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Original Articles

Insulin Attenuates the Systemic Inflammatory Response to Thermal Trauma

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Abstract

Background: Insulin has been recently shown to decrease mortality and prevent the incidence of multi-organ failure in critically ill patients. The molecular mechanisms by which insulin improves survival have not been defined. The purpose of the present study was to determine the effect of insulin therapy on the systemic inflammatory response. In vivo we determined the effect of insulin therapy on the inflammatory cascade, which was induced by thermal injury.
Materials and Methods: Thermally injured rats (30% TBSA) were randomly divided into two groups to receive either saline ($n = 28$) or insulin ($n = 28$). Our outcome measures encompassed the effect of insulin on pro-inflammatory cytokines, anti-inflammatory cytokines, and hepatic signal transcription factor mRNA expression.
Results: Insulin significantly decreased dose dependently serum pro-inflammatory cytokines IL-1β at 1, 5, and 7 days, IL-6 at 1 day, MIF at 5 and 7 days, and TNF at 1 and 2 days after injury when compared with controls ($p < 0.05$). Insulin increased anti-inflammatory cytokines IL-2 and IL-4 at 5 and 7 days after trauma, and IL-10 at 2, 5 and 7 days after trauma when compared with controls ($p < 0.05$). Pro-inflammatory signal transcription factors STAT-5 and C/EBP-β mRNA were significantly decreased 1 and 2 days posttrauma; insulin increased anti-inflammatory signal transcription factor mRNA expression of SOCS-3 and RANTES 7 days after the injury ($p < 0.05$).
Conclusions: Our data provide insight that insulin attenuates the inflammatory response by decreasing the pro-inflammatory and increasing the anti-inflammatory cascade, thereby restoring systemic homeostasis, which has been shown critical for organ function and survival in critically ill patients.

Introduction

The systemic inflammatory response to trauma, surgery, critical illness, or bacterial infection encompasses the release of pro-inflammatory cytokines, such as interleukin-1β (IL-1β), IL-6, macrophage inhibitory factor (MIF) or tumor necrosis factor (TNF) that are potentially detrimental (1–3). Anti-inflammatory cytokines, such as IL-2, IL-4 or IL-10 counter the effects of pro-inflammatory cytokines and attempt to maintain homoeostasis (4,5). The expression and synthesis of pro- and anti-inflammatory cytokines are controlled by several pro- and anti-inflammatory signal transcription factors. Signal transducer and activator of transcription (STAT-3 and -5) and CCCAT-enhancer binding protein-β (C/EBP-β) belong to the pro-inflammatory signal transcription factors (6–8). Suppressors of cytokine signaling (SOCS-1, -2, -3) and regulated on activation, normally T cell-expressed and secreted (RANTES) belong to the group of signal transcription factors that exert anti-inflammatory effects by suppressing pro-inflammatory cytokine expression (e.g., SOCS-3) or regulating T-cell function (e.g., RANTES) (9–11).

Recently, intensive insulin therapy was shown to decrease mortality in critically ill patients (12). Insulin given at doses to maintain blood glucose below 110 mg/dl prevented the incidence of multi-organ failure and thus improved clinical outcome and rehabilitation (12). The study, however, did not provide any insights on the mechanisms by which insulin improves survival. Furthermore, the question rose whether insulin improved mortality directly through modulating the inflammatory response and molecular signals, or indirectly through affecting hyperglycemia and thus metabolism. The purpose of the present study was to determine the effect of insulin on the systemic inflammatory response, pro- and anti-inflammatory cytokines, and several signal

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transcription factors. To induce the systemic inflammatory response, we chose the burn injury model, which induces a sustained and severe systemic inflammatory response and hypermetabolism (13).

Material and Methods

Male Sprague-Dawley rats (350–375 g) were placed in wire-bottom cages housed in a temperature-controlled room with a 12-hr light–dark cycle. Rats were acclimatized to their environment for 7 days before the study. All rats received water ad libitum throughout the study. Each rat received a 30% total body surface area (TBSA) full-thickness scald burn under general anesthesia (pentobarbital 50 mg/kg body weight) and analgesia (buprenorphin 1 mg/kg body weight) following a modified procedure as previously described (14). Rats were anesthetized, shaved, and received a 30% TBSA scald burn (99°C water; water contact 10 sec to the back). This model ensures that both groups are similar in metabolic rates. After thermal injury, rats were immediately resuscitated by intraperitoneal injection of Ringer's lactate (50 ml/kg body weight).

Dose-Response and Main Study

Prior to the main experiment we performed a dose-response study in which rats received a thermal injury plus 0.5, 1, 2.5, 5, or 10 U/kg insulin subcutaneously injected in an area of uninjured skin ($n = 4$ per group). The insulin used was protamine insulin (Berlininsulin H, Berlin-Chemie AG, Berlin, Germany), a form of insulin that is released over a 24-hr period. Burn plus NaCl injected subcutaneously served as controls ($n = 4$). The outcome measures encompassed serum pro-inflammatory cytokines 24 hr after thermal injury.

In the main study, animals received a thermal injury as described and were randomly divided into two groups: burn plus 5 U/kg insulin injected subcutaneously in uninjured skin ($n = 28$) (insulin); or burn plus NaCl subcutaneously ($n = 28$) (saline). Animals were killed at 1, 2, 5 and 7 days postburn by an overdose of anesthesia. Blood was collected by puncture of the vena cava inferior and separated into serum and plasma separators, spun at 1000 g for 15 min. The supernatant and pellet were separated and stored at −73°C. Samples of liver were harvested, snap-frozen in liquid nitrogen, and stored at −73°C for analysis.

Nutrition

Rats were fed with a liquid diet, riche in vitamins, protein, and carbohydrate (Fresubin, Fresenius Medical Care, Bad Homburg, Saarland, Germany) and had a caloric distribution of 24% protein, 21% fat and 55% carbohydrate, resulting in an energy intake of 1.01 cal/ml. Both groups of rats were pair fed according to the caloric intake. The feeding protocol was as follows: 25 calories on the day of burn, 51 calories on the first postburn day, 76 calories on the second and 101 calories from the third day postburn on. It was ensured that the nutritional intake was the same in all groups.

Normal

Eight rats received no injury, no treatment, no anesthesia, and no analgesia. Rats received the same liquid diet (Fresubin, Fresenius Medical Care, Bad Homburg, Saarland, Germany) and were pair fed relative to the treatment animals. Animals were killed at the same time points as treated animals 1, 2, 5, and 7 days. These rats served as unburned, untreated, time-matched sham rats to establish baseline levels in the present study.

Serum Glucose and Electrolytes

Serum glucose and electrolytes levels were determined by standard laboratory techniques (Böhring, Ingelheim, Germany).

Serum Cytokines

Pro-inflammatory cytokines IL-1β, IL-6, MIF and TNF, and anti-inflammatory cytokines IL-2, IL-4 and IL-10 were determined by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN, USA).

Hepatic Transcription Factor mRNA Expression

Isolation of RNA Total RNA was prepared from rat liver samples according to the method of Chomczynski and Sacchi using Trizol reagent (Gibco BRL, Gaithersburg, MD, USA) (15). Total RNA was quantified spectroscopically (OD 260 nm) or fluorometrically using Pico green-dye, and equilibrated to an absolute quantity of 0.5 μg/μl. Subsequently, reverse transcription–polymerase chain reaction (RT-PCR) was performed. Total liver RNA (0.5 μg) was introduced to synthesize cDNA in a 60-μl reaction mixture using 2.5 μM random hexamers (Amersham Pharmacia, Freiburg, Germany) and Superscript II reverse transcriptase (Gibco BRL). The following primers were used to amplify specific rat transcripts for: 18S rRNA (QuantumRNA, Ambion, Austin, TX, USA) (488 bp) forward 5′-CAAGAACGAAAGTCGGAGG-3′, reverse 5′-GGACATCTAAGGGCATCACA-3′. C/EBP-ß (according to EMBL Acc. Nr. M84011) (190 bp) forward 5′-GAGCGACGAGTACAAGA-3′; reverse 5′-CTGCTTGAACAAGTTCCG-3′. RANTES (according to EMBL Acc. Nr. U06436) (178 bp) forward 5′-TGCCTCCCCATATGGCTC-3′, reverse 5′-AACCCACTTCTTCTCTGGGTTG-3′. STAT-3 (according to EMBL Acc. Nr. X91810) (436 bp) forward 5′ACCAGATGCGGAGAAG, reverse 5′– AATTTGACCAGCAACCTGAC. STAT-5 (according to EMBL Acc. Nr. X91988) (317 bp) forward 5′-TCATCATCGAGAAGCAGCC-3′, reverse 5′-TTCCGTCACAGACTCTGCAC-3′. SOCS-3 (according to EMBL Acc. Nr. AJ249240) (300 bp) forward 5′-AAGACCTTCAGCTCCAAGAGC-3′, reverse 5′-CTTGAGTACACAGTCAAAGCGG-3′.

The predicted size of each RT-PCR product is assigned in parentheses. Each PCR was initially performed in a thermal cycler (Biometra, Göttingen, Germany) as previously described using standardized

amplification programs. Five microliters of each reaction was subsequently subjected to agarose gel electrophoresis followed by ethidium bromide staining. Absolute transcript concentrations were quantified introducing external cDNA standards by use of a real-time PCR cycler (LightCycler, Roche Diagnostics, Mannheim, Germany). Each gene-specific standard was prepared using the corresponding gel-purified amplicon followed a spectroscopic nucleic acid concentration determination. After serial dilutions of resulting DNA standards final sensitivity levels between 0.1 pg and 1 ng specific transcript per sample were performed during real-time PCR as follows: Using 1 μl of each cDNA the Master SYBR Green protocol was performed (Roche Diagnostics) in 10-μl sample volume in glass capillaries using the experimental conditions as follows: (a) 95°C 10 min preincubation; (b) amplification 95°C 5 sec, 55°C 10 sec with fluorescence detection, 72°C 18 sec, 45 cycles; (c) melting curve: 94°C 10 sec, 50°C 60 sec, than 0.1°C/sec up to 90°C under continuous fluorescence detection. Confirmation of each amplicon identity was obtained through melting curve analysis as well as by sequencing of resulting RT-PCR products (TOBLAP, Munich, Germany). Sequence determining of each PCR product confirmed a 100% homology to the published rat sequences. As negative controls, water instead of RNA was used.

Ethics and Statistics

These studies were reviewed and approved by the Animal Care and Use Committee of the University Texas Medical Branch, ensuring that all animals received humane care. Statistical comparisons were made by analysis of variance (ANOVA) and Student's *t*-test with Bonferroni's correction. Data are expressed as means ± standard deviation (SD) or means ± standard error of the mean (SEM), where appropriate. Significance was accepted at $p < 0.05$.

Results

Dose Response

We determined the optimal dose for the insulin administration after a 30% TBSA thermal injury without causing electrolyte imbalance. Insulin decreased serum IL-1β, IL-6, MIF, and TNF dose dependently, whereas 5 U/kg body weight of depot insulin demonstrated the greatest effect (Fig. 1). Therefore, all further experiments were performed using insulin in a dose of 5 U/kg body weight, subcutaneously injected every 24 hr.

Serum Glucose and Electrolytes

Insulin at a dose of 5 U/kg body weight significantly decreased rat serum glucose at a range of 120 mg/dl on all days after thermal injury. Controls demonstrated blood glucose levels at a range of 150 mg/dl ($p < 0.05$) (Table 1). Serum glucose, sodium, potassium, calcium and phosphate levels

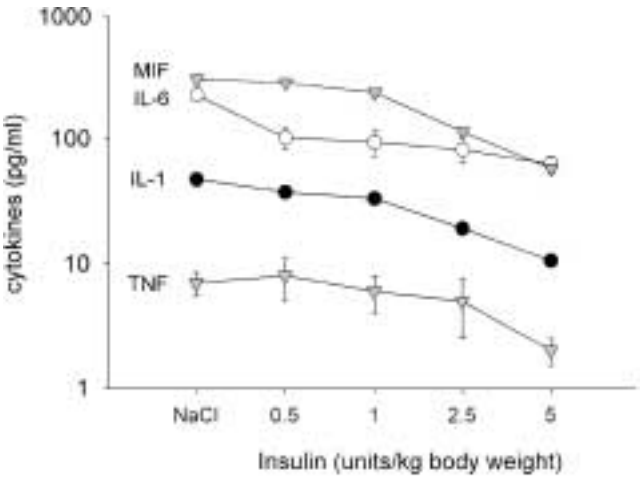

Fig. 1. Dose-response study performed 1 day after thermal injury. The optimal dose for the insulin administration after a 30% TBSA thermal injury was defined. Insulin decreased dose dependently serum IL-1β, IL-6, MIF, and TNF, whereas 5 U/kg body weight insulin demonstrated the greatest effect. Data are presented as means ± SEM, $n = 4$ for each group.

demonstrated lower concentrations 1 and 2 days after burn, when compared to values 5 and 7 days after burn, which is probably due to dilution of the initial resuscitation. Electrolytes were similar in the saline and insulin group at 1, 2, 5 and 7 days after injury (Table 1).

Pro-inflammatory Cytokines

Thermal injury caused an increase in serum TNF concentrations on days 1 through 5 after trauma. Insulin attenuated the increase of serum TNF on days 1 and 2 when compared with saline ($p < 0.05$) (Fig. 2a). Serum IL-1β concentrations also increased during the first day after burn. Levels remained elevated until 7 days after trauma. Insulin prevented the increase of serum IL-1β and significantly decreased serum IL-1β concentration on days 1, 5 and 7 after trauma when compared with animals receiving saline ($p < 0.05$) (Fig. 2b). In addition, insulin significantly decreased serum IL-6 concentrations 1 day after burn compared to controls, which were almost 300 times elevated above normal levels ($p < 0.05$) (Fig. 2c). MIF demonstrated a different pattern compared to IL-1β, IL-6 and TNF. In contrast to other pro-inflammatory cytokines, MIF was found to be elevated immediately after burn, but further increased over the study period by almost 100% in the saline group. Insulin significantly decreased serum MIF 5 and 7 days after trauma by almost 200% ($p < 0.005$) (Fig. 2d).

Anti-inflammatory Cytokines

To determine the effect of insulin on systemic homoeostasis, we measured anti-inflammatory cytokines, IL-2, IL-4, and IL-10. Similar to pro-inflammatory cytokines, anti-inflammatory cytokines IL-2 and IL-10 were elevated after the trauma. IL-4 was found to be decreased with the burn trauma.

Table 1. Serum glucose, sodium, potassium, calcium, and phosphate in insulin-treated and control rats after thermal trauma

	Controls				Insulin			
	Day 1	Day 2	Day 5	Day 7	Day 1	Day 2	Day 5	Day 7
Glucose (mg/dl)	149 ± 3	148 ± 3	149 ± 3	150 ± 3	122 ± 5*	121 ± 5*	125 ± 7*	122 ± 5*
Sodium (mg/dl)	146 ± 3	148 ± 3	150 ± 3	150 ± 3	145 ± 3	150 ± 3	151 ± 3	151 ± 3
Potassium (mg/dl)	6.4 ± 0.8	6.1 ± 0.8	6.8 ± 0.5	6.8 ± 0.5	6.4 ± 1.1	6.0 ± 1.1	6.7 ± 0.8	6.7 ± 0.8
Calcium (mg/dl)	2.70 ± 0.11	2.70 ± 0.08	2.70 ± 0.08	2.70 ± 0.13	2.60 ± 0.11	2.70 ± 0.21	2.80 ± 0.13	2.80 ± 0.08
Phosphate (mg/dl)	3.2 ± 0.3	3.1 ± 0.6	3.5 ± 0.3	3.4 ± 0.3	3.3 ± 0.8	3.3 ± 0.3	3.6 ± 0.3	3.8 ± 0.8

Data are presented as means ± SD.

*Significant difference between controls and insulin ($p < 0.05$).

Normal values: glucose, 135 ± 6 mg/dl; sodium, 150 ± 1 mg/dl; potassium, 6.8 ± 0.3 mg/dl; calcium, 2.9 ± 0.04 mg/dl; phosphate, 3.5 ± 0.2 mg/dl.

The anti-inflammatory cytokine IL-2 increased after the thermal injury and remained increased in the saline group during the study period. Insulin significantly increased serum IL-2 levels at 5 and 7 days after trauma ($p < 0.05$) (Fig. 3a). In contrast to IL-2, IL-4 significantly decreased after burn compared to normal levels. In the saline group, IL-4 further decreased over time. Insulin significantly increased IL-4 at 5 and 7 days after trauma when compared with saline ($p < 0.05$) (Fig. 3b). The anti-inflammatory cytokine IL-10 increased immediately after trauma. Saline-treated animals showed a decrease of IL-10 at 7 days after injury. Insulin administration significantly increased serum IL-10 at 2, 5 and 7 days after trauma when compared to saline ($p < 0.05$) (Fig. 3c).

Insulin increased dose- and time-dependent anti-inflammatory cytokines and decreased pro-inflammatory cytokines. Thus, ratios of pro-inflammatory to anti-inflammatory cytokines, which are predictors for organ function and systemic homoeostasis, significantly improved toward normal in the insulin treatment group ($p < 0.05$).

Hepatic mRNA Expression of Signal Transcription Factors

To define whether insulin alters pro- and anti-inflammatory cytokines directly or through signal transcription factor pathway, hepatic signal transcription factor mRNA expression was determined. Hepatic mRNA expression of C/EBP-β increased after trauma compared to normal. Insulin decreased the pro-inflammatory hepatic signal transcription factor C/EBP-β mRNA on the first day after burn, which remained at normal concentration when compared with saline ($p < 0.05$) (Fig. 4a). STAT-5 mRNA expression increased in the saline and insulin groups after trauma. Insulin decreased the hepatic mRNA expression of STAT-5 on days 1, 2 and 7 after trauma ($p < 0.05$) (Fig. 4b). Insulin had no effect on hepatic mRNA expression of STAT-3, which also increased after burn (Fig. 4c).

In contrast to pro-inflammatory signals, RANTES was found to be decreased after the burn trauma. Insulin significantly increased hepatic RANTES mRNA expression 7 days after trauma and approached normal levels ($p < 0.05$). Rats receiving saline demonstrated decreased RANTES levels throughout the study period (Fig. 4d). Hepatic SOCS-3 mRNA was found to be increased after the thermal injury, but rapidly decreased over the 7-day study period in the saline group. Insulin increased hepatic SOCS-3 mRNA expression at days 2, 5 and 7 after trauma when compared with controls ($p < 0.05$) (Fig. 4e).

Discussion

The systemic inflammatory response after trauma leads to protein degradation, catabolism and hypermetabolism. As a consequence, the structure and function of essential organs, such as muscle, heart, immune system and liver, are compromised and contribute to multi-organ failure and mortality (16,17). Pro-inflammatory cytokines were thought to trigger and enhance this response and to mediate the catabolic effects, also by the inhibition of the growth hormone–insulin-like growth factor-I (IGF-I)—insulin axis (18–20). Therefore, research focused on the attenuation of the inflammatory response using anti-inflammatory agents or antibodies against pro-inflammatory cytokines such as TNF, IL-1β or their receptors (21–23). These approaches showed promising results in vitro and in animal models by increasing survival rates in the state of septicemia (21–23). However, when these approaches entered clinical trials it became evident that these promising animal data could not be replicated in humans.

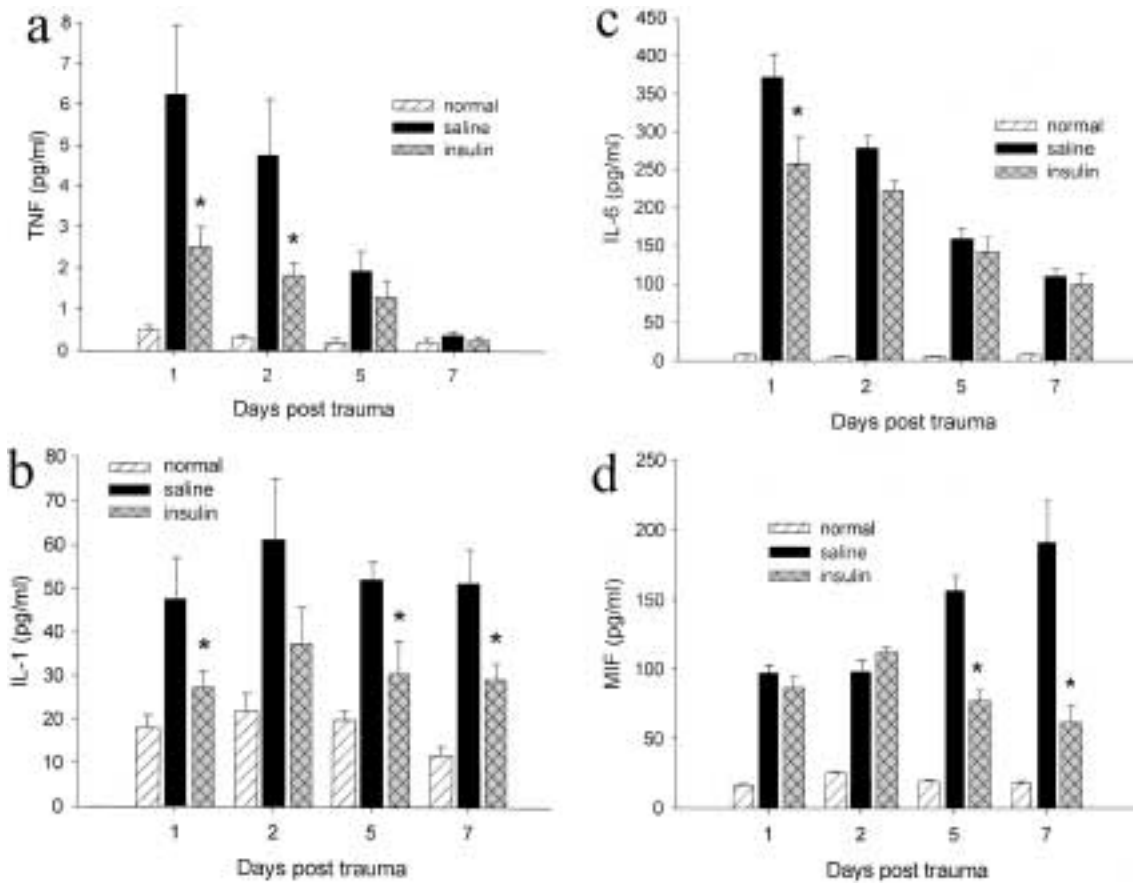

Fig. 2. Serum pro-inflammatory cytokines after a 30% TBSA thermal injury. (a) Serum TNF increased approximately 7-fold after thermal trauma and decreased over time. Insulin prevented an increase of serum TNF on days 1 and 2 postinjury when compared with saline ($p < 0.05$). **(b)** Serum IL-1β increased after burn and remained elevated during the study period. Insulin decreased serum IL-1β concentration on days 1, 5, and 7 after trauma when compared with animals receiving saline ($p < 0.05$). **(c)** Serum IL-6 increased 300-fold immediately after burn trauma and remained elevated even after 7 days. Insulin significantly decreased serum IL-6 concentrations 1 day after burn compared to saline ($p < 0.05$). **(d)** MIF was found to be increased immediately after burn, but further increased over the study period by almost 100% in the saline control group. Insulin significantly decreased serum MIF 5 and 7 days after trauma ($p < 0.05$).*Significant difference between insulin and control ($p < 0.05$). Data are presented as means ± SEM.

Others and we, as shown in the present study, chose a different approach by investigating the hypothesis that an endogenous hormone (insulin) must be locally or systemically present to attenuate the pro-inflammatory cascade (12,13).

Recently van den Berghe et al. (12) showed that insulin administration at a dose that kept blood glucose below 110 mg/dl decreased mortality in critically ill patients. The authors showed further that insulin prevented the incidence of multi-organ failure. In the present study, we investigated the effect of insulin on the systemic inflammatory response cascade to determine a possible explanation of how insulin improves survival in critically ill patients. Insulin administered at a dose that kept blood glucose below 110 mg/dl decreased pro-inflammatory mediators IL-1, IL-6 and TNF, as well as MIF. At the same time, insulin significantly increased anti-inflammatory cytokines IL-2, IL-4 and IL-10. Clinical studies demonstrated that nonsurvivors with pancreatitis had decreased IL-6 to IL-10 ratios when compared with survivors (24). Our group found that ratios of pro-inflammatory to anti-inflammatory cytokines correlate with organ function and can be used as predictors for mortality in severely burned pediatric patients (25). Hence, by decreasing pro-inflammatory and increasing anti-inflammatory cytokines, insulin equilibrates the balance between pro- and anti-inflammatory cytokines and may improve organ function and mortality after trauma.

To determine whether insulin exerts its effects on cytokines through changes in blood glucose or directly by modulating signal transcription factors, we measured hepatic signal transcription factor mRNA expression. We found that insulin alters the intracellular signal cascade in the liver. Insulin decreased the pro-inflammatory signal transcription factors STAT-5 and C/EBP-β. An up-regulation of both transcription factors led to impaired organ function and protein synthesis, such as albumin (6,7). Therefore, it appears that insulin improves organ function and protein synthesis during the

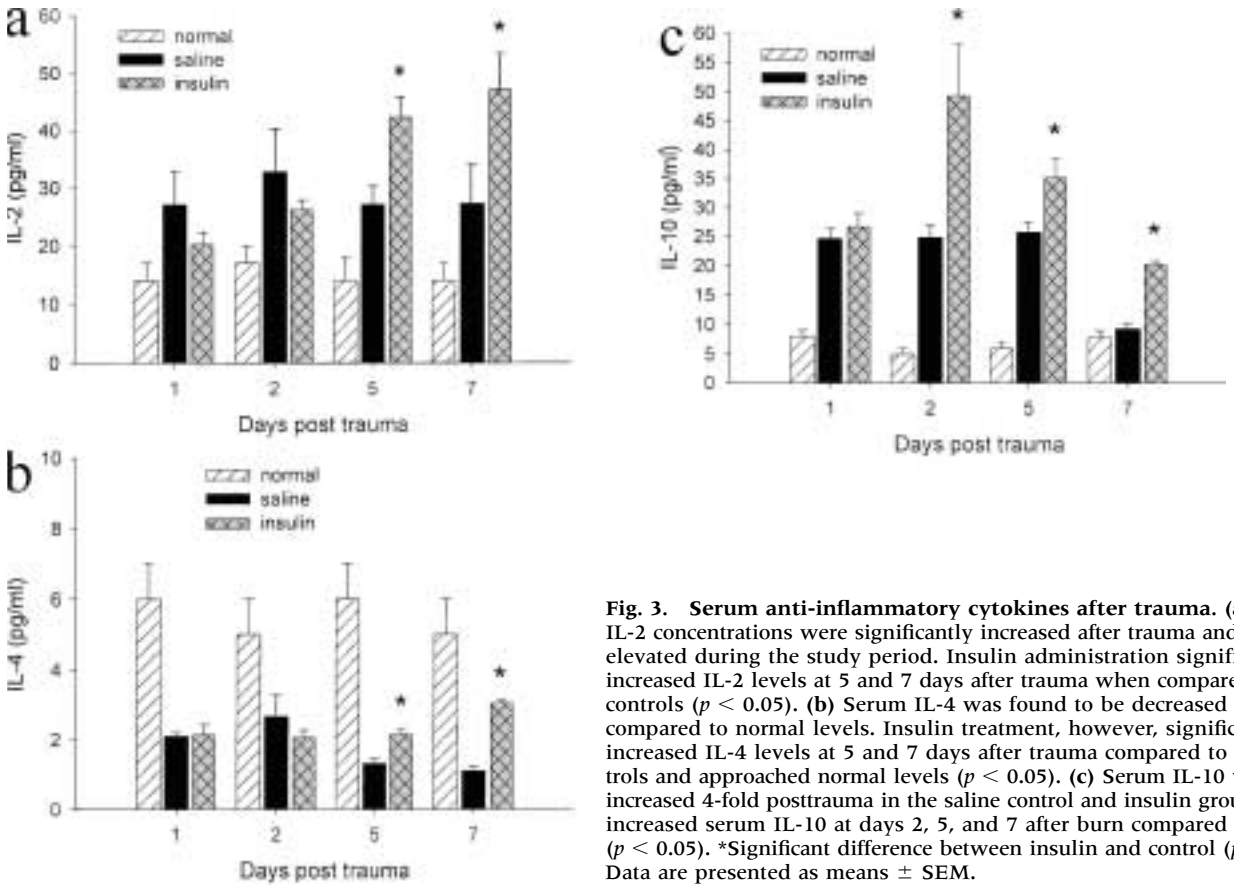

Fig. 3. Serum anti-inflammatory cytokines after trauma. (a) Serum IL-2 concentrations were significantly increased after trauma and remained elevated during the study period. Insulin administration significantly increased IL-2 levels at 5 and 7 days after trauma when compared to saline controls ($p < 0.05$). **(b)** Serum IL-4 was found to be decreased after burn compared to normal levels. Insulin treatment, however, significantly increased IL-4 levels at 5 and 7 days after trauma compared to saline controls and approached normal levels ($p < 0.05$). **(c)** Serum IL-10 was increased 4-fold posttrauma in the saline control and insulin group. Insulin increased serum IL-10 at days 2, 5, and 7 after burn compared to controls ($p < 0.05$). *Significant difference between insulin and control ($p < 0.05$). Data are presented as means ± SEM.

hypermetabolic response through these signal transcription factors. In addition to pro-inflammatory transcription factors, we determined signal transcription factors that were identified to either suppress cytokine signaling (SOCS) or regulate the T-cell function (RANTES). Members of the SOCS family of proteins play key roles in the negative regulation of cytokine signal transduction, by acting in a negative feedback loop and inhibiting the cytokine-activated Janus kinase/signal transducers and activators of transcription (JAK/ STAT) signaling pathway to modulate cellular responses (25). Direct interaction of SOCS SH2 domains with the JAK kinases or cytokine receptors allows their recruitment to the signaling complex, where they inhibit JAK catalytic activity or block access of the STAT's to receptor binding sites (25). Because we have shown that insulin decreased STAT-5 and increased SOCS-3, major players during the aftermath of a thermal injury (9), it remains to be defined whether insulin decreases cytokines, STAT-5, and C/EBP-β in a direct fashion or indirectly through SOCS-3. The relevance of SOCS-mediated regulation of cytokine responses has been demonstrated by the phenotypes of mice lacking these regulators. Several factors have been identified to affect SOCS expression, such as interferon gamma, growth hormone, and erythropoietin (25). The data from the present study strongly suggest that to the threshold of growth factors another one has to be added, insulin.

RANTES is a member of a large supergene family of pro-inflammatory cytokines called CC chemokines that appear to play a fundamental role in the inflammatory processes. Although expression of RANTES was first thought to be limited to activated T cells, recent data have shown that it is produced by a variety of tissue types in response to specific stimuli. Deletion analysis of the promoter region indicates that different transcriptional mechanisms control expression of RANTES in the various tissues studied. Posttrauma the immune system plays a crucial role in survival and clinical outcome (11). Growth factors, such as growth hormone, can affect the Th-1 and Th-2 by restoring the Th-1 response and improving the immune system after a thermal injury (26). We did not define the function of the immune system in the present study; however, it appears that insulin may have some beneficial effects by modulating the T-cell response because insulin significantly increased hepatic RANTES mRNA expression 7 days after trauma.

The results presented in the present study provide insight by which mechanisms insulin may improve the clinical outcome. We showed that insulin given at doses to maintain blood glucose around

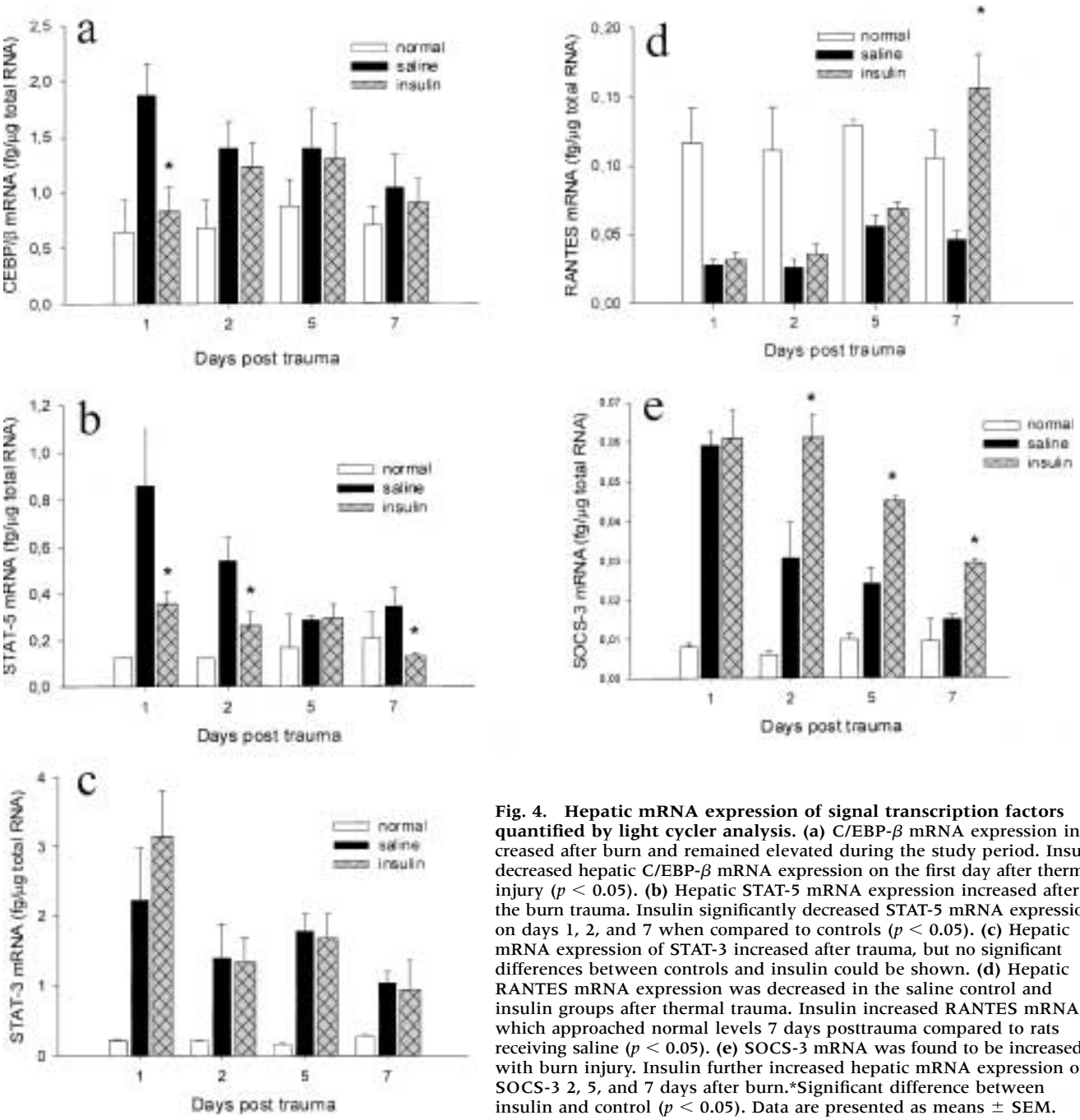

Fig. 4. Hepatic mRNA expression of signal transcription factors quantified by light cycler analysis. (a) C/EBP-β mRNA expression increased after burn and remained elevated during the study period. Insulin decreased hepatic C/EBP-β mRNA expression on the first day after thermal injury ($p < 0.05$). **(b)** Hepatic STAT-5 mRNA expression increased after the burn trauma. Insulin significantly decreased STAT-5 mRNA expression on days 1, 2, and 7 when compared to controls ($p < 0.05$). **(c)** Hepatic mRNA expression of STAT-3 increased after trauma, but no significant differences between controls and insulin could be shown. **(d)** Hepatic RANTES mRNA expression was decreased in the saline control and insulin groups after thermal trauma. Insulin increased RANTES mRNA, which approached normal levels 7 days posttrauma compared to rats receiving saline ($p < 0.05$). **(e)** SOCS-3 mRNA was found to be increased with burn injury. Insulin further increased hepatic mRNA expression of SOCS-3 2, 5, and 7 days after burn.*Significant difference between insulin and control ($p < 0.05$). Data are presented as means ± SEM.

120 mg/dl attenuated the inflammatory response by decreasing pro-inflammatory and increasing anti-inflammatory cytokines, thus restoring systemic homeostasis. We further showed that the effect of insulin is probably due to direct binding of insulin on signal transcription factors, rather than indirectly through changes in blood glucose and metabolism. Based on our data and the data of van den Berghe, we suggest that insulin may represent an important and safe therapeutic option in the treatment of critically ill patients.

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