the 6-month postburn time point significantly improves physical function.<sup>22–24</sup>

We would like emphasize that the present cohort study is heterogeneous because we included, in our cohort study, male and female patients with the latter containing both pre- and postmenarche patients, patients with and without inhalation injury, patients with and without infection/sepsis, patients with and without multiorgan failure, and patients who died and did not die. We did not eliminate patients with inhalation injury, sepsis, and multiple organ failure and death to smoothen out the trajectory of change of the variables measured because we wanted to achieve a large patient cohort to perform robust statistics. We propose that the development of trajectories or patterns of these detrimental outcomes will be the focus of future studies. Another concern could be the fact that the time to admission after injury averaged 6.7 days, which means that the population of study patients was biased towards survival since the mortality rate of extensively burned patients decreases across time and plateaus after the tenth postburn day. We, however, suggest that we did not bias our study population because we attempted to get patients as soon as possible to our center. Transfer of patients from Central and South America shortened over the past years to times comparable within the United States, and the majority of our patients now arrive at our center within the first 72 hours postburn.

Based on our findings, we suggest that a burn injury involving more than 30% to 40% of the total body surface causes marked and prolonged inflammation, marked increases in hypermetabolism, catabolism, cardiac dysfunction, hormonal changes, and subsequently prolonged morbidity and mortality in 10% of this patient population. We suggest that these events occur in a timely manner. Treatment should focus on several aspects of the pathophysiologic events postburn, such as anti-inflammation, attenuate hypermetabolism and improve glucose metabolism, immune system, and cardiac function.

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## Discussions

DR. BASIL A. PRUITT JR. (SAN ANTONIO, TEXAS): The authors have presented a wealth of data that confirm the pervasive multisystem effects of extensive burn injury and have identified long term functional impairment that influences outcomes in children with extensive burn injury.

To evaluate these data in detail, the authors need to provide us with some additional information. It is stated that protein net balance was measured in a subset of 60 of the 242 study patients, but the authors do not tell us how these patients were selected, and the possibility exists that unique selection criteria would limit the applicability of those data to other patients. DEXA technology was used to measure lean body mass, but it is unclear how tissue mass was differentiated from edema fluid to eliminate the possibility that the observed changes in body composition were simply changes in water retention. Both total fat and percent fat increased in the patients, but how did the authors decide whether that was a response to the injury or an effect of high carbohydrate feeding in children receiving insulin to reduce blood glucose levels, as may have been the case in at least some of the patients?

There was considerable variability in the measured serum components in Figure 4, the hormones in Figure 6, and particularly the cytokines in Figure 7. How do the authors account for that variability, for example, the biphasic change of significance in free fatty acid levels, the triphasic change of significance in  $\beta$  estradiol and progesterone levels that appear to move in reciprocal fashion, and the multiple changes in significance in the case of interferon  $\gamma$  and IL 6? In that same vein, how do you account for the wide swings in measured levels of IL 6 and IL 12p70? Are those variations a reflection of different sampling times or possibly the effect of superimposed sepsis? What was the effect of sepsis on the measured variables and was it evaluated in a subset of septic patients?

In the past, the authors emphasized gender differences in the response to burn injury, but now they combine data for males with data from females. What does such amalgamated data mean?

I have always considered the burn patient to be the universal trauma model, and I wonder whether these data will now permit us to identify both commonalities and differences in the response to different injuries and to that end, I ask the authors if they have explored such an extension of their data. Additionally, is it possible to use these data to refine predic-

tions of mortality that are based on age and burn size alone? Similarly, are any of the physical capability measures useful in predicting special needs for physical therapy and monitoring the success of physical therapy regimens?

In summary, the authors presented what constitutes a repository of data that offers the promise of being useful in comparing burn populations, identifying early sepsis, differentiating a favorable from an unfavorable trajectory, and revealing correlations that will guide us to interventions that will ameliorate the response to severe injury in burned children.

DR. JOE BESSY (NEW YORK, NEW YORK): I have 2 questions.

The first is that there are indeed many techniques that you and your group have developed to support these kinds of massive injuries, and I wonder if you have any sense of how much of the changes you documented so nicely reflect our therapy as opposed to reflecting the disease.

Then, in a bit more philosophic way, I believe in front of this Association almost 60 years ago, Bull and Squire (*Ann Surg.* 1949;130:160–173) presented their summary that documented the importance of age and burn size in survival from burns. In that paper, almost none of these patients would have survived, and here you have a mortality of about 8%. Is the response to burn injury good or bad?

DR. ANTHONY A. MEYER (CHAPEL HILL, NORTH CAROLINA): I feel that the great benefit of this study is not only to show some of the changes and the variability, but also to document, finally, the prolonged nature of the response to burn injury in addition to its magnitude. Everyone has always understood how sick these patients are, it is a question of how long they are so sick.

DR. WILLIAM P. SCHECHTER (SAN FRANCISCO, CALIFORNIA): I want to ask you a question about the insulin resistance that you noted. We also see this clinically in septic patients. How did you distinguish insulin resistance from the burn injury per se and sepsis, which many, if not most of them, experience? I would also like you to comment on the mechanism of insulin resistance if you would. At the end of your paper, you also said you wanted to address treatment of abnormalities in glucose metabolism. Can you expand a bit on that thought?

DR. ROGER W. YURT (NEW YORK, NEW YORK): Two questions. One, it appears that you are, perhaps, looking at a subset of patients in that the average time to admission was 6 days postinjury. So the question is, are there some patients that you are not looking at that did not survive to reach you?

The second question, you and your group have shown that there are certain interventions such as propranolol growth hormones and anabolic steroids that contribute to excellent outcomes. How do you reconcile this with the fact that at least, as you have explained it, you used any of those interventions in this group of patients?

DR. MARC G. JESCHKE (GALVESTON, TEXAS): Dr. Yurt, regarding your point about the subsets of the selection of survivors and nonsurvivors, this study was designed to take all of our control patients receiving no propanolol, and receiving no anabolic agent, to do exactly what you and Dr. Pruitt suggested. What we do with this data now is to develop trajectory models. As for the second question, none of these patients received catabolic or anabolic treatment.

In answer to Dr. Schechter's question on insulin resistance, that is, how did we differentiate burn sepsis, sepsis now is a subset of these patients. We will start looking at this. We are trying to determine what this identifies for patients with sepsis and to see what this difference is. However, your question about insulin resistance is very interesting. What we found is that burns produce a stress and protein response that leads via activation of the JNK-pathway to a blockade of the insulin receptor substrate, leading to impaired insulin signaling.

Dr. Meyer asked about therapeutic standards and what we are doing to achieve this, and again, nobody received an anabolic agent. We changed our regimen to early grafting. We are very aggressive about taking all patients back to the O.R. As soon as the donor site heals, we go back to the O.R. We try to operate on patients as early as possible once we feel the grafts have taken. We adjusted our nutrition to resting energy expenditure, and give them high protein feeding rather than fat feeding because we believe fat increases infiltration of the liver. And, one major factor is early extubation. Therefore, these all contribute to the improvement in complications and support.

Dr. Bessy asked about age and burn size. Again, we wanted to combine all of our patients to maintain power. What we would like to know is, why do these patients die rather than the other patients, and with 8%, or 19 patients, it is difficult to achieve statistical power. Therefore, we combined them all. Age surely has an effect, but we know that kids under 4 years of age are most prone to, and have the

highest mortality due to increased cardiac dysfunction. We have also seen that burns cause cardiac dysfunction.

To the questions that Dr. Pruitt asked. For the muscle studies, we selected our patients based on the completeness of the data set. We wanted to make sure that we included data from the first week and third week postburn. There was no specific selection for control patients.

Regarding DEXA technology, we submitted the paper to *JPEN* (*the Journal of Parenteral and Enteral Nutrition*) showing almost 90% correlation, indicating that with the DEXA technique, we can detect real changes in muscle volume.

As to increasing fat in patients, I think you are right, when we have to give insulin we increase hepatic storage. However, Dr. Hart and Dr. Herndon's study in critical care medicine found that feeding at 1.2 to 1.4 the resting energy expenditure, 4 times the ideal supplemental support for nutrition somewhat attenuated the catabolic and hypermetabolic response. And this is now our current standard.

You are absolutely right about the biologic variability. For all proteins, all hormones, we try to be very consistent and adhere to the timelines. We obtain blood for hormones from 6:00 to 8:00 AM as well as cytokines pre-O.R. However, you still have significant biologic variability, even with modern techniques, and that is why we have 242 patients and use all patients. In answer to your question about universal trauma, you are absolutely right; burn is considered the severe model of trauma. For part of the trial, one of the goals of the group is to look at protein and genomic changes due to trauma and burn, and one outcome study that will be done is to compare burn to trauma, look at the genomic and protein profile, and then see if there is a distinct difference in these responses?

Lastly, Dr. Pruitt, we are working with the data to perhaps develop trajectories to say what causes all patients to do poorly, what causes all patients to do better, why do some patients get 80% burn in 2 weeks and some do not, to come up with predictors and trajectories.