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Retinol Binding Protein: Marker for Insulin Resistance and Inflammation Postburn?

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Abstract

Introduction—Burn injury leads to vast changes in both metabolic and inflammatory responses and is associated with increased morbidity and mortality. Insulin resistance (IR) and hyperglycemia are major components of the hypermetabolic response found in burn-injured patients and subsequently contribute to adverse outcomes. Studies have shown that increased systemic retinol binding protein (RBP) levels are associated with IR and hyperinflammation in diabetic and obese patients. The aim of this study was to determine RBP profiles and to test the hypothesis that elevated RBP levels are associated with both IR and the inflammatory response in burned patients.

Methods—RBP was measured in 372 patients during the acute stay postburn. Patients' demographics, glucose levels, and insulin administration were recorded. Cytokines, hormones, plasma proteins, and organ markers were measured. The average of all measurements of RBP (2.1 mg/dL) was used to divide patients into high and low groups. Statistical analysis was performed by Student *t* test. Statistical significance was accepted at $P < .05$.

Results—Fifty-one patients (high group) had elevated RBP levels during acute hospitalization and demonstrated a significant higher incidence of multiorgan failure, sepsis, and mortality ($P < .05$). Moreover, in the high group, a significant increase of IR, inflammatory cytokines, and catabolic and organ-specific markers were detected ($P < .05$).

Conclusions—Increased RBP levels postburn correlate with increased IR, inflammatory and catabolic responses, incidence of multiorgan failure, and mortality. RBP may be a novel biomarker to monitor these detrimental responses postburn.

Keywords

insulin resistance; burn injury; retinol binding protein (RBP); renal function; inflammation; catabolism

Clinical Relevancy Statement

Posttraumatic, stress-induced insulin resistance (IR) is a major complication following burn injury that is associated with several clinical complications. The study investigates the use of retinol binding protein (RBP) as a possible target of monitoring the hyperglycemic response and inflammatory processes postinjury. Moreover, the findings of molecular biological studies are put into a clinical context depicting possible relationships of RBP levels and organ-specific complications. The relevance of our study is that we were able to show that RBP plays a more important role than previously thought and may predict IR and hyperglycemia in trauma or severely injured patients.

Introduction

Retinol binding protein (RBP), mainly expressed by liver cells and adipocytes, not only is the only known carrier for the important antioxidant vitamin A (retinol)¹ but has also been shown to be involved in the pathways of insulin resistance found in type 2 diabetes.^{1,2} This is supported by evidence that knockout rodents deficient for GLUT 4 receptors in adipocytes exhibit increased systemic levels of RBP.³ It has been shown that injection of RBP impairs the insulin signaling pathway and induces the gluconeogenic pathway of phosphoenolpyruvate decarboxylase in the liver, leading to enhanced insulin resistance (IR).³

Insulin resistance is commonly found postburn.⁴⁻⁷ Impaired insulin sensitivity results in reduced glucose uptake into the cells and subsequent hyperglycemia postburn.^{8,9} Although discussion of IR and hyperglycemia in critically ill patients has been controversial, studies in burn patients have frequently shown that hyperglycemia is associated with increased morbidity and mortality.^{4,5,10-14}

A study conducted by Graham et al² showed a positive correlation between IR and serum RBP levels in diabetic patients. In addition, studies are providing further evidence that RBP plays an important role in glucose metabolism.^{3,15} It has been shown that systemic RBP4 expression in humans is associated with inflammatory processes.^{16,17}

However, to date, no large studies have examined the involvement of RBP in a stress-induced IR scenario. We chose to examine the relationship between RBP, clinical parameters, and outcomes in our pediatric burn population. The various underlying cellular mechanisms and the involved pathways of trauma-induced IR are currently the subject of several investigations.¹⁸⁻²⁰ On a cellular level, current research is focused on the insulin receptor, insulin receptor substrates, and phosphatidylinositol 3-kinase/Akt pathways.^{21,22} The complete mechanisms in burns are unknown, and it is suspected that different underlying mechanisms exist.¹⁸ However, using the fact that stress-induced IR in burn patients is a predominant factor in hyperinflammatory and hypermetabolic processes, this patient population is ideal to examine a possible correlation between systemic RBP levels, IR, metabolic response, and inflammation.²³ We hypothesize that elevated systemic RBP levels reflect insulin resistance and inflammation and might serve as a surrogate marker for IR and inflammation in severely burned patients.

Patients and Methods

Patients were resuscitated according to the Galveston formula with 5000 cc/m² total body surface area (TBSA) burned + 2000 cc/m² TBSA lactated Ringer's solution given in increments over the first 24 hours. Within 48 hours of admission, all patients underwent total burn wound excision, and the wounds were 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 nutrition treatment according to a standardized protocol. The intake was calculated as 1500 kcal/m^2 body surface + 1500 kcal/m^2 area burn as previously published.²⁴ The nutrition route of choice in our patient population was enteral nutrition via a duodenal (Dobhof) or nasogastric tube.

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 a causative 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 ($>38.5^\circ\text{C}$ or $<36.5^\circ\text{C}$), tachycardia (>150 bpm in children), refractory hypotension (systolic blood pressure <90 mm Hg), thrombocytopenia (platelets $<50,000/\text{mm}^3$), hyperglycemia (serum glucose >240 mg/dL), and enteral feeding intolerance (residuals >200 cc/h or diarrhea >1 L/d) as previously published.²⁵ Multiorgan failure was assessed continuously during the length of stay using the DENVER2 score.²⁶

Cytokines and Proteins

Blood and/or urine were collected from burn patients at admission, preoperatively, and consecutively up to 60 days postinjury for serum protein and 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.

Serum proteins C-reactive protein (CRP), osteocalcin, RBP, and prealbumin were determined using high-performance liquid chromatography, nephelometry (BNII; Plasma Protein Analyzer, Dade Behring, Bowie, MD), and enzyme-linked immunosorbent assay 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 17 inflammatory cytokines (interleukin [IL]-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-17), monocyte chemoattractant protein-1 (MCP-1), and tumor necrosis factor. 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°C . Serum samples were then incubated with microbeads labeled with specific antibodies to one of the aforementioned cytokines for 30 minutes. Following 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-phycoerythrin for 10 minutes and washed, and the concentrations of each cytokine were determined using the array reader.

Body Mass Index

Body mass index (BMI) was calculated according to the formula recommended by the World Health Organization (WHO): $\text{BMI} = \text{weight (kg)} / \text{height}^2 \text{ (m)}$. To validate these results for age, we used percentile ranks for stratification.²⁷

Ethics and Statistics

The study was reviewed and approved by the Institutional Review Board of the University of Texas Medical Branch, Galveston, Texas. Prior to the study, each patient, parent, or child's legal guardian had to sign a written informed consent form. Data collection and analysis were performed using Microsoft Excel (Microsoft, Redmond, WA) and SigmaStat version 3.5 and SigmaPlot (Systat Software, San Jose, CA). Analysis of variance with post hoc Bonferroni correction, paired and unpaired Student *t* test, and χ^2 analysis were used

where appropriate. Correlations between patient characteristics were analyzed by using multiple linear regression analysis. Data are expressed as means \pm SD or SEM, where appropriate. Significance was accepted at $P < .05$.

Results

Three-hundred seventy-two patients were included in the study. Patients were divided in 2 groups according to their average RBP levels over the length of stay in the intensive care unit. To date, no landmark for the predictive value of RBP exists. The average systemic RBP level of 2.1 mg/dL for all patients was chosen to serve as a cutoff level and then to assign the patients to high (H) and low (L) RBP according to their average RBP levels during the first 10 days. Stratification of the individuals resulted in 321 patients to the low group and 51 patients to the high group.

Patient Demographics and Outcomes

Patient demographics in both gender and ethnicity were similar in both groups. Patients with RBP high levels had a significantly older average age of 10.3 years ($P < .05$) than patients in the low group (7.8 years). Regarding injury characteristics, patients with high RBP levels had a slightly higher incidence of inhalation injury (RBP low, 45%; RBP high, 47%) combined with a larger burn size (third-degree TBSA: RBP low, 48%; RBP high, 51%; Table 1). Patients with high RBP showed an increased incidence of mortality (RBP low, 7%; RBP high, 29%; $P < .001$), multiorgan failure (RBP low, 17%; RBP high, 51%; $P < .05$), maximum DENVER2 score (RBP low, 3.5; RBP high, 5.1; $P < .05$), and sepsis (RBP low, 10%; RBP high, 39%; $P < .001$; Table 2). The length of stay per TBSA was higher in the patients with high RBP than in patients with low RBP (RBP low, 0.6 days; RBP high, 1.1 days; $P < .05$; Table 2).

Multiple linear regression analysis revealed a significant positive relationship between the severity of injury and systemic RBP levels during the first 10 days after burn injury. Third-degree TBSA burn showed positive correlation with systemic RBP levels ($r = 0.205$, $P < .05$). Total burn size and the incidence of inhalation injury failed to predict RBP levels in this model and therefore did not correlate significantly (Table 3). An independently conducted analysis exposed a positive correlation for age and systemic RBP levels ($r = 0.173$, $P < .05$).

RBP Cutoff Value and Profile

The distribution of the individual RBP levels among the groups is shown in the box plot (Figure 1). The low group showed an equally distributed pattern among the quartiles, whereas patients in the high group skewed toward the upper quartile with maximum values around 4 mg/dL and 5 outliers above the 98th percentile.

Average RBP values increased during hospitalization at a similar rate in both groups, whereas values remained significantly lower in the low group during the studied period (Figure 2).

Glucose Insulin

Patients in the low RBP group required significantly less ($P < .05$) exogenous insulin and had significantly lower ($P < .05$) plasma glucose levels than the patients in the high RBP group during the first 5 weeks of study. In the subsequent days, levels were similar in both groups (Figure 3).

Cytokines

By analyzing inflammatory cytokines, we found that IL-6 and IL-8 levels were significantly higher in the high RBP group than in the low RBP during the first 3 to 4 weeks after injury (Figure 4A,B; $P < .05$). Similarly, MCP-1 and osteocalcin plasma levels were significantly increased in the high RBP during the first 5 weeks postburn (Figure 4C,D; $P < .05$). Other analyzed cytokines showed no statistical difference between both groups (data not shown).

Organ Markers and Liver-Synthesized Proteins

Organ markers such as blood urea nitrogen, bilirubin, and creatinine were also significantly elevated in the high RBP group (Figure 5A–C; $P < .05$). Liver-synthesized prealbumin, the acute-phase protein CRP, and plasma level triglycerides were also significantly higher in the high RBP group than in the low RBP group during the total period of study (Figure 5D–F; $P < .05$).

BMI

Patients with high RBP levels had a remarkably higher BMI corrected for age (RBP low, 20; RBP high, 23; $P < .005$) and a significantly higher absolute BMI (RBP low, 78th percentile; RBP high, 82nd percentile) compared to the low-level group ($P < .05$; Figure 6). This result corresponds with the on average older patients in the RBP high group.

Discussion

Hypermetabolism and inflammation are determinants for outcomes after severe burn injury.^{28,29} The detrimental effects of these pathophysiological processes lead to a higher incidence of comorbidities, infectious complications, and a prolonged need for rehabilitation.^{30,31} A portion of this response is attributed to the effects of hyperglycemia and insulin resistance, but the discussion of these processes and their effects in critically ill patients is controversial. The underlying mechanisms and links between inflammation, hypermetabolism, and IR are the current subject of several investigations.^{4,6,21,23,28}

The analysis of this patient collective revealed that elevated RBP levels correspond with higher systemic glucose levels and increased exogenous insulin requirements during the acute hospital stay. In addition, patients with high RBP levels showed prolonged inflammatory responses of higher magnitude.

Hyperinflammation in burn patients is expected.³² However, the significantly elevated levels of proinflammatory cytokines and the significantly prolonged elevation of CRP and IL-8 in the high RBP group at a similar infection rate may be attributed to systemic circulating RBP. This result supports the hypothesis that RBP may serve as a marker for endogenous noninfective inflammation, which is also a characteristic of IR.

Previously published results of studies conducted in obese and diabetic patients showed that increased systemic levels of RBP correlated with a decreased expression of GLUT4 in adipocytes.^{2,3,33} It is thought that GLUT4 deficiency contributes to hyperglycemia via diminished glucose sensing seen in adipocytes.³ This finding was also reflected in our study population by a significantly older patient population with both higher body fat percentages and BMI.

RBP is an important carrier for vitamin A, which acts as an important antioxidant in several metabolic processes.³⁴ Inflammation, a known oxidizing process, leads to increased needs of reducing agents, and therefore the requirements of vitamin A are increased.^{35,36} Measured elevated RBP levels can therefore also be attributed to the increased needs of retinol

(vitamin A) due to inflammatory processes. Further investigation needs to be done to address this interesting aspect of the role of both vitamin A and RBP in acute and chronic inflammatory processes.

We were able to show that MCP-1 is significantly increased in individuals with high levels of RBP. MCP-1 stimulates macrophage infiltration into tissue and is involved in immunologic processes and lipolysis.^{37,38} RBP has likewise been shown to be involved in catabolic processes such as lipolysis and bone mineral content (BMC) breakdown.^{39,40}

The patients with elevated RBP levels also showed increased triglyceride and osteocalcin levels. High plasma triglyceride levels can be either attributed to the effects of IR leading to a decreased uptake of triglycerides in peripheral tissue or, in this context, to an increased lipolysis caused by macrophage activity. Osteocalcin inhibits the calcification in extracellular bone matrix and leads to BMC loss.⁴¹ In addition, osteocalcin is suspected to have positive effects on the attenuation of IR.^{42,43} Newer studies have shown that osteocalcin stimulates insulin production in pancreatic cells and increases the effects of insulin in peripheral tissue via stimulation of adiponectin.^{43,44} Increased levels of osteocalcin therefore may be reflective of insulinresistance.⁴⁵

Metabolic changes are also reflected by organ-specific markers. Liver function in RBP high patients was impaired, shown by significantly elevated levels of serum bilirubin. Interestingly, the production of prealbumin, a sensitive marker for protein synthesis, was restored in the RBP high group.⁴⁶ This result may be explained by the selective production of prealbumin as it is known that an RBPprealbumin transporter system exists.³⁴ The transporter system may be triggered by elevated systemic RBP levels.

Significantly elevated markers for kidney function in the high RBP level group can be attributed to the resulting higher incidence of comorbidities and the worsened hospital course. However, it also can be hypothesized that impaired renal function contributes to elevated systemic RBP levels, as it has been shown that impaired renal function leads to a diminished excretion of RBP.⁴⁷

The results of our study suggest that serum RBP levels correlate with stress-induced IR as well as hypermetabolic and inflammatory responses. More mechanistic research needs to be done to investigate a possible involvement in these processes and the underlying mechanisms. On the basis of our results, we propose that systemic RBP levels may serve as a clinical marker for stress-induced IR and inflammatory processes during the acute phase postburn. Impaired renal function contributes to elevated systemic RBP levels, as it has been shown that impaired renal function leads to a diminished excretion of RBP.⁴⁷ The results of our study suggest that serum RBP levels correlate with stress-induced IR as well as hypermetabolic and inflammatory responses. More mechanistic research needs to be done to investigate a possible involvement in these processes and the underlying mechanisms. On the basis of our results, we propose that systemic RBP levels may serve as a clinical marker for stress-induced IR and inflammatory processes during the acute phase postburn.

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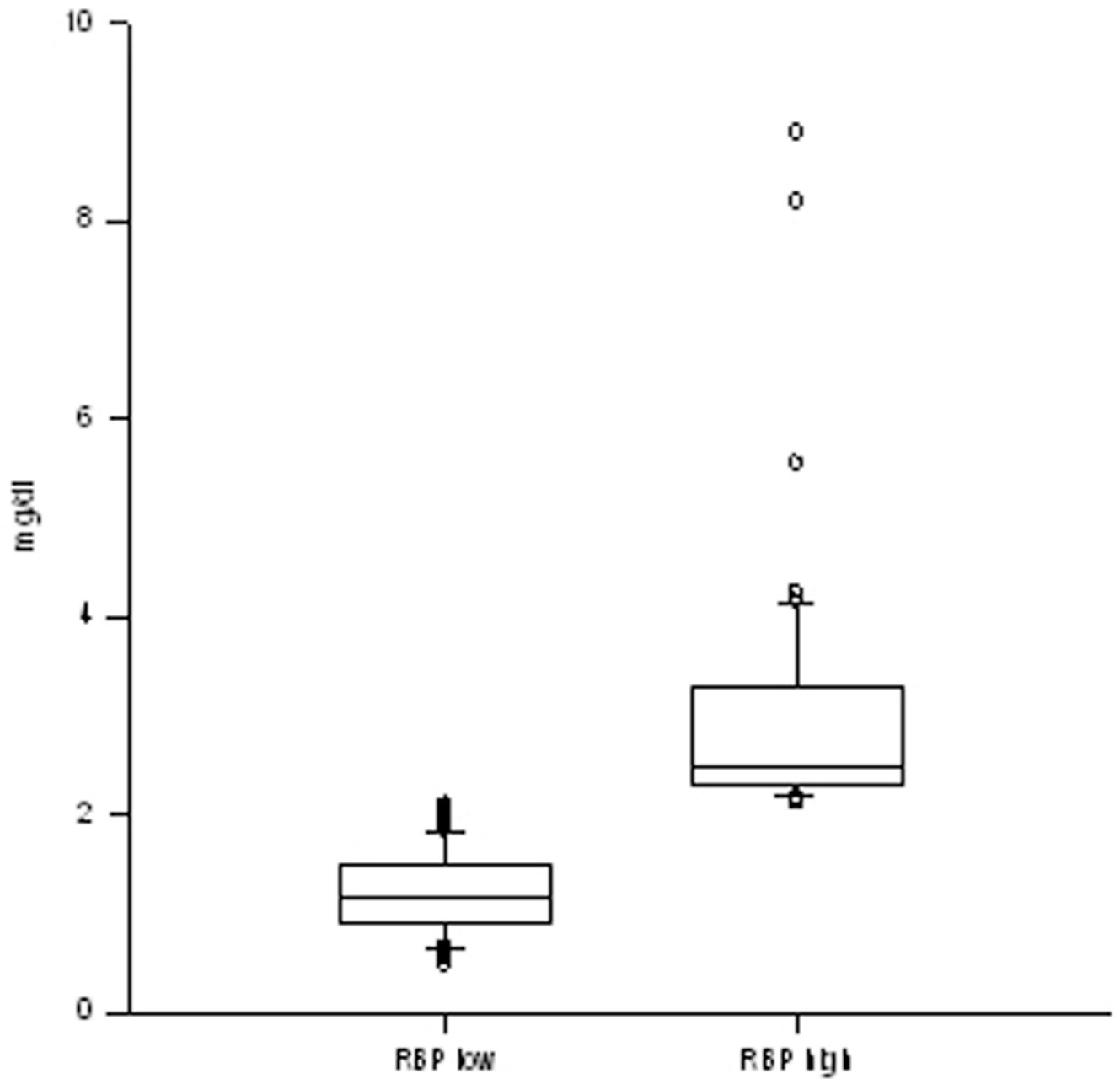


Figure 1.

Box plot of the retinol binding protein (RBP) values of both groups. Both groups showed consistency in their RBP levels. The RBP high group skewed toward the upper quartile with a maximum of 4 mg/dL, whereas in the RBP low group, the values are nearly equally distributed among the patients.

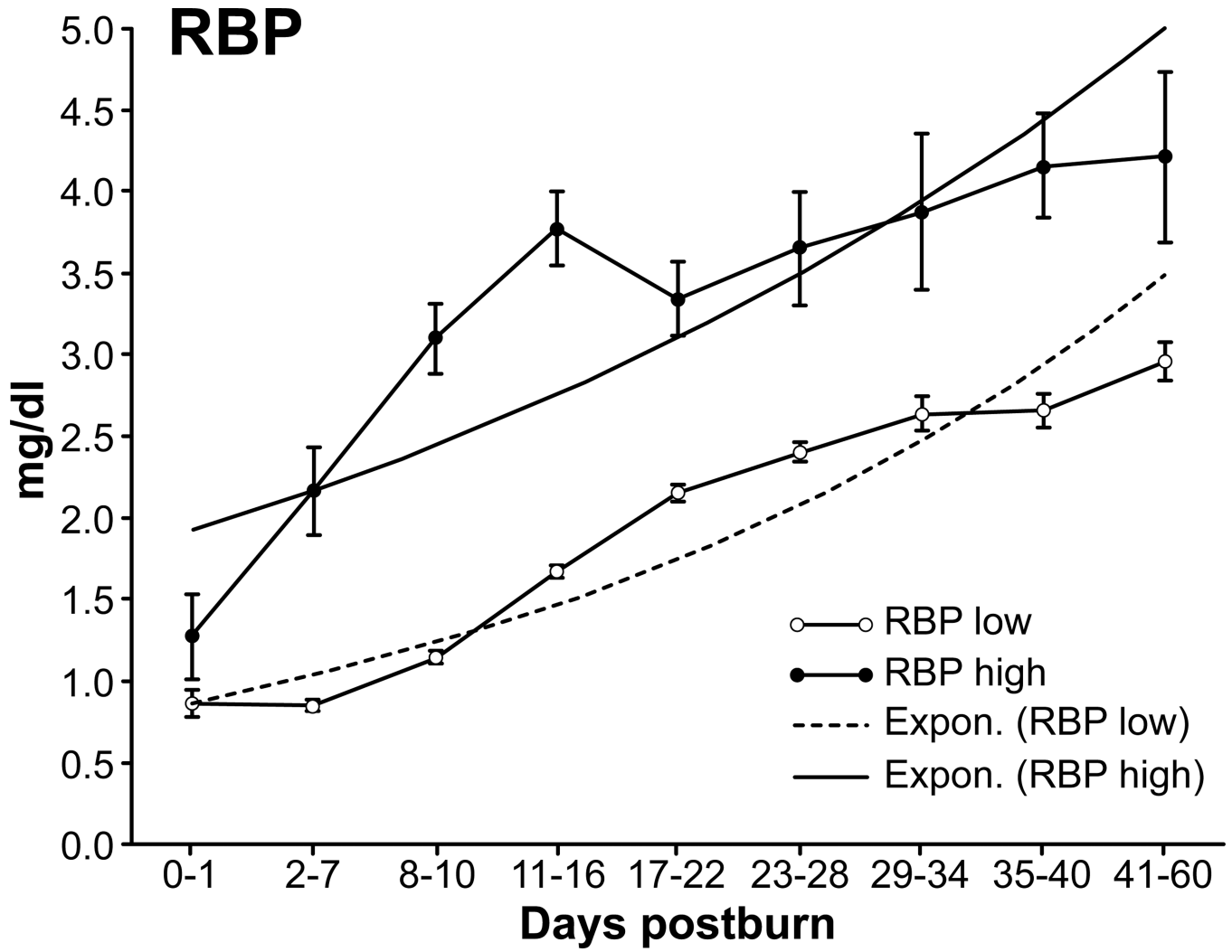


Figure 2. Retinol binding protein (RBP) levels shown over time. The high group showed an immediate onset in the early phase, whereas the low group had a remarkable increase after day 10. Exponential trend lines indicate a persistent increase over time in both groups (data presented as mean ± SEM).

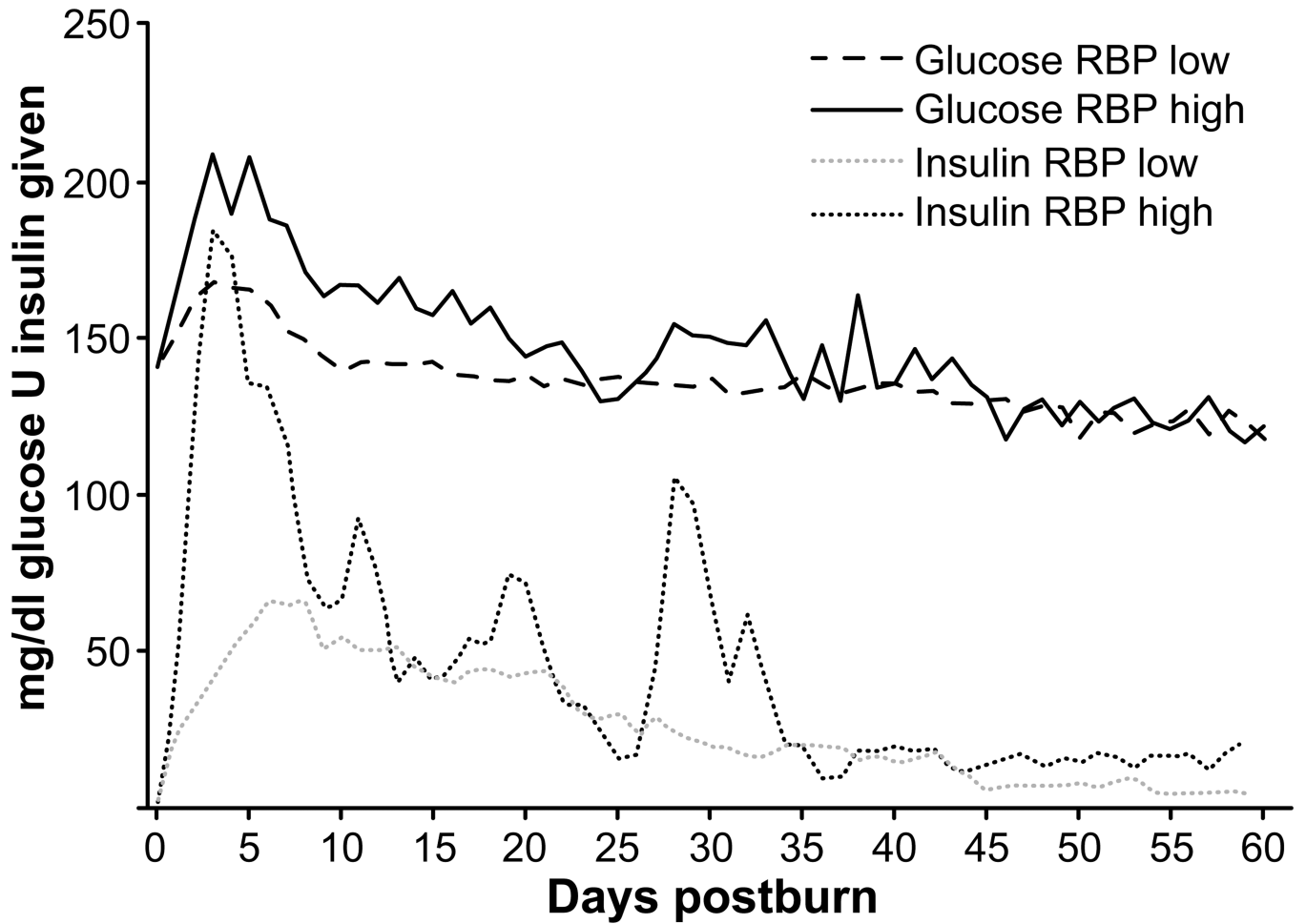


Figure 3.

Glucose levels and units of insulin given. Patients with high systemic retinol binding protein (RBP) levels had significantly higher glucose levels combined with a significantly higher insulin demand, indicating insulin resistance (data presented as mean \pm SEM).

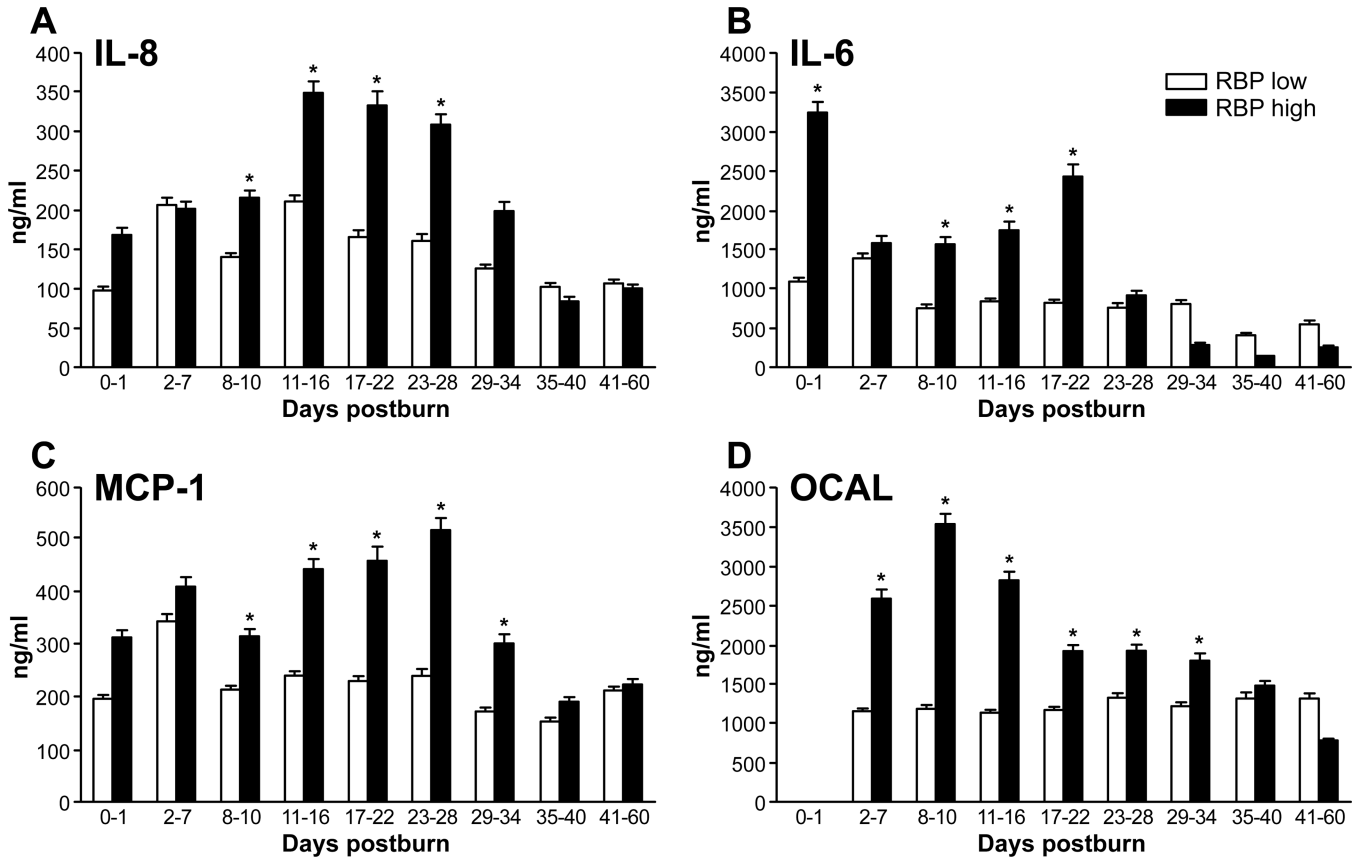


Figure 4. Inflammatory markers. Cytokine response demonstrated by the established and key regulatory proteins (A) interleukin (IL)–8 and (B) IL-6. Catabolic regulators for bone mineral content and lipolysis (C) monocyte chemoattractant protein-1 (MCP-1) and (D) osteocalcin (OCAL) (data presented as mean ± SEM). Significance: p<0.05

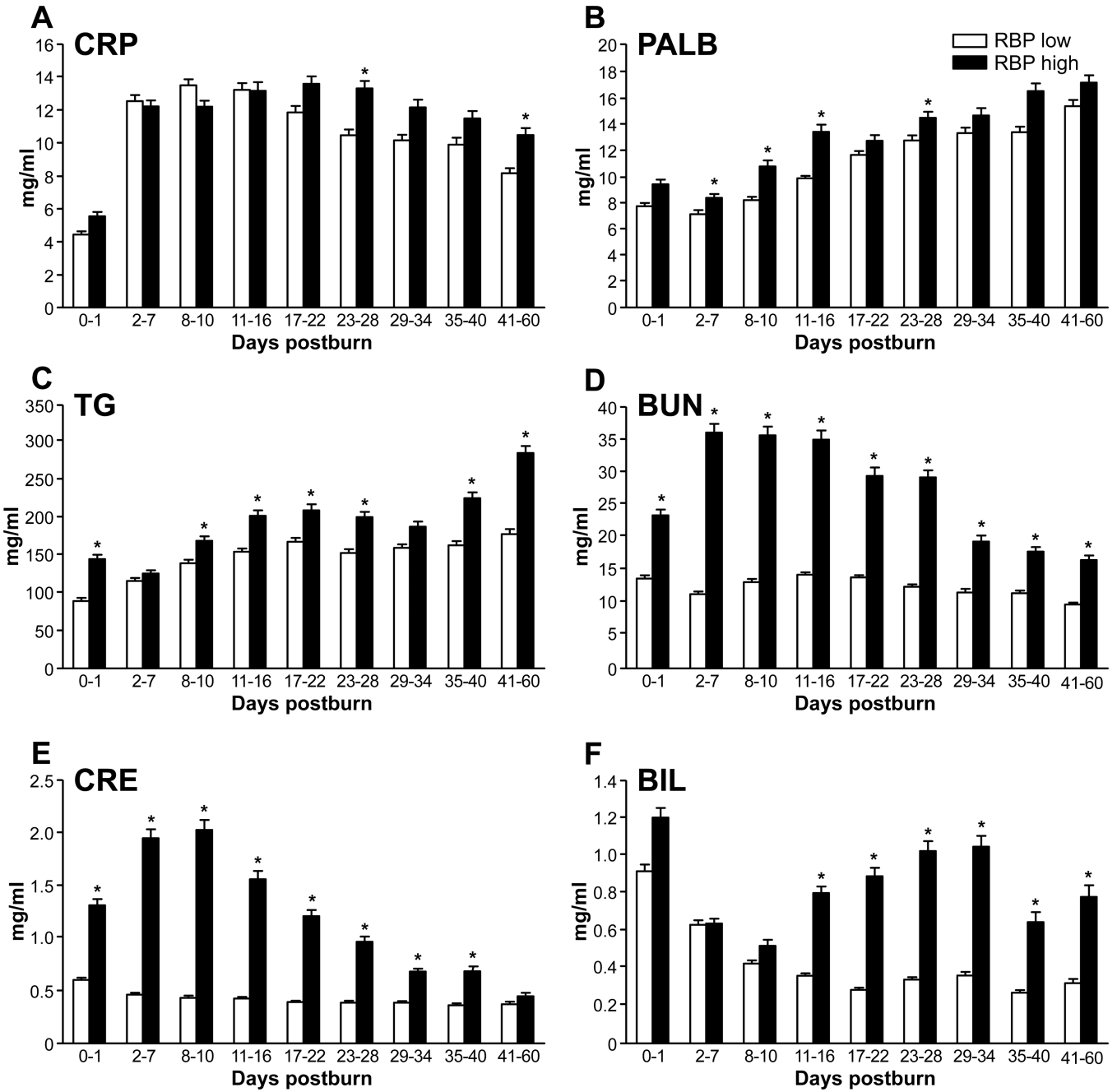


Figure 5. Liver-synthesized proteins and triglycerides. Higher (A) C-reactive protein (CRP) levels after day 17, demonstrating a prolonged inflammatory response in the retinol binding protein (RBP) high group. (B) Prealbumin (PALB) as an important molecule in the carrier system for RBP is correlating with systemic high RBP levels. (C) Significantly higher triglyceride (TG) levels in the high group indicating diminished metabolism and lipolysis. Organ-specific markers for (D, E) renal and (F) liver (F) function were significantly elevated in the RBP high group (data presented as mean ± SEM). BUN, blood urea nitrogen; CRE, creatinine; BIL, bilirubin. Significance: $p < 0.05$

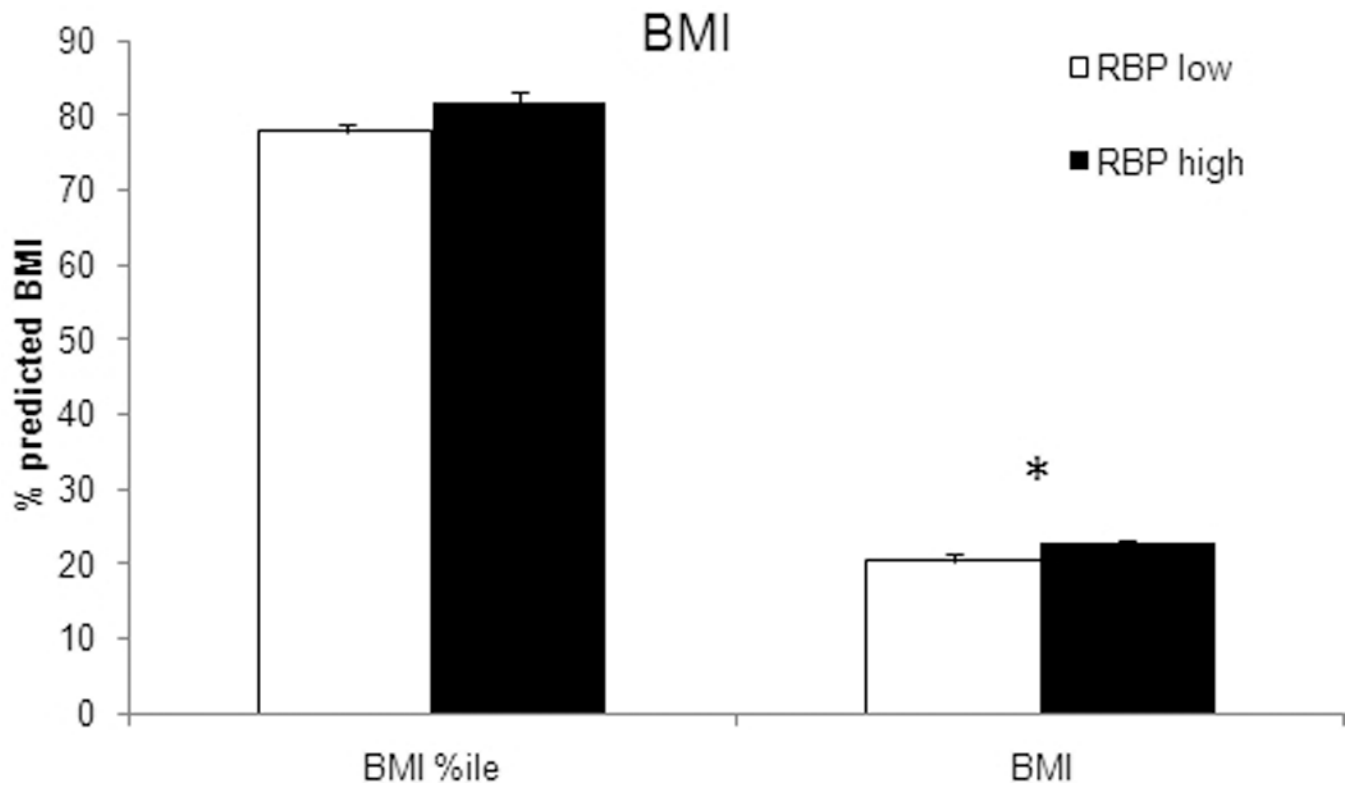


Figure 6. Comparison of the body mass index (BMI) at admission. Patients in the high retinol binding protein (RBP) group had a significantly higher absolute BMI corrected for age according to their percentile, indicating an elevated body fat content. Significance: $p < 0.05$

Table 1

Patient Characteristics of the Stratified Groups

	All	RBP Low	RBP High	P Value
No.	372	321	51	
Gender, No.				
Male	247	214	33	
Female	125	107	18	
Ethnicity, No.				
African American	23	21	2	
White	55	46	9	
Hispanic	292	252	40	
Other	2	2	0	
Age at admittance, y	8.1 ± 5.3	7.8 ± 5.1	10.3 ± 5.9	<.05
Inhalation injury, No. (%)	169 (45.4)	145 (45.2)	24 (47.1)	NS
Type of burn, No. (%)				
Flame	306 (82.3)	263 (81.9)	43 (84.3)	
Scald	41 (11.0)	38 (11.8)	3 (5.9)	
Other	25 (6.7)	20 (6.2)	5 (9.8)	
TBSA burn, second and third degree, %	59.5 ± 17.7	59.3 ± 16.8	60.4 ± 23.1	NS
TBSA, second degree, %	11.5 ± 15.8	11.9 ± 16.2	9.1 ± 13.0	NS
TBSA, third degree, %	48.0 ± 24.4	47.5 ± 23.5	51.2 ± 29.9	NS
Time, burn to admission, d	2.6 ± 2.3	2.5 ± 2.0	3.0 ± 3.7	NS

Both groups showed similar characteristics in demographics and severity of injury.

NS, not significant; RBP, retinol binding protein; TBSA, total body surface area.

Table 2

Patients' Clinical Outcome Parameters

	All	RBP Low	RBP High	P Value
No.	372	321	51	
Number OR	5.1 ± 3.3	5.1 ± 3.4	5.2 ± 3.0	NS
Time between OR	5.3 ± 1.7	5.3 ± 1.7	5.1 ± 2.0	NS
LOS ICU, d	37.8 ± 27.1	36.1 ± 24.0	38.8 ± 29.2	NS
LOS/TBSA	0.7 ± 0.8	0.6 ± 0.3	1.1 ± 2.2	<.05
Died, No. (%)	38 (10.2)	23 (7.2)	15 (29.4)	<.001
Max DENVER2	3.7 ± 1.8	3.5 ± 1.5	5.1 ± 2.4	<.05
MOF, No. (%)	81 (21.8)	55 (17.1)	26 (51.0)	<.05
Sepsis, No. (%)	54 (14.5)	34 (10.6)	20 (39.2)	<.001
No. infections	2.7 ± 2.5	2.6 ± 2.4	3.1 ± 3.1	NS

Data presented as mean ± SD unless otherwise indicated. Patients with low RBP levels showed a significantly lower incidence of clinical complications (MOF, sepsis) and mortality.

ICU, intensive care unit; LOS, length of stay; MOF, multiorgan failure; NS, not significant; OR, operations; RBP, retinol binding protein; TBSA, total body surface area.

Table 3

Logistic Regression Analysis of RBP Levels, Injury Characteristics, and Age

	Coefficient	Standard Error	t	P Value	VIF
Injury severity	0.657	0.18	3.654	<.001	
Inhalation injury	-0.12	0.0941	-1.276	.203	1.049
TBSA burn	0.00171	0.00395	0.434	.665	2.235
TBSA third degree	0.00596	0.00288	2.068	.039	2.302
Age and systemic RBP levels	1.221	0.083	14.705	<.001	
Age at admittance	0.0289	0.00855	3.377	<.001	1

The analysis revealed a significant positive relationship between injury severity (inhalation injury, TBSA third-degree burn), and systemic RBP levels during the hospital stay. Increasing age has a positive correlation to systemic RBP levels.

RBP, retinol binding protein; TBSA, total body surface area; VIF, variance inflation factor.