Acute Hyperinsulinemia Restrains Endotoxin-induced Systemic Inflammatory Response

An Experimental Study in a Porcine Model

Vibeke Brix-Christensen, M.D., Ph.D.,* Søren Kæseler Andersen, M.D.,* René Andersen, M.B.,* Annette Mengel,†
Thomas Dyhr, M.D.,‡ Niels Trolle Andersen, Ph.D.,§ Anders Larsson, M.D., D.M.Sc.,‖ Ole Schmitz, M.D., D.M.Sc.,#
Hans Ørskov, M.D., D.M.Sc.,** Else Tønnesen, M.D., D.M.Sc.††

Background: Intensive insulin therapy in critically ill patients reduces morbidity and mortality. The current study elucidates whether acute hyperinsulinemia *per se* could attenuate the systemic cytokine response and improve neutrophil function during endotoxin (lipopolysaccharide)-induced systemic inflammation in a porcine model.

Methods: Pigs were anesthetized, mechanically ventilated, randomized into four groups, and followed for 570 min: group 1 (anesthesia solely, n = 10), group 2 (hyperinsulinemic euglycemic clamp [HEC], n = 9), group 3 (lipopolysaccharide, n = 10), group 4 (lipopolysaccharide–HEC, n = 9). Groups 3 and 4 were given a 180-min infusion of lipopolysaccharide (total, 10 μg/kg). Groups 2 and 4 were clamped (p-glucose: 5 mM/l, insulin 0.6 mU · kg^{-1} · min^{-1}) throughout the study period. Changes in pulmonary and hemodynamic function, circulating cytokines, free fatty acids, glucagon, and neutrophil chemotaxis were monitored.

Results: Tumor necrosis factor α and interleukin 6 were significantly reduced in the lipopolysaccharide–HEC group compared with the lipopolysaccharide group (both $P = 0.04$). In the lipopolysaccharide–HEC group, the glucagon response was diminished compared with the lipopolysaccharide group ($P < 0.05$). Serum free fatty acid concentrations were decreased in animals exposed to HEC. Animals receiving lipopolysaccharide showed an increase in pulmonary pressure ($P < 0.001$), but otherwise, there were no major changes in pulmonary or hemodynamic function. Neutrophil function was impaired after lipopolysaccharide administration.

Conclusion: Hyperinsulinemia concomitant with normoglycemia reduces plasma concentrations of tumor necrosis factor α and the catabolic hormone glucagon in lipopolysaccharide-induced systemic inflammation in pigs. The finding strongly supports the role of insulin as an antiinflammatory hormone. Whether the effect to some extent operates *via* a reduced free fatty acid concentration is unsettled.

SEVERE sepsis, defined as sepsis associated with impaired organ function, is a leading cause of death in critically ill patients. The pathophysiology of sepsis seems to be extremely complex, but the prevailing the-

ory has been that sepsis represents a generalized uncontrolled or "malignant" inflammatory response.

Since 2001, a number of trials involving immune-modulating agents have shown promising efficacy in patients with severe sepsis.[1–3] Van den Berghe *et al.*[3] demonstrated that intensive insulin therapy maintaining a blood glucose concentration between 4.4 and 6.1 mM resulted in lower morbidity and mortality in a group of critically ill patients than during conventional therapy accepting a higher glycemic level. The majority of patients were surgical patients, and it remains to be clarified whether the beneficial effects of intensive insulin therapy also include medical critically ill patients.

The mechanisms behind the protective effect of insulin and strict metabolic control in severe sepsis are unknown.[4] Recently, Van den Berghe *et al.*[5] suggested that the beneficial effect on morbidity and mortality was associated with the metabolic control, *i.e.*, normoglycemia, rather than the effect of insulin. In clinical practice, however, it is difficult to distinguish between the effect of exogenous insulin, prevention of hyperglycemia, or both. In addition, higher concentrations of free fatty acids (FFAs) could also be launched as important players in the devastating scenario.

Hyperglycemia associated with insulin resistance is common in critically ill patients.[6] Furthermore, patients with severe sepsis are characterized by a systemic inflammatory response composed by cellular and hormonal mediators. Activation and paralysis of immunocompetent cells occur simultaneously, and increased secretion of proinflammatory and antiinflammatory cytokines are other characteristic manifestations of sepsis.[7]

The function of neutrophils is impaired during hyperglycemia, and correcting hyperglycemia improves bacterial phagocytosis.[8] Insulin has been proposed as a potent antiinflammatory molecule with the ability to suppress the production of proinflammatory cytokines[4] and acute phase proteins.[9]

We hypothesized that hyperinsulinemia during lipopolysaccharide-induced systemic inflammation in addition to its anabolic effect had an antiinflammatory effect manifested as changes in cytokine concentrations in peripheral blood and improved neutrophils function *ex vivo*. Consequently, the current study was undertaken to investigate the effect of insulin *per se* on the metabolic and inflammatory response to endotoxemia. This was done
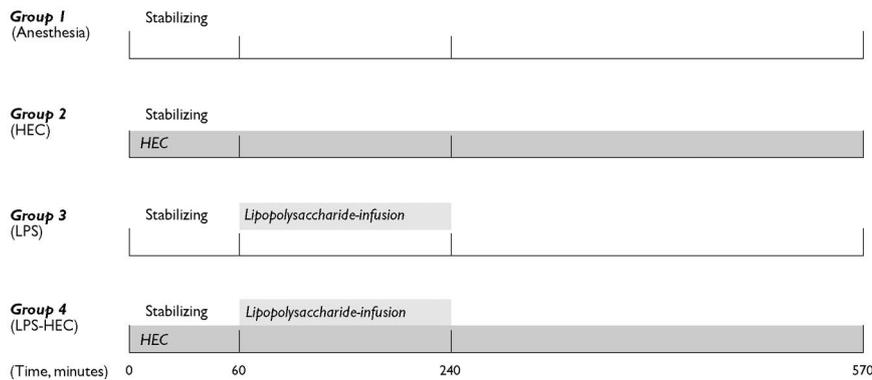
Fig. 1. Schematic presentation of experimental protocol. See text for details. HEC = hyperinsulinemic euglycemic clamp; LPS = lipopolysaccharide.

by performing a hyperinsulinemic euglycemic clamp (HEC) in a porcine model exposed to lipopolysaccharide-induced systemic inflammation. In addition, we also found it of importance to elucidate whether concentrations of FFA and the catabolic hormone glucagon could be involved in potentially beneficial effects of insulin.

Materials and Methods

The National Committee on Animal Research Ethics (Copenhagen, Denmark) approved the protocol, and the work was performed according to the guidelines in the *Guide for the Care and Use of Laboratory Animals.*[10] Thirty-eight female Landrace pigs (weight, 35–40 kg) were fasted overnight but were allowed free access to water. They were premedicated with intramuscular ketamine (10 mg/kg) and midazolam (0.25 mg/kg). Anesthesia was induced with intravenous ketamine (5 mg/kg). The pigs were orally intubated, and anesthesia was maintained with a continuous intravenous infusion of fentanyl (60 $\mu g \cdot kg^{-1} \cdot h^{-1}$) and midazolam (6 mg · $kg^{-1} \cdot h^{-1}$). Neuromuscular blocking drugs were not used. The animals were ventilated with a volume-controlled ventilator (Servo 900 ventilator; Siemens Elema, Solna, Sweden) with a positive end-expiratory pressure of 5 cm H_2O. Tidal volume was kept at 10–15 ml/kg, and the respiratory rate was adjusted (20–25 breaths/min) to maintain normocapnia (arterial carbon dioxide tension [Pa_{CO_2}] in the range of 34–45 mmHg). Ventilation was performed with oxygen in air aiming at an arterial oxygen tension (Pa_{O_2}) greater than 105 mmHg. After dissection of the neck vessels, a Swan-Ganz catheter (Edwards Lifescience Corp., Irvine, CA) was inserted in the pulmonary artery *via* the right cava superior vein. Localization of the balloon-tipped catheter was determined by observing the characteristic pressure trace on the monitor as it was advanced through the right side of the heart into the pulmonary artery. Another catheter (5 French; St. Jude Medical Company, St. Paul, MN) was inserted into the left carotid artery for continuous blood pressure monitoring and blood sampling. A urine catheter was inserted for urine collection.

Respiratory Monitoring

Expired minute volume, tidal volume, respiratory rate, peak and mean inspiratory pressure, and inspired oxygen fraction (F_{IO_2}) were monitored continuously throughout the study. Blood samples were collected from the arterial catheter every 30 min and more frequently in relation to lipopolysaccharide infusion for analysis of blood gases and acid–base status.

Hemodynamic Monitoring

Continuous observations were performed of arterial blood pressure, heart rate (from the electrocardiogram), and pulmonary artery pressure (PAP). Pulmonary capillary wedge pressure was measured intermittently. Cardiac output was continuously monitored using an Edwards Vigilance Monitor (Edwards Lifescience Corp.).

Experimental Design

Animals were allocated into four groups in random order as shown in figure 1. After induction of anesthesia and tracheal intubation, the animals were left to stabilize for 60 min. The animals in group 1 (anesthesia, n = 10) were subjected to general anesthesia for 570 min, solely. In group 2 (HEC, n = 9), the animals were in addition exposed to HEC for 570 min. In group 3 (lipopolysaccharide, n = 10), the animals were given a 180-min infusion of lipopolysaccharide. Finally, the animals in group 4 (lipopolysaccharide–HEC, n = 9) were subjected to a combination of HEC and lipopolysaccharide infusion. During the 570-min study period, the animals in group 1 (anesthesia) and group 2 (HEC) received 10 ml · $kg^{-1} \cdot h^{-1}$ isotonic saline, whereas the animals in the two other groups received 20 ml · $kg^{-1} \cdot h^{-1}$ to maintain blood pressure during and after lipopolysaccharide infusion. At study termination, the animals were killed by evisceration of the heart preceded by a fentanyl bolus of 1 mg.

Lipopolysaccharide Infusion. *Escherichia coli* lipopolysaccharide endotoxin, (*E. coli* 026:β6, Bacto Lipopolysaccharides; Difco Laboratories, Detroit, MI) was dissolved in saline 120 min before each experiment to dissolve any precipitate. After a stabilization period, li-

popolysaccharide infusion was started at baseline at a rate of $2.5 \ \mu g \cdot kg^{-1} \cdot h^{-1}$, and it was increased stepwise to $15 \ \mu g \cdot kg^{-1} \cdot min^{-1}$ during 30 min. After this, the fusion was kept at a rate of $2.5 \ \mu g \cdot kg^{-1} \cdot h^{-1}$ during 150 min and was thereafter discontinued.

Hyperinsulinemic Euglycemic Clamp. Insulin (Insulin Actrapid; Novo-Nordisk, Copenhagen, Denmark) was infused intravenously at a constant rate of $0.6 \ mU \cdot kg^{-1} \cdot min^{-1}$ for 570 min. Plasma glucose was clamped at 5 mM by infusion of 20% glucose.

Measurement Sequence and Laboratory Analyses

Blood for determination of insulin, glucagon, and FFAs was drawn hourly throughout the study, and blood for cytokines was drawn at 0 (baseline), 240, 480, and 570 min. Blood gases, plasma lactate, and base excess were analyzed by an ABL 700 (Radiometer, Copenhagen, Denmark) hourly.

Plasma glucose concentrations were measured every 5–10 min. They were determined in duplicate immediately after sampling (Beckman Instruments, Palo Alto, CA). Serum insulin concentrations were measured in duplicate by a two-site immunospecific insulin enzyme-linked immunosorbent assay (DAKO Diagnostics, Cambridgeshire, United Kingdom). Plasma glucagon was determined by an in-house radioimmunoassay, and serum FFA was assayed by a colorimetric method using a commercial kit (Wako Chemicals, Neuss, Germany).

Cytokines. Fresh frozen plasma samples ($-80°C$) obtained from EDTA-stabilized blood were used for cytokine measurements with enzyme-linked immunosorbent assays. Commercially available kits specific for porcine tumor necrosis factor (TNF) α and interleukin (IL) 10 (Biosource International, Camarillo, CA) were used according to the manufacturer's instructions. Determinations were performed in duplicate. The detection levels for the assays were TNF-α less than 6 pg/ml and IL-10 less than 3 pg/ml.

Concentrations of IL-6 and IL-8 were determined in EDTA plasma from arterial blood samples in a sandwich immunoassay. Porcine specific matched pairs of anticytokine antibodies along with recombinant cytokine standards were obtained from R&D Systems (Abingdon, United Kingdom). All detecting antibodies were biotinylated. A time-resolved fluorometric assay using an Eu^{3+}-streptavidin complex for detection in a Wallac Victor 2 Fluorometer (Wallac, Turku, Finland) was used. After appropriate titration of antibody concentrations, control parameters regarding assay sensitivity, precision, recovery from spiking, and dilutional linearity were produced. All standard curves, controls, and samples were analyzed in a dilution of 50% EDTA plasma. Standardized pooled cytokine-free sterile filtered porcine EDTA plasma was used for standards and controls. A dilution buffer of phosphate-buffered saline, 2% bovine serum albumin, and 0.05% Tween-20 was used. All samples were run in triplicates and analyzed in a blinded, randomized manner. Standard curves were created using a four-parameter logistic curve fit (Microcal Origin; Universal Imaging Corporation, Downingtown, PA). Median sample concentrations were calculated from this.

Brief Assay Protocol. Microtiter plates (Nunc, Copenhagen, Denmark) were coated with capture antibody in phosphate-buffered saline and incubated for 18 h at 40°C. Blocking unspecific binding sites was done with phosphate-buffered saline, pH 7.4, containing 5% nonfat dried milk and 0.1% Tween-20 for 2 h. Plates were washed three times in 50 mM Tham, pH 7.3, containing 0.2% Tween-20 between all steps hereafter. Standards, samples, and controls were loaded and incubated for 2 h at ambient temperature. Plates were incubated with secondary antibody in dilution buffer for 2 h at ambient temperature. Eu^{3+}-streptavidin (1:2,000) in assay buffer (Wallac) was added and incubated for 30 min. One hour of signal enhancement (Enhancement Solution; Wallac) was performed, followed by analysis in the fluorometer and registration of light intensities by the assay software (Multicalc; Wallac).

The protocol yielded assay sensitivities of 6 pg/ml (IL-8) and 12 pg/ml (IL-6). An acceptable precision was achieved with both interrun and intrarun variations ranging between 5 and 10% (percent coefficient of variation).

Chemotaxis of Polymorphonuclear Neutrophils. Neutrophils were harvested for chemotaxis in EDTA-Vacutainers (Becton, Dickinson, Franklin Lakes, NJ) at baseline, 240 min, and 480 min, and their ability to perform chemotaxis *ex vivo* was determined by an under-agarose assay as described by Kongstad *et al.*[11] Briefly, polymorphonuclear neutrophils were isolated immediately after blood collection by erythrocyte sedimentation in 5% dextran (T-500; Pharmacia, Uppsala, Sweden), centrifuged over Lymphoprep® (Nycomed, Oslo, Norway) and hypotonic lysed and washed. An agarose medium consisting of 1.2% agarose and 0.25% gelatin in RPMI was added to specially constructed chemotaxis chambers with a gelatin-coated microscope slide at the bottom of each chamber. Six series of three wells were cut in each gel, with a well diameter of 2 mm and an interwell distance of 3 mm. Ten microliters suspension with approximately 5×10^5 cells was added to the middle well in each series. To the outermost wells, 10 μl RPMI was added as control, and to the opposite outermost wells, 10 μl chemotactic factor, zymosan-activated serum (10 mg/ml), was added. The chambers were immediately sealed, incubated at 37°C for 135 min, and terminated by flooding the agarose with methanol overnight followed by formaldehyde for 60 min. The gel was removed, and the cells were stained. The chemotactic migration of the polymorphonuclear neutrophils was measured as the linear distance they had moved from the margin of the well containing cells toward the chemoattractant well. The spontaneous migration was

measured as the linear distance from the well containing cells and the control well. Measurements were performed in duplicate in two different chambers.

Statistical Analysis

All statistical analyses were performed using Intercooled Stata 8.1 (Stata Corporation, College Station, TX).

Clinical and Metabolic Variables. Data were log transformed (the natural logarithm, base e) when appropriate. If raw data were used in the analysis, the arithmetic mean and the SD were stated for each group at each time point, and if the log-transformed data were used, the geometric mean and the SD on the log scale (an estimate of the coefficient of variation) were stated.

A two-way repeated-measurement analysis (three measurements for each pig: 60, 240, and 570 min) of each variable was performed using lipopolysaccharide ($\pm$) and HEC ($\pm$) as explaining factors. Generally, this design allowed us to test the interacting effects of lipopolysaccharide and HEC, in both cases with and without dependency of time. The assumptions of the models were verified by inspecting scatter plots of the residuals *versus* fitted values and normal quantile plots of the residuals.

General ANOVA Model. The general ANOVA model was as follows:

Lipopolysaccharide HEC Time Lipopolysaccharide ·

HEC Lipopolysaccharide · Time HEC ·

Time Lipopolysaccharide · HEC · Time

Comparison of changes over time were first performed. If the interaction among lipopolysaccharide, HEC, and time was statistically significant, we restricted the comparison to pairwise testing of selected groups. If the interaction among lipopolysaccharide, HEC, and time was not statistically significant, we continued to compare the effect of lipopolysaccharide and HEC (with regard to changes over time) independent of each other. If there was no statistical significance between lipopolysaccharide and time (with regard to changes over time), we tested whether there was a general difference (with regard to independent of time) between lipopolysaccharide ($\pm$) animals. The same was done for HEC. In addition, we tested whether there was an interaction between lipopolysaccharide and HEC independent of time.

In terms of insulin, glucagon, and FFA, the assumptions of the model were not fulfilled. Consequently, all four groups were compared, with relation to the relative rate of change between 60 and 240 min, by Kruskal-Wallis test, and if a statistical significant difference was found, the groups were compared pairwise with multiple testing.

Polymorphonuclear Neutrophil Chemotaxis. The same general ANOVA model as above was used. Only the time points changed.

Cytokines. Data regarding TNF-α and IL-10 were tested with *t* tests between lipopolysaccharide and lipopolysaccharide–HEC groups at the 240-min time point because these were the only groups with measurable TNF-α and IL-10 signals. Normality was assumed on the basis of normal quantile plots. Data regarding IL-6 and IL-8 were not assumed to be normally distributed on the basis of normal quantile plots. The relative rates of change in cytokine concentration between 240 and 480 min and 240 and 570 min, respectively, were tested with use of the Mann–Whitney two-sample rank sum test.

Results

Effects on Cytokine Concentrations

Data of the effects on cytokine concentrations are shown in figure 2. In the lipopolysaccharide pigs (group 3) and the lipopolysaccharide–HEC pigs (group 4), TNF-α and IL-10 plasma concentrations increased during the lipopolysaccharide infusion and peaked at 240 min after the start of infusion. The TNF-α concentrations in the lipopolysaccharide–HEC group were significantly lower than in the lipopolysaccharide group ($P = 0.04$). Although there was a trend toward a lesser increase in IL-10 concentrations in the lipopolysaccharide–HEC group at 240 min, there was no significant difference between the two groups. In the anesthesia group (group 1) and the HEC group (group 2), TNF-α and IL-10 were not detectable in plasma.

Interleukin 6 and IL-8 were detectable in plasma only after lipopolysaccharide infusion (groups 3 and 4). The peak concentrations of both cytokines were observed 240 min after the start of lipopolysaccharide infusion. The decrease in plasma IL-6 from 240 to 480 min after lipopolysaccharide infusion was significantly higher in lipopolysaccharide–HEC pigs (group 4) compared with lipopolysaccharide pigs (group 3) ($P = 0.04$). There was no statistically difference in plasma IL-8 at any time between lipopolysaccharide pigs and lipopolysaccharide–HEC pigs.

Circulating Glucose, Insulin, FFA, and Glucagon

Circulating glucose, insulin, FFA, and glucagon data are shown in figure 3. Plasma glucose was comparable in all four groups. We found a statistically significant difference between the four groups with relation to relative change from 60 to 240 min for insulin ($P = 0.001$), FFA ($P = 0.032$), and glucagon ($P = 0.009$). The pairwise comparisons yielded the following results:

1. The HEC group showed a higher serum insulin response compared with the anesthesia group (P < 0.05), and the lipopolysaccharide–HEC group
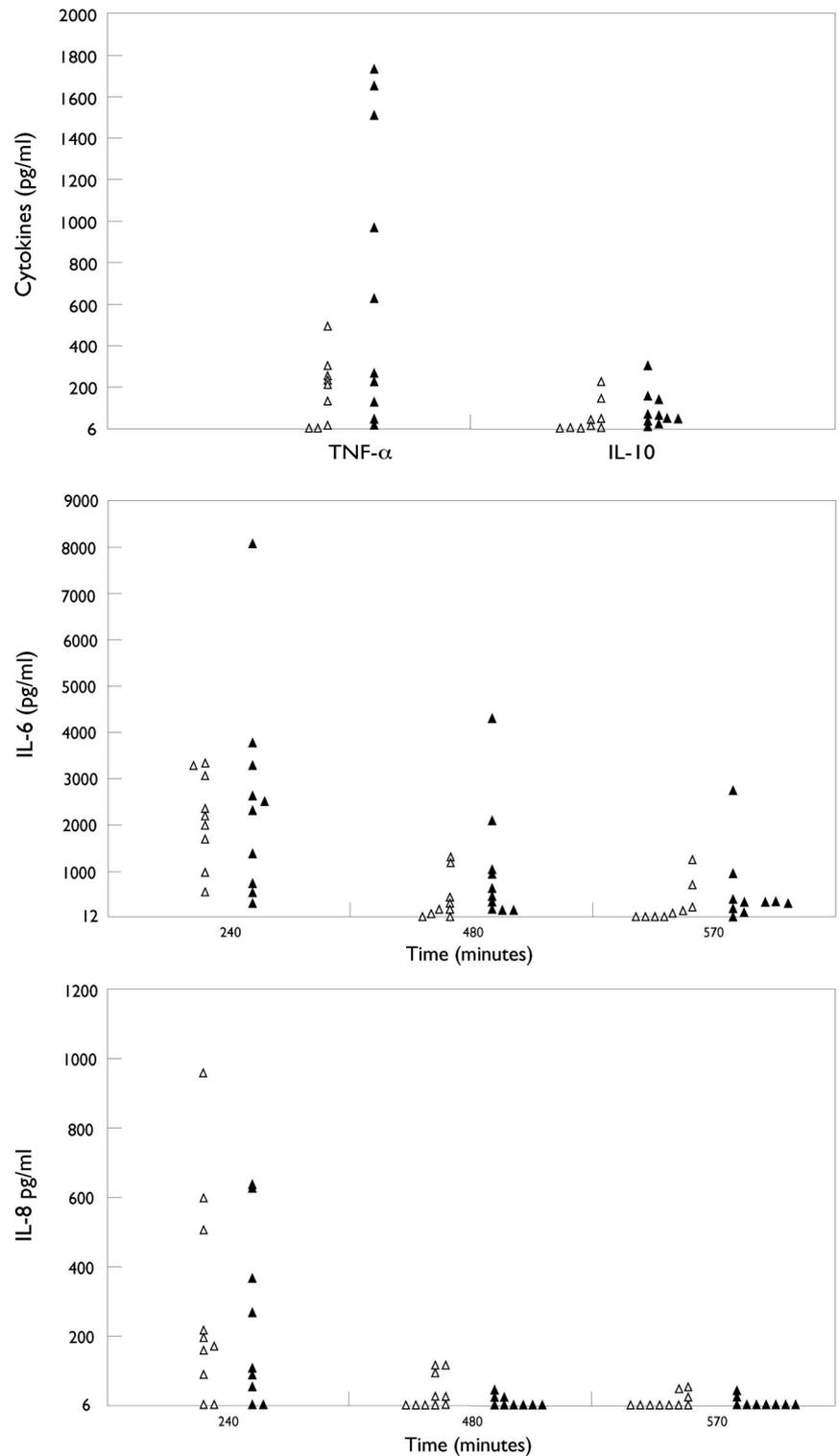
Fig. 2. Plasma cytokines. (*A*) The individual concentrations of plasma tumor necrosis factor (TNF) *α* and interleukin (IL) 10. (*B*) The individual concentrations of plasma IL-6. (*C*) The individual concentrations of plasma IL-8. Each *symbol* represents one pig. △ = lipopolysaccharide–hyperinsulinemic euglycemic clamp; ▲ = lipopolysaccharide.

showed a higher serum insulin response than the HEC group ($P < 0.05$).

2. Serum FFA was higher in the lipopolysaccharide group compared with the HEC group ($P < 0.05$).

3. In the lipopolysaccharide–HEC group, the glucagon response was diminished compared with the lipopolysaccharide group ($P < 0.05$), and the lipo-

polysaccharide group showed a marked plasma glucagon response compared with the HEC group ($P < 0.05$).

Base Excess and Lactate

Base excess and lactate data are shown in figure 4. Lipopolysaccharide infusion resulted in decreased levels
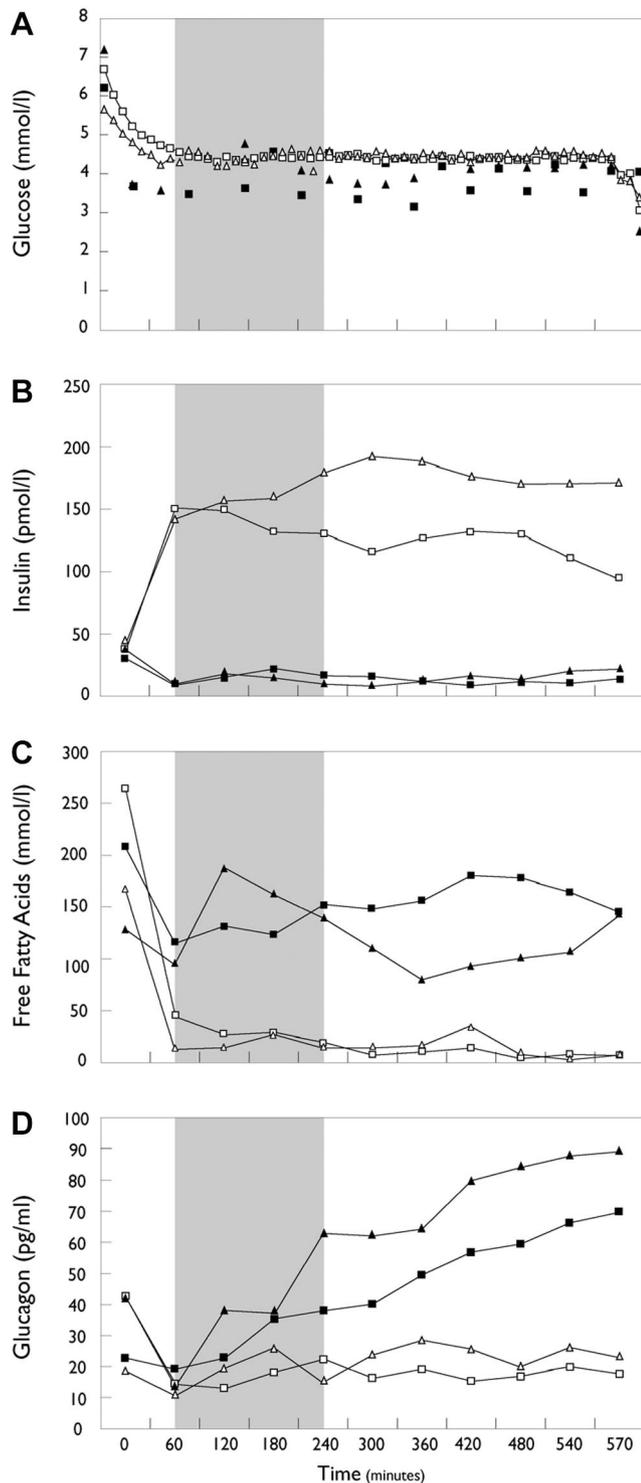
Fig. 3. Circulating (*A*) glucose; (*B*) insulin (within-animal SD, 0.45; total SD, 0.47); (*C*) free fatty acids (within-animal SD, 0.85; total SD, 0.95), and (*D*) glucagon (within-animal SD, 0.67; total SD, 0.52). The groups are represented as the geometric mean value in each group at each time point. Lipopolysaccharide (LPS) infusion was given 60–240 min. ■ = anesthesia; □ = hyperinsulinemic euglycemic clamp (HEC); △ = LPS-HEC; ▲ = LPS.

in base excess over time ($P < 0.001$) and increased levels of lactate over time ($P = 0.001$).

Chemotaxis

Chemotaxis data are shown in figure 5. Lipopolysaccharide infusion resulted in decreased chemotaxis over time ($P = 0.01$). Furthermore, there was an effect of the HEC independent of time ($P = 0.03$).

Hemodynamic Parameters

Hemodynamic parameter data are shown in table 1. Pigs remained hemodynamically stable throughout the study period in the HEC and anesthesia groups. Pigs in the lipopolysaccharide and lipopolysaccharide–HEC groups showed a steep increase in PAP over time ($P = 0.00$).

Neither lipopolysaccharide infusion nor the combination of HEC and lipopolysaccharide infusion had any major effects on mean blood pressure, pulmonary capillary wedge pressure, or cardiac output. Lipopolysaccharide infusion decreased the ratio of Pao_2:Fio_2 over time ($P = 0.00$).

The lipopolysaccharide-induced inflammatory condition was treated with increased rate of fluid infusion (saline), increased ventilation (tidal volume and respiratory rate), and increased Fio_2. In the lipopolysaccharide group, four animals developed arrhythmias/ventricular fibrillation/cardiac arrest. These conditions were successfully treated with defibrillation.

Discussion

In this experimental study, we demonstrated that short-term hyperinsulinemia, together with normoglycemia, vastly reduced the systemic inflammatory and metabolic responses to endotoxemia in a porcine model. It was clearly demonstrated that maintenance of hyperinsulinemia reduced plasma concentrations of glucagon and TNF-α, the latter supporting the hypothesis that insulin has antiinflammatory effects.

The infused amounts of insulin far exceeded the amounts necessary to maintain normoglycemia. This is a major difference from the clinical situation where normoglycemia usually is established with much lower insulin doses.[3] However, the beneficial effects of hyperinsulinemia in sepsis seem to be analogs to the effect of glucose–insulin–potassium solutions used for improvement of cardiac performance in patients with acute myocardial infarction,[12] where insulin and glucose are administered in large doses without targeting normoglycemia. The aim of insulin–glucose–potassium is to stimulate myocardial metabolism of glucose instead of fatty acids when oxygen supply is compromised in the ischemic myocardium. Furthermore, the ischemic myocardium produces high amounts of TNF-α.[13] This production is suppressed by insulin,[4] probably through decreased con-
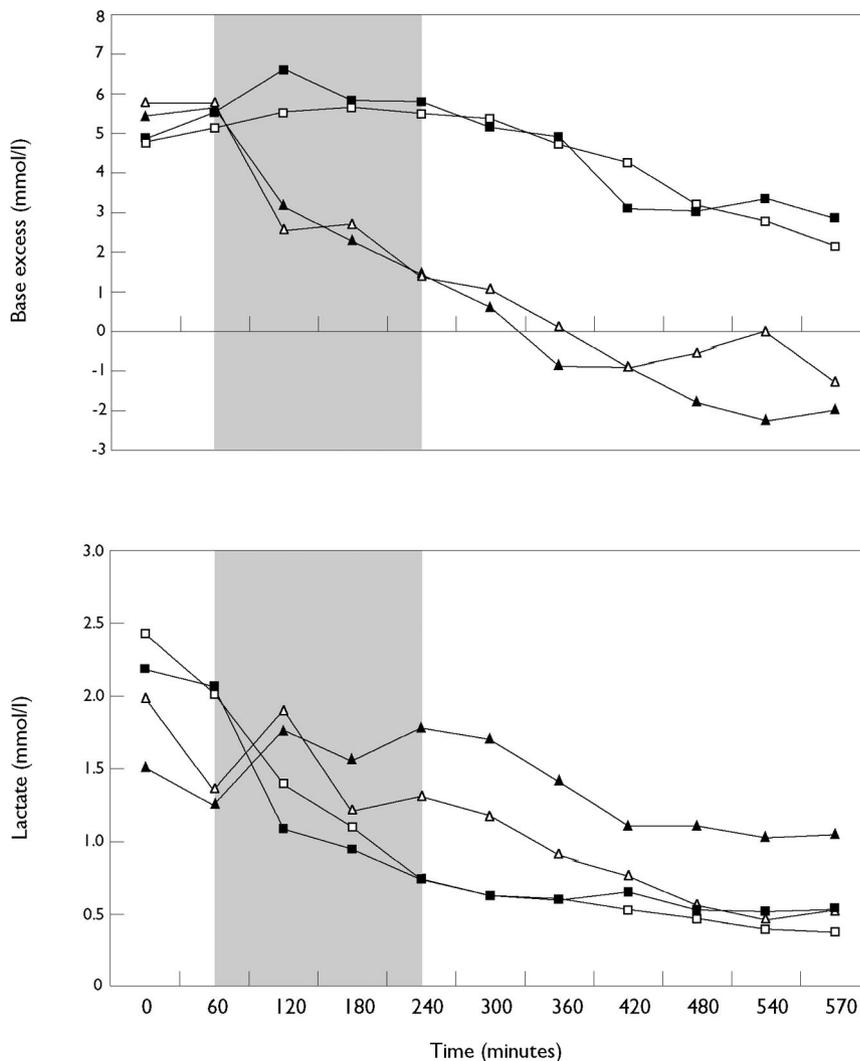
Fig. 4. Lactate and base excess. The groups are represented as mean values in each group at each time point. Lipopolysaccharide (LPS) infusion was given 60–240 min. Within-animal SD, 0.60; total SD, 0.73. ■ = anesthesia; □ = hyperinsulinemic euglycemic clamp (HEC); △ = LPS-HEC; ▲ = LPS.

centration of nuclear factor κB, which is an important regulator of cytokine-inducible gene expression.[14]

High concentrations of plasma TNF-α and the ratio TNF-α:IL-10[15] have previously been reported to correlate with poor outcome in humans with severe sepsis and to link with the presence of insulin resistance. However, TNF-α is only one of an orchestra of proinflammatory cytokines that initially can be found in plasma during
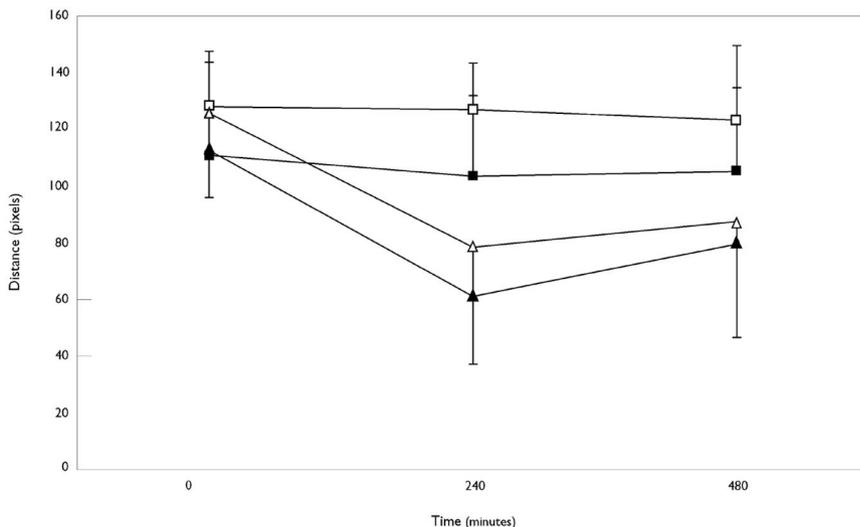
Fig. 5. Chemotactic response. The calculated chemotactic response of neutrophils (chemotaxis migration − random migration) is presented as mean ± SD for each group. ■ = anesthesia; □ = hyperinsulinemic euglycemic clamp (HEC); ▲ = lipopolysaccharide (LPS); △ = LPS-HEC.

Table 1. Hemodynamic Parameters

Group	Baseline	60 min	240 min	570 min
Heart rate				
1	59 ± 16	57 ± 12	49 ± 12	48 ± 8
2	60 ± 14	63 ± 17	61 ± 15	54 ± 13
3	48 ± 13	55 ± 14	55 ± 8	55 ± 11
4	64 ± 18	64 ± 18	65 ± 31	71 ± 28
Mean blood pressure				
1	58 ± 5	59 ± 8	59 ± 8	55 ± 7
2	58 ± 8	61 ± 8	65 ± 7	61 ± 10
3	60 ± 10	63 ± 10	69 ± 10	69 ± 12
4	76 ± 19	73 ± 12	74 ± 13	70 ± 14
Cardiac output				
1	2.5 ± 0.9	2.9 ± 1.0	2.6 ± 0.9	3.1 ± 0.8
2	2.9 ± 1.0	3.0 ± 0.7	3.0 ± 0.7	3.4 ± 0.7
3	2.6 ± 0.9	3.0 ± 0.7	3.2 ± 0.9	3.26 ± 0.7
4	3.1 ± 0.8	3.4 ± 0.7	3.2 ± 0.5	4.3 ± 1.6
Pulmonary capillary wedge pressure				
1	10 ± 1.6	8.4 ± 1.6	10.1 ± 2.1	8.7 ± 1.2
2	9.8 ± 2.2	9.7 ± 2.5	11.4 ± 2.6	9.6 ± 1.9
3	10.3 ± 1.9	10.1 ± 2.3	11.1 ± 3.3	10 ± 2.1
4	10 ± 2.2	10.3 ± 1.6	12.3 ± 4.0	10.7 ± 1.7
Pulmonary artery pressure				
1	16.3 ± 1.6	16.5 ± 2.3	16.5 ± 1.3	16.8 ± 2.9
2	14.4 ± 2.4	15.9 ± 2.6	19.4 ± 3.0	20.0 ± 2.7
3	16.4 ± 3.0	18.8 ± 3.5	32.5 ± 9.4	25.6 ± 8.8
4	16.8 ± 1.3	17.1 ± 1.6	30.9 ± 5.5	28.1 ± 5.9
$Pa_{O_2}/F_{I_{O_2}}$				
1	553 ± 58	575 ± 29	555 ± 35	531 ± 47
2	519 ± 100	517 ± 55	524 ± 51	492 ± 69
3	517 ± 53	537 ± 60	499 ± 109	376 ± 123
4	483 ± 75	544 ± 48	510 ± 102	359 ± 101

Group 1 received anesthesia solely, group 2 received anesthesia and were exposed to hyperinsulinemic euglycemic clamp, group 3 received an infusion of lipopolysaccharide, and group 4 received a combination of hyperinsulinemic euglycemic clamp and lipopolysaccharide infusion.

$F_{I_{O_2}}$ = fraction of inspired oxygen; Pa_{O_2} = arterial oxygen tension.

sepsis. TNF-α is believed to play an important role as a mediator of multiorgan dysfunction and as an inducer of the secretion of other proinflammatory cytokines, such as IL-1β, IL-6, and IL-8. However, a certain amount of TNF-α seems to be necessary for optimal function of the immune system. Blockade of the TNF-α response to sepsis was associated with reduced survival in animals, just as a clinical trial has shown increased mortality in patients treated with a TNF-α antagonist.[16]

Hyperinsulinemia was also associated with a diminished response of IL-6, another proinflammatory molecule. It has previously been shown that the proinflammatory cytokines TNF-α,[17] IL-6,[18] and IL-8 increase blood lipids, enhance gluconeogenesis, stimulate the production of catabolic hormones, and decrease insulin sensitivity as part of the initial inflammatory stress response. The modified proinflammatory cytokine response may thus have contributed to the diminished glucagon and FFA response.

In the current study, there was a already marked decrease in plasma FFA concentrations in the stabilization period, which illustrates how easily the animals were stressed although they were heavily premedicated before induction of anesthesia and intubation. The FFA concentrations in the animals exposed to anesthesia

alone were rather stable throughout the study. Animals receiving lipopolysaccharide had plasma FFA concentrations similar to those of pigs in the anesthetic group. One would have expected that circulating FFAs would be higher during lipopolysaccharide exposure than anesthesia *per se*; however, tiny and transient fluctuations in serum insulin may have obscured this finding because of the high efficacy of insulin on antilipolysis.[19] However, the key issue is that hyperinsulinemia in combination with lipopolysaccharide administration is characterized by suppressed FFA concentrations. In large amounts, FFAs contribute to metabolic acidosis and cellular and organ dysfunction. This goes along with the significant reduction in base excess observed in pigs receiving lipopolysaccharide in this study and the impaired neutrophils chemotaxis. It remains to be clarified whether lower FFA concentrations *per se* contribute to the beneficial effect of insulin treatment in critically ill surgical patients.

Free fatty acids are toxic for the ischemic myocardium and may lead to damaged cardiac cell membranes, calcium overload, and arrhythmias, particularly in the case of insulin deficiency.[20] The increase in lactate concentration and the subsequent metabolic acidosis are also advocated to explain the deleterious effect of hypergly-

cemia in cerebral tissue. Moreover, the ability of insulin to reduce the catabolic glucagon concentrations may also be part of the antiinflammatory effect of insulin because plasma concentrations of glucagon are normally enhanced during sepsis,[21] although the effect of glucagon itself on the antiinflammatory response is not well elucidated.

Through the observation period, parallel impairments in pulmonary and hemodynamic function were observed in pigs receiving lipopolysaccharide. The infusion of lipopolysaccharide had a major effect on PAP, manifested as an abrupt increase in PAP occurring 20–25 min after the start of the infusion. Within 5–10 min, the mean PAP increased from a range of 15–20 mmHg to a range of 45–50 mmHg, with peak levels during the period of infusion of lipopolysaccharide at an increasing rate. The PAP remained elevated in the lipopolysaccharide and lipopolysaccharide–HEC groups, although at a lower level throughout the study period. The increase in PAP was accompanied by discrete increases in partial pressure of carbon dioxide (Pco_2) and plasma lactate, whereas Pao_2 showed a decreasing tendency. Insulin did not, however, have a significantly beneficial effect on the respiratory and cardiovascular systems in the current study.

The function of neutrophils is impaired in patients with diabetes, proportionally according to the degree of hyperglycemia.[22,23] In the current short-term study, the hyperinsulinemia could not restore the impaired chemotactic response induced by endotoxin. Neutrophils play a key role in the innate immune system. Circulating neutrophils are activated by proinflammatory cytokines.[24] When activated, they transmigrate from the circulation to the tissue/organs (chemotaxis). Neutrophil infiltration with release of lysosomal enzymes and free oxygen radicals may cause tissue damage. The proinflammatory cytokine IL-8 plays a pivotal role for recruitment and activation of neutrophils. There was no difference in plasma IL-8 between the groups receiving lipopolysaccharide. However, this does not rule out any difference at organ level.[25]

Limitations of the Study

Endotoxin (lipopolysaccharide) is a membrane component of gram-negative bacteria and produces multiple endocrine and metabolic effects that mimic those seen in acute gram-negative sepsis. The animals received an infusion of lipopolysaccharide with gradual increase and a total infusion time of 180 min. This does not, however, mimic the clinical situation but allows us to monitor the acute inflammatory response to endotoxin. In contrast to most clinical courses of severe sepsis, the injury was well defined with respect to time and lipopolysaccharide dose and was controlled in this setup.

Conclusion

It seems that the physiologic response to acute endotoxemia in pigs is analogs to the systemic inflammatory response in humans and is composed by a transitory proinflammatory and antiinflammatory cytokine response and impaired neutrophil function. The exogenous insulin acutely modulates the innate immune system by decreasing an inappropriate proinflammatory response, restoring normal glucose concentrations and decreasing FFAs and glucagons, playing an antiinflammatory role. Longer term effects of hyperinsulinemia on the proinflammatory cytokine response remain to be determined.

References

1. Annane D, Sebille V, Charpentier C, Bollaert PE, Francois B, Korach JM, Capellier G, Cohen Y, Azoulay E, Troche G, Chaumet-Riffaut P, Bellissant E: Effect of treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. JAMA 2002; 288: 862–71

2. Bernard GR, Vincent JL, Laterre PF, LaRosa SP, Dhainaut JF, Lopez-Rodriguez A, Steingrub JS, Garber GE, Helterbrand JD, Ely EW, Fisher CJ: Efficacy and safety of recombinant human activated protein C for severe sepsis. N Engl J Med 2001; 344:699–709

3. Van den Berghe G, Wouters R, Weekers F, Verwaest C, Bruyninckx F, Schetz M, Vlasselaers D, Ferdinande P, Lauwers P, Bouillon R: Intensive insulin therapy in critically ill patients. N Engl J Med 2001; 345:1359–67

4. Das UN: Is insulin an antiinflammatory molecule? Nutrition 2001; 17:409–13

5. Van den Berghe G, Wouters PJ, Bouillon R, Weekers F, Verwaest C, Schetz M, Vlasselaers D, Ferdinande P, Lauwers P: Outcome benefit of intensive insulin therapy in the critically ill: Insulin dose versus glycemic control. Crit Care Med 2003; 31:359–66

6. Mizock BA: Alterations in fuel metabolism in critical illness: Hyperglycemia. Best Pract Res Clin Endocrinol Metab 2001; 15:533–51

7. Hotchkiss RS, Karl IE: The pathophysiology and treatment of sepsis. N Engl J Med 2003; 348:138–50

8. Gallacher SJ, Thomson G, Fraser WD, Fisher BM, Gemmell CG, MacCuish AC Neutrophil bactericidal function in diabetes mellitus: Evidence for association with blood glucose control. Diabet Med 1995; 12:916–20

9. Hansen TK, Thiel S, Wouters PJ, Christiansen JS, Van den Berghe G: Intensive insulin therapy exerts anti-inflammatory effects in critically ill patients and counteracts the adverse effect of low mannose-binding lectin levels. J Clin Endocrinol Metab 2003; 88:1082–8

10. Institute of Laboratory Animal Resources: Guide for the Care and Use of Laboratory Animals, 7th edition. Washington, D.C., National Academy Press, 1996

11. Petersen TK, Jensen AL, Aaes H: Chemotaxis of canine polymorphonuclear neutrophil granulocytes using under-agarose method applied to glass microscope slides. Comp Haematol Int 1998; 8:31–6

12. Diaz R, Paolasso EA, Piegas LS, Tajer CD, Moreno MG, Corvalan R, Isea JE, Romero G: Metabolic modulation of acute myocardial infarction. The ECLA Collaborative Group. Circulation 1998; 98:2227–34

13. Irwin MW, Mak S, Mann DL, Qu R, Penninger JM, Yan A, Dawood F, Wen WH, Shou Z, Liu P: Tissue expression and immunolocalization of tumor necrosis factor-alpha in post-infarction dysfunctional myocardium. Circulation 1999; 99: 1492–8

14. Christman JW, Lancaster LH, Blackwell TS: Nuclear factor-κB: A pivotal role in the systemic inflammatory response syndrome and new target for therapy. Intensive Care Med 1998; 24:1131–8

15. Gogos CA, Drosou E, Bassaris HP, Skoutelis A: Pro- versus anti-inflammatory cytokine profile in patients with severe sepsis: A marker for prognosis and future therapeutic options. J Infect Dis 2000; 181:176–80

16. Fisher CJ Jr, Agosti JM, Opal SM, Lowry SF, Balk RA, Sadoff JC, Abraham E, Schein RM, Benjamin E: Treatment of septic shock with the tumor necrosis factor receptor:Fc fusion protein. The Soluble TNF Receptor Sepsis Study Group. N Engl J Med 1996; 334:1697–702

17. Hrebicek A, Rypka M, Chmela Z, Vesely J, Kantorova M, Golda V: Tumor necrosis factor alpha in various tissues of insulin-resistant obese Koletsky rats: Relations to insulin receptor characteristics. Physiol Res 1999; 48:83–6

18. van Hall G, Steensberg A, Sacchetti M, Fischer C, Keller C, Schjerling P, Hiscock N, Møller K, Saltin B, Febbraio MA, Pedersen BK: Interleukin-6 stimulates lipolysis and fat oxidation in humans. J Clin Endocrinol Metab 2003; 88:3005–10

19. Jensen MD, Caruso M, Heiling V, Miles JM: Insulin regulation of lipolysis in nondiabetic and IDDM subjects. Diabetes 1989; 38:1595–601

20. Russell RO, Rogers WJ, Mantle JA, McDaniel HG, Rackley CE: Glucose-insulin-potassium, free fatty acids and acute myocardial infarction in man. Circulation 1976; 53:207–9

21. Lang CH, Dobrescu C, Bagby GJ: Tumor necrosis factor impairs insulin action on peripheral glucose disposal and hepatic glucose output. Endocrinology 1992; 130:43–52

22. Rassias AJ, Marrin CA, Arruda J, Whalen PK, Beach M, Yeager MP: Insulin infusion improves neutrophil function in diabetic cardiac surgery patients. Anesth Analg 1999; 88:1011–6

23. Cavalot F, Anfossi G, Russo I, Mularoni E, Massucco P, Burzacca S, Mattiello L, Trovati M: Insulin, at physiological concentrations, enhances the polymorpho-nuclear leukocyte chemotactic properties. Horm Metab Res 1992; 24:225–8

24. Brix-Christensen V, Petersen TK, Ravn HB, Hjortdal VE, Andersen NT, Tønnesen E: Cardiopulmonary bypass elicits a pro- and antiinflammatory cytokine response and impaired chemotaxis in neonatal pigs. Acta Anaesthesiol Scand 2001; 45:407–13

25. Brix-Christensen V, Vestergaard C, Chew M, Johnsen CK, Andersen SK, Engkilde K, Hjortdal V, Tønnesen E: Plasma cytokines do not reflect expression of pro- and anti-inflammatory cytokine mRNA at organ level after cardiopulmonary bypass in neonatal pigs. Acta Anaesthesiol Scand 2003; 47:525–31