Original Article

Survival Benefits of Intensive Insulin Therapy in Critical Illness

Impact of Maintaining Normoglycemia Versus Glycemia-Independent Actions of Insulin

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Tight blood glucose control with insulin reduces morbidity and mortality of critically ill patients. However, the relative impact of maintaining normoglycemia and of glycemia-independent actions of insulin remains unknown. We therefore independently manipulated blood glucose and plasma insulin levels in burn-injured, parentally fed rabbits over 7 days to obtain four study groups: two normoglycemic groups with either normal or elevated insulin levels and two hyperglycemic groups with either normal or elevated insulin levels. We studied the relative impact of glycemia and glycemia-independent effects of insulin on survival; myocardial contractility in an open chest preparation; endothelial function in isolated aortic rings; and liver, kidney, and leukocyte function in a rabbit model of critical illness. Mortality was significantly lower in the two normoglycemic groups independent of insulin levels. Maintaining normoglycemia, independent of insulin levels, prevented endothelial dysfunction as well as liver and kidney injury. To increase myocardial systolic function, elevated insulin levels and prevention of hyperglycemia were required concomitantly. Leukocyte dysfunction was present in the two hyperglycemic groups, which could in part be rescued by insulin. The results suggest that the observed benefits of intensive insulin therapy required mainly maintenance of normoglycemia; whereas glycemia-independent actions of insulin exerted only minor, organ-specific impact. *Diabetes* 55:1096–1105, 2006

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ACh, acetylcholine; CVP, central venous pressure; dp/dt, first derivative of left and right ventricular pressure over time; GIK, insulin infused together with glucose and potassium; L-NAME, L-nitro-arginine-methyl-ester; MAP, mean arterial pressure; SVRI, systemic vascular resistance index.

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Hyperglycemia in critically ill patients is brought about by hepatic and peripheral insulin resistance and by concomitant relative insulin deficiency due to limited compensatory ability of pancreatic β-cells, largely independent of the underlying disease (1). Hyperglycemia during critical illness has long been considered essential to provide fuel for vital organ systems and hence was interpreted as a beneficial adaptation. Evidence is now growing against this notion as hyperglycemia is identified as an independent risk factor for adverse outcome of numerous surgical and medical conditions (2–4), and avoiding hyperglycemia with intensive insulin therapy has been shown to improve outcome (5,6). The risks of hyperglycemia comprise increased vulnerability to infectious complications, impaired recovery of organ failure, myocardial dysfunction, and neuromuscular weakness (7–12). The cardiovascular and immune systems thus emerge as two important target systems of glycemic control in the critically ill (7,10,13).

It remains unclear, however, to what extent maintaining normoglycemia and glycemia-independent actions of insulin account for the different clinical benefits of intensive insulin therapy in the critically ill. A post hoc analysis of the randomized controlled study of intensive insulin therapy in surgical intensive care patients suggested that blood glucose control best explains the clinical benefits of the intervention (14). In contrast, insulin-induced promotion of glycolysis in cardiomyocytes and a diversion of fatty acids to adipocytes is thought to reduce oxygen consumption in the heart, which is proposed as the mechanism behind a cardioprotective effect of insulin infused together with glucose and potassium (GIK) (15). Clinical studies investigating outcome-effects of GIK, however, revealed controversial results. This could be due to either different doses of insulin and glucose or to the varying levels of blood glucose achieved with the different protocols (16).

In the current study, we aimed at defining the extent to which blood glucose control and glycemia-independent actions of insulin contribute to the major benefits of intensive insulin therapy in an animal model of prolonged critical illness. End point measures were mortality, liver and kidney function, hemodynamic function, endothelial function, and day-3 leukocyte function.

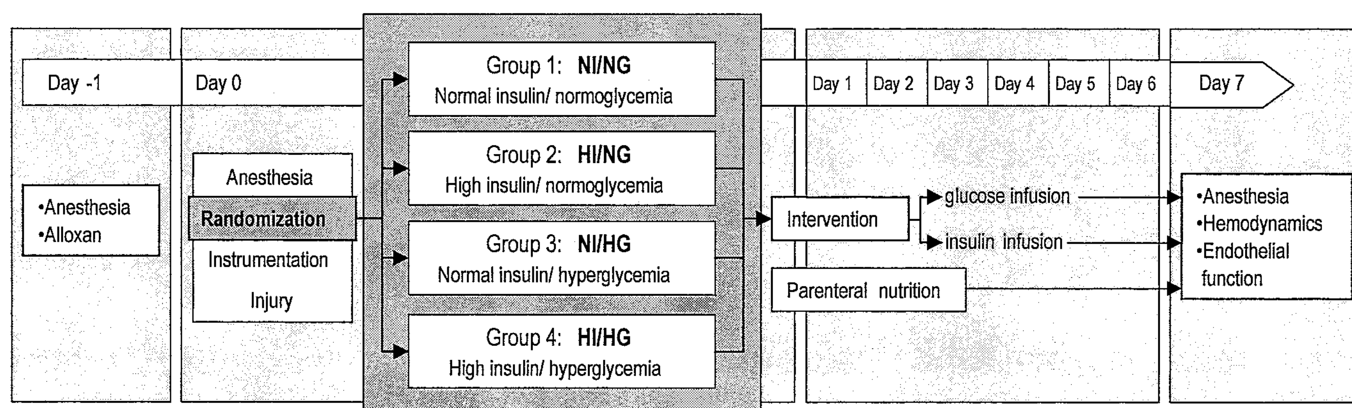

FIG. 1. Flowchart of the study design.

RESEARCH DESIGN AND METHODS

Animals were treated according to the Principals of Laboratory Animal Care formulated by the U.S. National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the National Institutes of Health. The protocol was approved by for animal research (protocol Nr P 04058). Adult male New Zealand White rabbits, weighing ±3 kg, were purchased from a local rabbitry, were housed individually with free access to water and regular rabbit chow, and were exposed to artificial light for 14 h per day.

In our animal model, critical illness was induced by the combination of placing intravascular catheters, selectively destroying pancreatic β-cells by alloxan, followed by burn injury, which has previously shown to reveal the dynamic endocrine and metabolic changes characteristic of critical illness, including hyperglycemia and endogenous insulin deficiency (10). Without exerting intrinsic effects on survival (10), alloxan limits endogenous insulin release and thus minimizes interindividual differences in insulin levels upon glucose load. This was necessary to control both blood glucose and plasma insulin levels independently.

Study protocol (Fig. 1)

Day −1. At 0800 ± 1 h, animals were weighed, and under general anesthesia (30 mg/kg ketamine i.m. [Imalgene 1000; Merial, Lyon, France]; 0.15 ml/kg medetomidine i.m. [Orion, Espoo, Finland]), an ice-cold 10% solution of alloxan-monohydrate (150 mg/kg; Alloxan; Sigma-Aldrich, Bornem, Belgium) was injected slowly via a marginal ear vein. Afterward, the animals had free access to regular rabbit chow and drinking water enriched with glucose to face alloxan-induced acute hypoglycemia.

Day 0. At 1600 ± 1 h, under general anesthesia (see above) supplemented with 1.5 volume % isoflurane (Isoba Vet.; Schering-Plough, Brussels, Belgium) inhalation, catheters were placed into the right jugular vein for intravenous infusion (4F; Vygon, Ecouen, France) and into the right carotid artery for blood sampling (5 Ch; Sherwood Medical, Tullamore, Ireland). Rabbits were randomized into four groups by sealed envelopes: group 1 (NI/NG), normal insulin, normoglycemia; group 2 (HI/NG), high insulin, normoglycemia; group 3 (NI/HG), normal insulin, hyperglycemia; and group 4 (HI/HG), high insulin, hyperglycemia. The protocol was designed to reach at least a target of eight rabbits per group surviving until day 7.

Because 80–110 mg/dl is commonly accepted as normoglycemia (5), this blood glucose was set as target glycemia in both normoglycemic groups. For both hyperglycemic groups, we selected target glycemia of 250–350 mg/dl, because this level is in the range commonly chosen by other authors in experimental (17–21) and clinical studies (6,22). Thus, these levels allow us to compare our results with those from previous clinical and experimental studies.

After shaving both flanks and performing a paravertebral block (5 ml Xylocaine 1%; Astra Pharmaceuticals, Brussels, Belgium), a full thickness burn injury of 20% body-surface area was imposed. Animals were then fitted to a homemade jacket to secure catheters and immediately returned to their cages. Continuous fluid resuscitation (18 ml/h Hartmann solution [Baxter, Lessiness, Belgium] supplemented with 17 g glucose/500 ml) was started via a volumetric pump (Infusomat secura; B.Braun, Melsungen, Germany) using a homemade swiffle device to allow free moving in the cage. Insulin (Actrapid; Novo Nordisk, Begsvaerd, Denmark) was continuously administered intravenously via a syringe pump (Perfusor secura; B.Braun), the daily doses of which are given in Table 1. The two preset levels of blood glucose were achieved by adjusting a continuous glucose infusion (50% glucose via a syringe pump; Baxter) supplementing basal glucose intake. Animals were deprived of oral feeding and received water ad libitum. In the evening, a supplementary dose of piritramid was given subcutaneously (0.2 mg/kg Dipidolor; Janssen-Cilag, Beerse, Belgium).

Day 1. At 1000 ± 1 h, Hartman solution was replaced by parenteral nutrition infused at 12 ml/h. We choose total intravenous nutrition because this is the only way to assure equal nutrient intake of the rabbits. To account for

TABLE 1
Dose and target of intervention, nutritional intake, and levels of plasma insulin, blood glucose, lactate, and blood pH

	Group 1: NI/NG	Group 2: HI/NG	Group 3: NI/HG	Group 4: HI/HG
Intervention				
Insulin dose (units · kg^{-1} · day^{-1})	1.2	4.0	1.2	4.0
Target glycemia (mg/dl)	80–110	80–110	250–350	250–350
Nutritional intake				
Glucose intake (g/day)*	27.4 ± 1.9	47.2 ± 2.4	64.3 ± 2.8	75.9 ± 3.1
Fat intake (g/day)	3.5 ± 0.05	3.3 ± 0.09	3.4 ± 0.07	3.5 ± 0.09
Amino acid intake (g/day)	3.5 ± 0.05	3.3 ± 0.09	3.4 ± 0.07	3.5 ± 0.09
Metabolic control				
Plasma insulin (mU/l)†	43.3 ± 3.4	188 ± 16.7	47.3 ± 3.5	199.8 ± 15.9
Glycemia (mg/dl)‡	93.0 ± 3.4	85.2 ± 3.5	328 ± 11.3	314 ± 10.6
Lactate (mmol/l)§	1.5 ± 0.2	1.9 ± 0.1	4.1 ± 0.3	6.2 ± 0.3
Blood pH	7.413 ± 0.01	7.430 ± 0.01	7.385 ± 0.01	7.350 ± 0.01‖

Data are means ± SE over 7 days. *Significant difference between groups ($P < 0.03$). †$P > 0.5$ NI/NG vs. NI/HG and HI/NG vs. HI/HG, $P < 0.0001$ normal insulin vs. high insulin groups. ‡$P > 0.3$ NI/NG vs. HI/NG and NI/HG vs. HI/HG, $P < 0.0001$ normoglycemic vs. hyperglycemic groups. §$P > 0.7$ NI/NG vs. HI/NG, $P < 0.003$ normoglycemic vs. hyperglycemic groups, $P < 0.003$ HI/HG vs. all other groups. ‖$P < 0.04$ HI/HG vs. HI/NG.

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different glucose needs to achieve the two levels of blood glucose in the four groups and to assure equal fat and amino acid intake (each at 1.2 g · kg^{-1} · day^{-1}), we composed two different parenteral nutrition solutions. For animals requiring <2 g glucose/h to achieve target blood glucose, we used parenteral nutrition containing 35% Clinomel N7 (Baxter; Clinitec, Maurepas Cedex, France) and 65% Hartman solution. For animals requiring >2 g glucose/h, we used parenteral nutrition containing 35% Clinomel, 35% Hartmann solution, and 30% glucose 50%. All intravenous infusions were prepared daily under sterile conditions and weighed before and after administration for exact quantification of intake.

Days 2–7. Parenteral nutrition was changed daily at 1000 ± 1 h, at which time the amount of parenteral nutrition and supplementary glucose given was recorded.

Day 7. At 1000 ± 1 h, animals were anesthetized using half of the above mentioned dose of anesthetics intravenously, and the animals were weighed. After tracheostomy, animals were normoventilated (small animal ventilator KTR4; Hugo Sachs, March-Hugstetten, Germany). Anesthesia was supplemented with 1.5 volume % isoflurane inhalation and 0.15 mg/kg piritramid i.v. Arterial blood pressure and central venous pressure (CVP) were monitored from the indwelling lines. Maintenance of normovolemia was achieved by infusing Hartman solution at 6 ml/h.

Hemodynamics

The carotid artery catheter was advanced into the left ventricle, guided by the pressure curve. This was technically impossible in three animals, so the number of animals for which these measurements were done was seven NI/NG, seven HI/NG, eight NI/HG, and eight HI/HG. Thereafter, via a partial median sternotomy, an electromagnetic flow probe (Transonic System, Ithaca, NY) was placed around the ascending aorta, and a fluid-filled catheter (24 GA; BD Insyte-W, BD Vialon; BD Medical Systems, Sandy, UT) was inserted into the right ventricle. Hemodynamics were continuously digitalized at 1,000 Hz and visualized on a computer by a custom-made system (Hemodyn W; Hugo Sachs Elektronik). After allowing 10 min of stabilization, left and right ventricular pressures, CVP, first derivative of left and right ventricular pressure over time (dp/dt), arterial pressure (mean arterial pressure [MAP]), and cardiac output (cardiac index) were measured. Systemic vascular resistance index (SVRI) was calculated as (MAP − CVP)/cardiac index. Before recording, optimal preload was assured judging CVP, systolic pressure variation, left ventricular end-diastolic pressure, and changes of cardiac output, dp/dt, and MAP in response to boluses of 3 ml Hartmann solution. Data were analyzed evaluating 10 consecutive successful beats.

Endothelial function

For evaluating endothelial function, we decided to examine nitric oxide (NO)-mediated endothelium-dependent vasodilatation in isolated perfused aortic rings as described previously (17). This method is most sensitive to detect endothelial dysfunction caused by alterations in the microenvironment (23).

After the hemodynamic measurements, thoracic aorta was carefully separated from connective tissue, and 5-mm segments were sliced. Suspended by stainless steel clips, they were immediately placed into an organ bath filled with modified Krebs solution (118.3 mmol/l NaCl, 4.7 mmol/l KCl, 2.5 mmol/l $CaCl_2$, 1.2 mmol/l $MgSO_4$, 25.0 mmol/l $NaHCO_3$, 0.026 mmol/l $Na_2CaEDTA$, 1.2 mmol/l KH_2PO_4, 100 mg/dl glucose, 40 mU/l insulin, and 10^{-5} mol/l indometacin, bubbled with 95%O_2/5%CO_2, pH 7.4 ± 0.05, 37.4°C). To exclude confounding effects exerted by the glucose and insulin content in the organ bath, the tests were run in parallel in a modified organ bath with glucose and insulin concentrations reflecting the animals' randomization (high insulin solution, 100 mU/l insulin; high glucose solution, 300 mg/dl glucose). We did not control for osmolality in the different organ baths because it is known that altered osmolality does not affect endothelium-dependent and -independent vasorelaxation (24). Connected to a force transducer (F30; Hugo Sachs Elektronik), isometric tension was recorded continuously. After 1 h of stabilization, pretension was adjusted to 2 g. Subsequently, norephedrine was added to the organ bath to a concentration of 10^{-6} mol/l. When reaching steady contraction state, changes in contraction were recorded in response to drugs added to the organ bath: *1*) acetylcholine (ACh) in stepwise cumulative doses of 10 nmol/l to 10 μmol/l. Because ACh induces NO release from NO synthetase activity, it marks endothelium-dependent vasorelaxation and thus endothelial function. The next dose was only administered after retaining stable conditions. *2*) ACh as described above in an organ bath supplemented with L-nitro-arginine-methyl-ester (L-NAME; 10^{-4} mol/l), a nonspecific NO synthetase blocker to exclude endothelium-independent actions of ACh, and *3*) sodium nitroprusside (nipruss, sodium-nitroferricyanide(III)dihydrate) to cumulative doses of 10 nmol/l to 10 μmol/l to assess NO-mediated endothelium-independent vasorelaxation. After each step, the organ bath was flushed three times, and pretension was readjusted; the system was allowed to equilibrate for 30 min (all chemicals from Sigma-Aldrich). Relaxation of the rings was expressed as percentage of developed tension induced by norephedrine. Measurements in sick animals were compared with healthy controls.

Assays

Daily at 0800 ± 1 h and 2000 ± 1 h, arterial blood was sampled and immediately analyzed on a blood gas analyzer (ABL-analyzer; Radiometer, Copenhagen, Denmark) to quantitate pH, hemoglobin, lactate, and glucose. Additional measurements were carried out whenever blood glucose was unstable to allow tight adjustment of glucose infusion. Supplementary, on days −1 (baseline, before injecting alloxan), 0 (randomization), 3, 5, and 7, 4 ml blood was collected and centrifuged for 10 min at 13,000 rpm, and plasma was stored at −80°C until further analysis.

Plasma insulin was analyzed in duplicate within a single assay run by radioimmunoassay using a guinea pig–derived antibody (provided by Prof. R. Bouillon, KU Leuven, Belgium [25]; lower detection limit of 5 mU/l, within assay variation <10%).

By commercial kits, alanine aminotransferase and aspartate aminotransferase were measured according to the guidelines of the International Federation of Clinical Chemistry, creatinine was measured by Jaffé method, and urea was measured by means of a kinetic UV assay with equipment-specific reagents; all were measured in plasma using Modular Roche and specific reagents by Roche (Roche/Hitachi, Bern, Swizerland; A.M.L bvba, Antwerp, Belgium) according to the user's manual.

Chemotaxis, phagocytosis, and oxidative burst of monocytes and granulocytes were each measured on 100 μl fresh whole blood on day 3 and in eight healthy controls. Chemotaxis was evaluated by measuring the ability to bind formyl-Nle-Leu-Phe-Nle-Tyr-Lys fluorescein derivative (fnlpntl; Molecular Probes Europe, Leiden, the Netherlands) as described previously (26). Phagocytosis and oxidative burst were evaluated using, respectively, Phagotest and Phagoburst (Orpegen Pharma, Heidelberg, Germany), according to the manufacturer's protocol. All samples were analyzed by flow cytometry (FACSort; Becton Dickinson, Erembodegem, Belgium), and results were expressed as percentage of fluorescein-positive monocytes and granulocytes. For graphic presentation, data were expressed as percentage of the capacity of healthy controls and data were logarithmically transformed for statistical analysis. Biochemical analyses were conducted blinded to randomization.

Statistical analysis

Results are expressed as means ± SE. Data were analyzed by multifactorial ANOVA, ANOVA for repeated measurement, and Fisher's protected least significant difference post hoc testing and for not normally distributed data Kruskal-Wallis and Mann-Whitney test, when appropriate. For survival analysis Mantel-Cox log-rank test was used. A P value <0.05 was considered significant.

RESULTS

Alloxan-induced insulin deficiency was confirmed in all animals (Fig. 2, day 0). After randomization, plasma insulin was maintained constant at a normal or an elevated (high-physiological [27]) level (Table 1) over the experimental period of 7 days, independently of the two levels of blood glucose (Fig. 2).

Plasma lactate levels rose in both hyperglycemic groups and were most pronounced in HI/HG. Blood pH remained normal in all groups (Table 1). Body weight did not change in any of the groups.

Mortality. Forty-seven animals were needed to reach designated group size and were used for survival analysis. Both normoglycemic groups revealed a similar mortality (11.1%), whereas mortality in both hyperglycemic groups was higher (NI/HG, 35.7%; HI/HG, 46.7%, $P = 0.03$ hyperglycemic vs. normoglycemic groups; Fig. 3). Plasma insulin levels did not contribute to the survival benefit ($P = 0.7$ high insulin vs. normal insulin groups).

Liver and kidney function. Parameters for liver and kidney function in plasma revealed a moderate injury after alloxan. The organ function recovered in both normoglycemic groups but not in both hyperglycemic groups (Fig. 4).

Hemodynamics. Heart rate, arterial pressure, CVP, left and right ventricular end-diastolic pressure, peak left and right ventricular pressure, SVRI (data not shown), and cardiac output (Fig. 5) did not differ among the groups and

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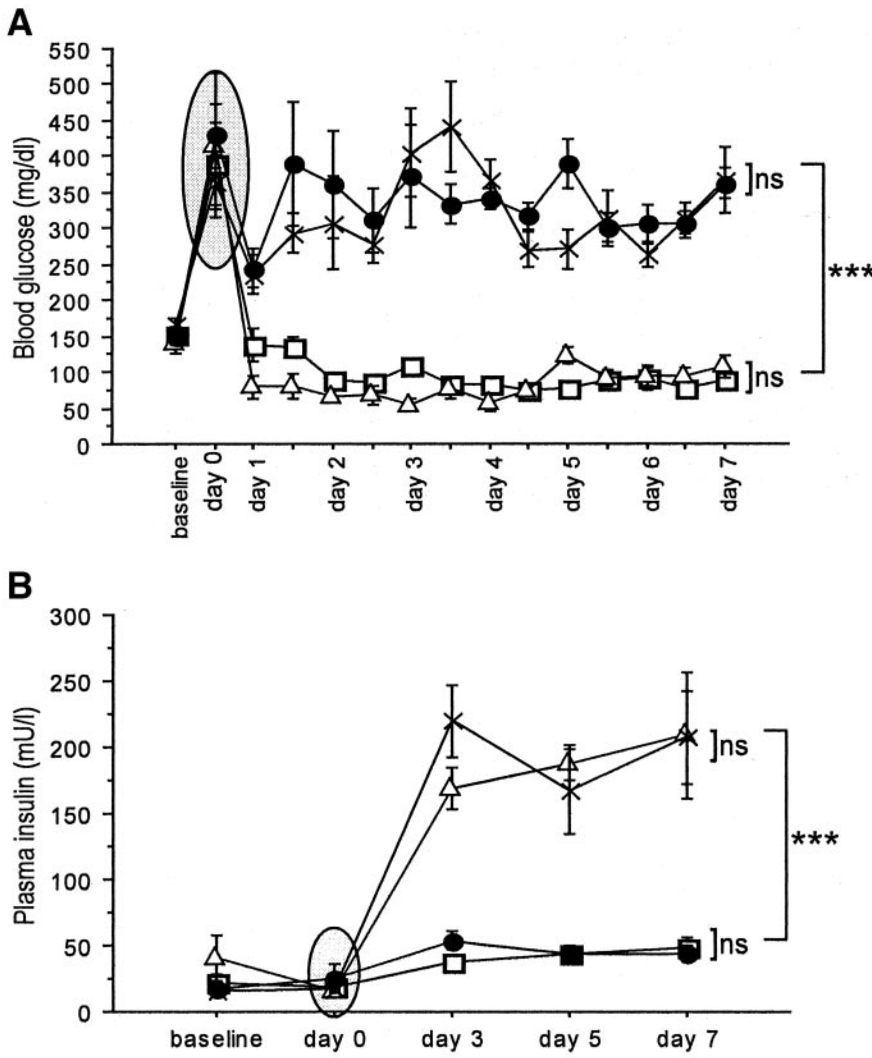
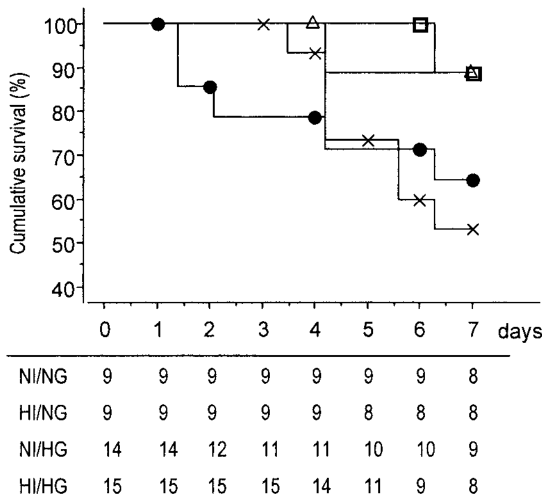


FIG. 2. Evolution of blood glucose (*A*) and plasma insulin (*B*) over time. □, NI/NG (*n* = 8); △, HI/NG (*n* = 8); ●, NI/HG (*n* = 9); ×, HI/HG (*n* = 8). Ovals represent the high blood glucose/low plasma insulin 30 h after alloxan injection. ****P* < 0.0001. ns, no significant difference between NI/HG and HI/HG.

healthy controls. HI/NG animals revealed a higher dp/dt_{max} in left and right ventricle compared with all other groups and with controls ($P < 0.05$) (Fig. 5). This reflected increased myocardial contractility with insulin only when normoglycemia was maintained concomitantly. There was a strong trend for higher dp/dt_{min}, reflecting diastolic dysfunction, in both hyperglycemic groups compared with NI/NG animals (NI/NG vs. NI/HG, $P = 0.05$, NI/NG vs. HI/HG, $P = 0.07$; all normoglycemic vs. all hyperglycemic animals, $P = 0.08$).



	0	1	2	3	4	5	6	7
NI/NG	9	9	9	9	9	9	9	8
HI/NG	9	9	9	9	9	8	8	8
NI/HG	14	14	12	11	11	10	10	9
HI/HG	15	15	15	15	14	11	9	8

FIG. 3. Cumulative survival. Numbers of animals at risk are depicted below the graph. □, NI/NG; △, HI/NG; ●, NI/HG; ×, HI/HG; *n* = 47 total animals used. *P* < 0.03 for both normoglycemic vs. both hyperglycemic groups; no significant difference between NI/HG and HI/HG.

Endothelial function. The endothelium-dependent relaxation of isolated aortic rings was impaired in all critically ill animals compared with healthy controls ($P < 0.05$). In both normoglycemic groups, relaxation to increasing doses of ACh was better than in the hyperglycemic groups ($P = 0.0003$), independent of insulin ($P = 0.6$) (Fig. 6). The ACh-induced relaxation was completely abolished by adding L-NAME. This indicated that maintaining normoglycemia largely prevented endothelial dysfunction observed during hyperglycemic critical illness. The endothelium-independent NO-mediated vasorelaxation was not impaired in any of the groups, and thus this mechanism appeared unaffected by critical illness or by the interventions. Changing the glucose and insulin concentration in the medium did not affect these results (data not shown), excluding a confounding effect of these compounds in the studied experimental setting.

Leukocyte function. Measured parameters of leukocyte function were similar to healthy controls in both normoglycemic groups and in HI/HG animals. However, in the NI/HG group, phagocytosis capacity of monocytes ($P =$

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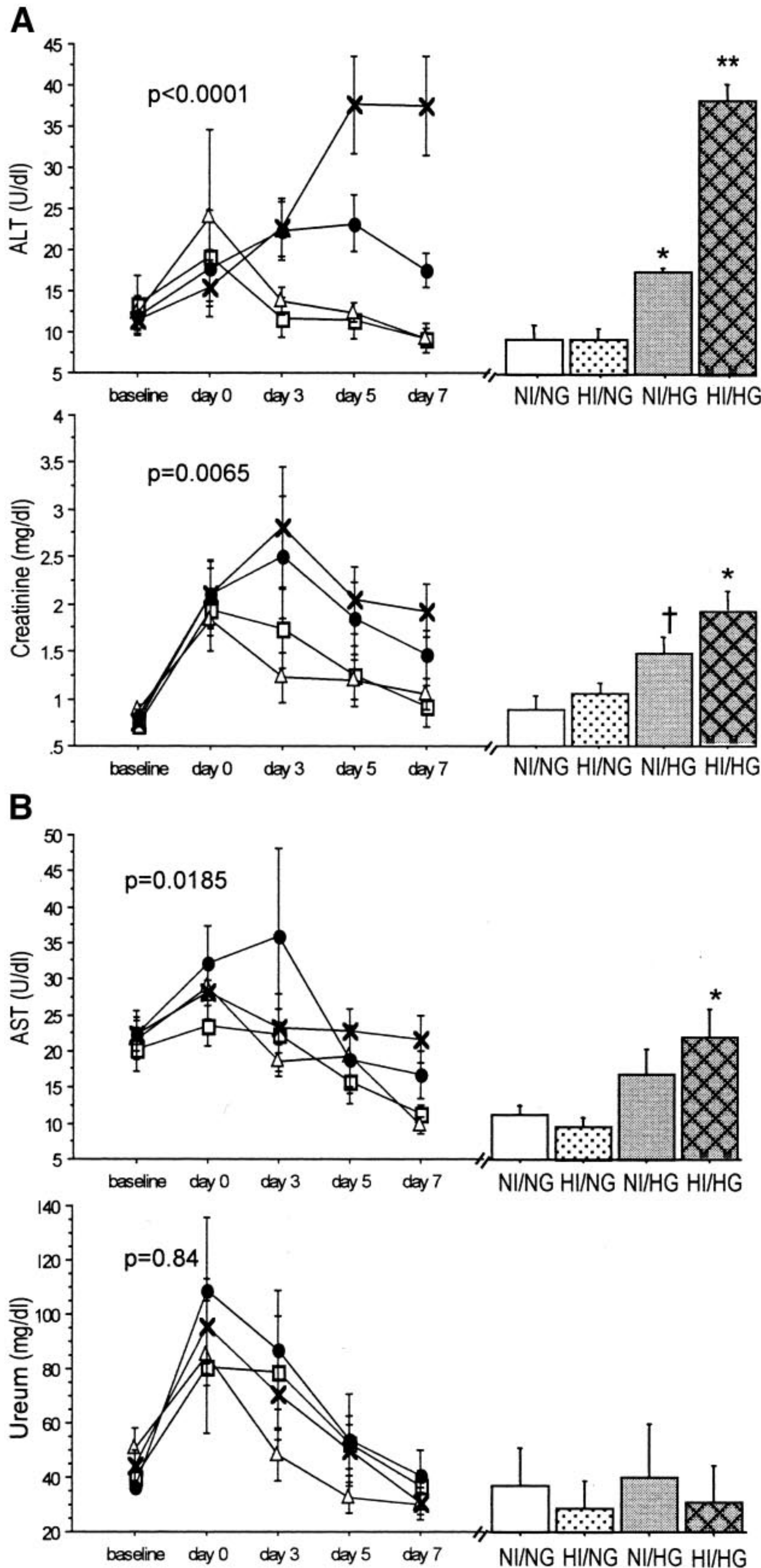

FIG. 4. Plasma markers for organ function. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, and urea in plasma over time (*A*) and on day 7 (*B*). □, NI/NG ($n = 8$); △, HI/NG ($n = 8$); ●, NI/HG ($n = 9$); ×, HI/HG ($n = 8$). ** $P < 0.0008$ vs. NI/NG and HI/NG; * $P < 0.05$ vs. NI/NG and HI/NG; † $P = 0.06$ vs. NI/NG.

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0.01), generation of oxidative bursts in monocytes ($P = 0.03$), and phagocytosis capacity of granulocytes ($P = 0.008$) were reduced by almost 50% compared with healthy controls (Fig. 7).

Maintaining these functions appeared important for survival as already on day 3, the animals who did not survive until day 7 revealed worse phagocytosis capacity (monocytes, survivors 92.9 ± 7.9%, nonsurvivors 44.0 ± 15.8%, $P = 0.004$; granulocytes, survivors 90.9 ± 6.2%, nonsurvivors 52.5 ± 9.3%, $P = 0.05$) and generation of

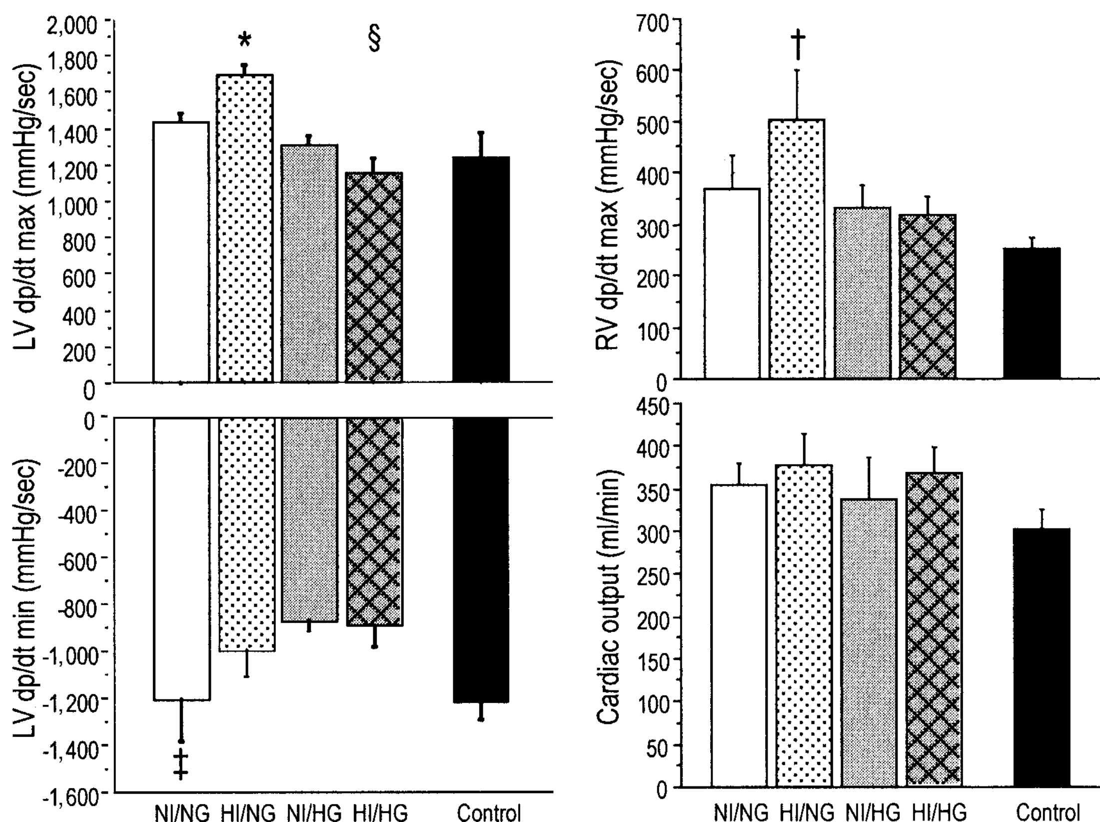

FIG. 5. Hemodynamics. LV, left ventricle; RV, right ventricle (n = 7 for NI/NG and HI/NG, n = 8 for NI/HG and HI/HG). *P < 0.05 vs. all other groups; §P < 0.05 vs. both normoglycemic groups; †P < 0.05 vs. both hyperglycemic groups; ‡P = 0.05 vs. NI/HG, P = 0.07 vs. HI/HG.

oxidative bursts (monocytes, survivors 94.7 ± 8.6%, nonsurvivors 42.6 ± 9.0%, P = 0.02; granulocytes, survivors 96.0 ± 6.5%, nonsurvivors 58.8 ± 13.6%, P = 0.02) compared with survivors regardless of randomization.

Chemotaxis of granulocytes was lower in the NI/HG group compared with healthy controls (P = 0.03), but because chemotaxis on day 3 was not different between survivors and nonsurvivors (monocytes, survivors 123.4 ± 7.6%, nonsurvivors 127.7 ± 15.3%, P = 0.7; granulocytes, survivors 88.3 ± 4.7%, nonsurvivors 82.9 ± 10.2%, P = 0.7), this did not appear to affect survival.

Neither of the interventions resulted in a higher level of these leukocyte functions compared with healthy controls, excluding overstimulation.

The number of animals was too small to perform statistical testing to assess whether the observed effects on leukocyte function are independently associated with survival.

DISCUSSION

In this animal model of critical illness, preventing hyperglycemia but not glycemia-independent actions of insulin explained the improved survival with intensive insulin therapy. Also, prevention of liver, kidney, and endothelial dysfunction was attributable to glycemic control, whereas a stimulatory effect on myocardial function required both glycemia-independent actions of insulin and those of glycemic control. Innate immune function appeared to be negatively affected by hyperglycemia during critical illness, as indicated by impaired phagocytosis and oxidative burst generation in monocytes and granulocytes, which could be rescued in part by glycemia-independent actions of insulin.

Our results for the first time provide clear evidence for the notion that maintaining strict blood glucose control is the crucial factor to reduce mortality of critical illness with intensive insulin therapy (6,14). In line with recent findings in patients, we observed that also for preventing kidney, liver, and endothelial dysfunction, insulin therapy required strict glycemic control in this animal model (28). The protective effect on the endothelium has been suggested to play a key role in the survival benefit of intensive insulin therapy (28). Endothelial protection is likely to improve microcirculation in vital organs, whereby their function may be protected and hence risk of death reduced. Avoiding direct toxicity of glucose to endothelial cells, but perhaps also to other cells that take up glucose independently of insulin, such as neurons (11) and hepatocytes (9), may contribute to the survival benefit achieved with tight blood glucose control.

The cardiovascular system appears to be crucially affected by intensive insulin therapy (5). In the current study, we demonstrated that high levels of insulin ameliorated myocardial contractility but only when normoglycemia was maintained concomitantly. This observation is of relevance to the controversy surrounding GIK therapy (15). GIK traditionally focuses on insulin as the key component of a metabolic cocktail, which is thought to beneficially modulate myocardial metabolism. Insulin is thought to have direct inotropic properties by economizing and ameliorating myocardial performance (22,29). However, in our model of prolonged critical illness, such an effect of insulin on the heart was abolished by concomitant hyperglycemia. This observation may explain the different outcomes of clinical studies investigating GIK. In the DIGAMI (Diabetes Mellitus Insulin-Glucose Infusion in

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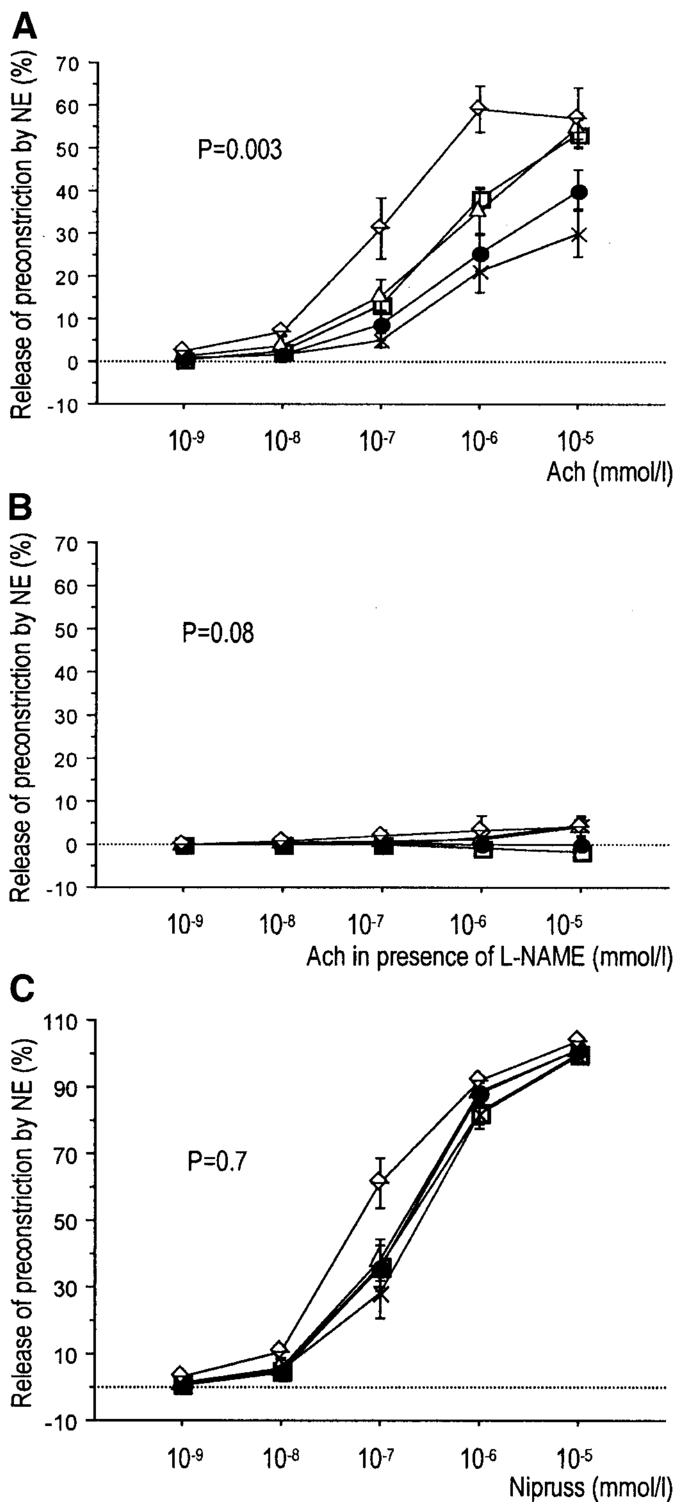

FIG. 6. Endothelial function. Relaxation of norephedrine (NE)-induced contraction of isolated aortic rings to cumulative doses of ACh (*A*), relaxation to ACh in presence of L-NAME (*B*), and sodium nitropusside (nipruss, *C*); ◇, control (n = 4); □, NI/NG (n = 8); △, HI/NG (n = 8); ●, NI/HG (n = 9); ×, HI/HG (n = 8). *A*: NI/NG vs. HI/NG P = 0.8, NI/NG vs. NI/HG P = 0.04, NI/NG vs. HI/HG P = 0.002, HI/NG vs. NI/HG P = 0.02, HI/NG vs. HI/HG P = 0.001, NI/HG vs. HI/HG P = 0.2.

Acute Myocardial Infarction)-1 study, GIK promoted survival of patients with diabetes and acute myocardial infarction (30). In this study, patients in the GIK arm had significantly lower blood glucose levels than those receiving placebo. The consecutive DIGAMI-2 trial was designed to distinguish between effects of glycemic control and glycemia-independent actions of insulin. However, this study did not succeed in separating the blood glucose levels between the groups and hence did not reveal a difference in outcome (31). Likewise, the most recent CREATE-ECLA (Clinical Trial of Reviparin and Metabolic Modulation in Acute Myocardial Infarction Treatment and Evaluation–Estudios Clinicos Latino America), conducted in >20,000 patients, not only did not bring about a lowering of blood glucose levels but even increased blood glucose levels in the GIK-treated patients and also did not show a beneficial effect on outcome (32). Glycemic control thus appears to be a prerequisite for cardioprotection with insulin. A number of clinical studies have shown that in myocardial dysfunction, such as after acute myocardial infarction or cardiac surgery, hyperglycemia independently predicts adverse outcome (4,33), whereas controlling blood glucose with insulin ameliorated myocardial performance (34) and reduced morbidity and mortality (5,30). Taking together these results and our current findings in the animal model, it appears that insulin can only exert beneficial effects on the myocardium when concomitant normoglycemia is guaranteed.

Besides these effects on the myocardium, alterations in glucose homeostasis may affect microcirculation, possibly contributing to ischemic damage of vital organs (35). The regulation of microcirculation, not only in the heart but also in other vital organs, is largely dependent on a functional vascular endothelium (36). The endothelial cells release vasoactive substances, such as NO, under the control of a number of regulating factors (37). Insulin resistance and hyperglycemia have been shown to be such regulators (38), because in acute hyperglycemia (17) and in chronic models of type 2 diabetes (24), endothelial dysfunction has been documented. Especially when insulin resistance, rather than deficiency, is the cause of hyperglycemia, endothelial dysfunction is present (38). In critical illness, endothelial dysfunction, evidenced by increased levels of adhesion molecules (28,39), insulin resistance, and excessive NO release are all present, and intensive insulin therapy has been shown to prevent excessive NO release and to protect the endothelium (28). Most serum markers of endothelial activation, however, only reflect a small part of endothelial function and may also be affected by the disease itself, antibody cross-reactivity, and altered clearance of serum proteins. Hence, assessing the endothelial capability to induce vasorelaxation in isolated vascular rings is the most reliable method to quantify integrated endothelial function (23). With this approach, we showed that it is specifically hyperglycemia that aggravates endothelial dysfunction in critical illness, and conversely, maintenance of normoglycemia during the course of illness can prevent this. Glycemia-independent actions of insulin did not affect this phenomenon.

Similar vulnerability to glucose toxicity may apply to other cellular systems taking up glucose independent of insulin, such as the immune cells (10). Our current data indicate that persistent hyperglycemia may impair leukocyte function, most specifically the capacity of monocytes and neutrophils to phagocytose and to generate oxidative bursts, functions that appear crucial for survival, but also that these can be rescued to a certain extent by glycemic-independent actions of insulin.

Cellular "immunoparalysis" (40) has shown to contribute to organ failure and mortality of patients with sepsis

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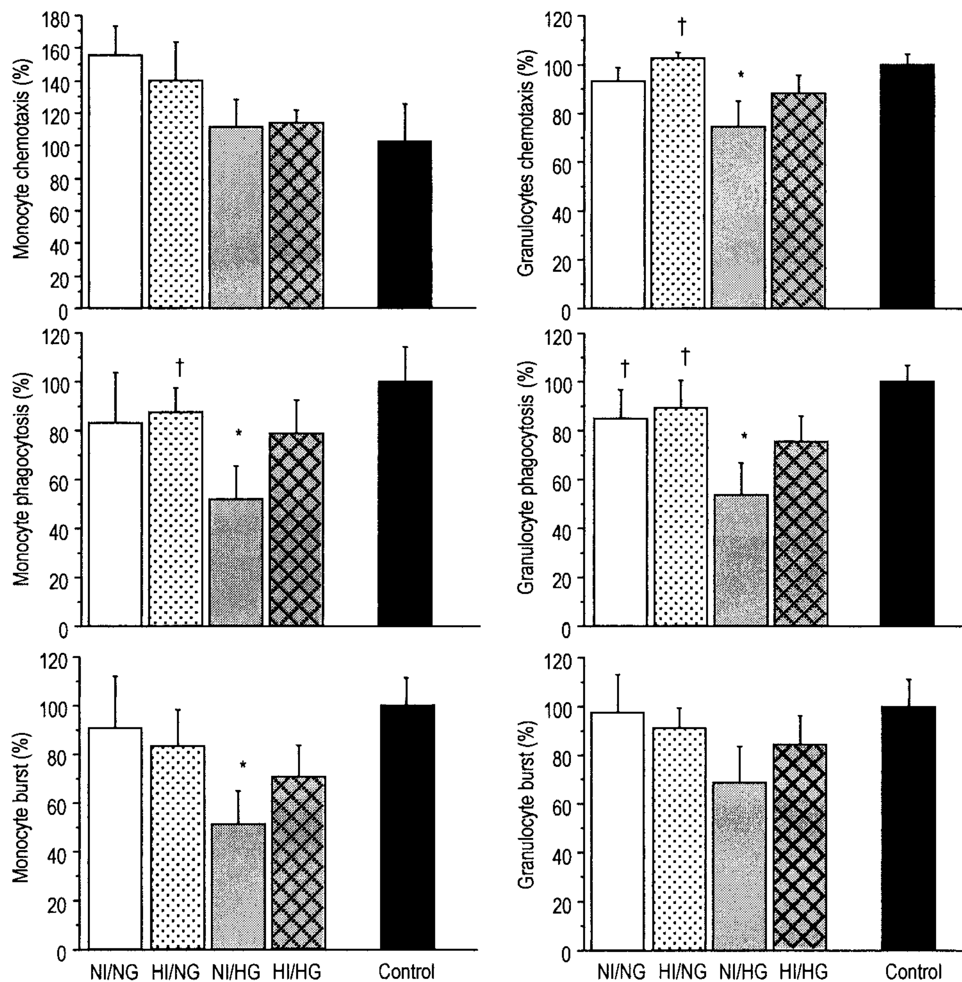

FIG. 7. Leukocyte function on day 3. Data are given as percentage of the activity of healthy controls. *P < 0.05 vs. healthy controls, †P < 0.05 vs. NI/HG.

(41). There is ample evidence from studies in diabetes that prolonged hyperglycemia is associated with leukocyte dysfunction (42–45). In contrast, acute hyperglycemia in nondiabetic animals has been shown to improve leukocyte function (46). However, in this acute in vivo experiment in healthy animals, to achieve hyperglycemia, glucose was infused, which, unlike in our study, evoked a substantial rise of plasma insulin. In our study, high plasma insulin counteracted the immunosuppressive effects evoked by hyperglycemia. This is in line with immune-stimulating effects of insulin observed in healthy volunteers (47) and in patients during elective cardiac surgery (48,49). With circulating levels in the high physiological range, as achieved in our study, insulin infusion did not result in excessive stimulation of leukocytes. This is an important safety aspect because an overwhelming inflammatory response would also be deleterious.

Some limitations of our study need to be highlighted. The study design resulted in different amounts of caloric intake among the four groups (Table 1). This is inevitable if one wants to achieve different levels of blood glucose for a certain level of insulin in an in vivo setting. However, because the caloric intake in all four groups remained within the ranges considered normal for rabbits (27) and because bodyweight did not differ among the study groups, we can exclude the extremes of severe overfeeding and starvation. Additionally, lactate levels were elevated in both hyperglycemic groups. Although acidosis did not occur, we thus cannot rule out potential effects of hyperlactatemia on the observed organ function parameters. The quantification of myocardial contractility by dp/dt suffers from dependency on heart rate, preload, and afterload. However, because normovolemia was achieved before measuring all of these parameters at once, equal preload, heart rate, and markers of afterload (arterial pressure and SVRI) were assured in all groups, whereby erroneous interpretation of the dp/dt results was avoided. Furthermore, because substantial mortality occurred in both hyperglycemic groups, the animals with the most pronounced organ dysfunction in those groups were not available for measurements on day 7. This may have caused bias. However, the resulting bias would weaken rather than accentuate the observed differences and thus would be against our conclusions. Therefore, the risk of overstating our findings is minimal. Finally, our animal model of burn injury–induced critical illness may mirror only part of the complex entity of human critical illness, and thus extrapolating to the human situation or to other illnesses should be done with great caution.

In conclusion, in our experimental model, the benefits of intensive insulin therapy primarily required maintenance of normoglycemia, and glycemia-independent actions of insulin exerted only minor, organ-specific impact. For the reduction of mortality and for the prevention of liver, kidney, and endothelial dysfunction, maintenance of normal blood glucose levels was crucial. Glycemia-independent actions of insulin evoked increased myocardial contractility only when normoglycemia was maintained

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concomitantly, which emphasizes the importance of glycemic control. Hyperglycemia may negatively affect cellular immune function, but this was rescued in part by glycemia-independent actions of insulin. These observations may explain the contradictory results of previous clinical trials. Further studies are needed to dissect out the exact molecular pathways underlying the different effect of blood glucose control and eventual glycemia-independent actions of insulin on the different organ systems.

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