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Hyperinsulinemia predicts survival in a hyperglycemic mouse model of critical illness*

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Abstract

Objectives—The mechanisms by which correcting hyperglycemia with exogenous insulin improves mortality and morbidity in critically ill patients remain unclear. We designed this study to test the hypothesis that relative endogenous insulin deficiency is associated with adverse outcomes in critical illness related to hyperglycemia.

Design—Prospective controlled animal study.

Setting—University research laboratory.

Subjects—Male C57BL/6J mice, 8–12 wks old.

Interventions—Spontaneously breathing mice were instrumented with chronic indwelling arterial and venous catheters. After a postoperative recovery period, endotoxemia was initiated with intra-arterial lipopolysaccharide (1 mg/kg) in the presence of dextrose infusion (100 μ L/hr). Insulin secretion was blocked with diazoxide (2.5–30 mg/kg/day). Mice were monitored continuously for 48 hrs with blood sampled serially for blood glucose and plasma insulin determinations.

Measurements and Main Results—In both saline- and glucose-infused mice, lipopolysaccharide administration induced transient hemodynamic instability without significant impact on mortality. In the saline-infused group, lipopolysaccharide administration caused a transient reduction in blood glucose and in circulating insulin. However, in glucose-infused mice, lipopolysaccharide induced a large and unexpected increase in circulating insulin without significant alteration in blood glucose. Blockade of insulin secretion in response to lipopolysaccharide in the presence of exogenous glucose precipitated marked hyperglycemia and resulted in >90% mortality. In a subanalysis of animals matched for the degree of hyperglycemia, nonsurvivors had markedly lower insulin levels compared with survivors (3.5 ± 0.8 ng/dL vs. 9.3 ± 1.4 ng/dL; $p < .004$).

*See also p. 2665.

Conclusions—Endogenous insulin deficiency in the face of hyperglycemia is associated with mortality in a mouse model of lipopolysaccharide-induced critical illness.

Keywords

metabolism; sepsis; glucose control; hyperglycemia; critical illness; mortality

Hyperglycemia has received much recent attention as a confounding factor that may exacerbate morbidity and mortality in critically ill patients. Treating hyperglycemia with exogenous insulin infusion in a surgical intensive care unit (ICU) setting has been shown to improve mortality (1, 2). Similarly, intensive glycemic control with exogenous insulin infusion improved inhospital mortality in patients with prolonged length of stay in a single-center medical ICU population (3). However, other studies including the more recent multicentered VISEP trial and a subsequent meta-analysis failed to demonstrate a clinical benefit from intensive glucose control in patients with sepsis or other critical illness (4–6). Discrepancies between these studies may arise from differences in patient population, nutritional support, general care protocols, or early study termination. Furthermore, none of these trials have reported circulating insulin levels either before or during randomization, making it difficult to interpret any relative benefits of increasing insulin or lowering glucose.

Potentially, either reversing hyperglycemia or increasing insulin could improve clinical outcomes in critically ill patients. Hyperglycemia can be cytotoxic due to induction of apoptosis and inflammation, and elaboration of reactive oxygen species (7–9), whereas insulin has cytoprotective and anti-inflammatory actions independent of its glucose-lowering effect (10–13). However, to date, neither clinical studies nor animal models have systematically manipulated circulating insulin to evaluate its glucose-dependent or glucose-independent benefits in critical illness.

Therefore, the purpose of the current study was to examine the impact of altering glucose and insulin on mortality, using a chronically instrumented, unhandled, long-term model of endotoxemia in which hyperglycemia was induced with exogenous glucose infusion and insulin secretion was controlled pharmacologically. Previous animal studies in this area have utilized pancreatic destruction to induce a Type 1 diabetic state before onset of critical illness (14–16). However, as nondiabetics experienced the greatest mortality benefit from exogenous insulin administration in the clinical ICU setting (2, 3), we conducted our studies in animals with intact and functioning pancreatic β cells. We hypothesized that blocking endogenous insulin secretion in the presence of hyperglycemia would increase mortality from endotoxemia in our animal model. Portions of the results of this study have been previously published in abstract form (17, 18).

METHODS

Animal protocols were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh. Male C57BL/6J mice (20–25 g, 8–10 wks old) underwent surgical insertion of indwelling femoral arterial and venous catheters (19). Blood pressure and heart rate were monitored continuously, recorded, and stored for off-line analysis, using Windaq Acquisition software (v2.34, Akron, OH).

Experimental Protocol

After 3 days recovery from surgery, control mice received intravenous (IV) infusion of either sterile saline (100 μ L/hr, $n = 10$) or 50% dextrose (D50) (100 μ L/hr, $n = 10$) (Fig. 1). Blood (80 μ L) was withdrawn through the arterial catheter in unhandled, unanesthetized animals at times = -24 hrs, 0 hr, 8 hrs, 24 hrs, and 48 hrs for blood glucose measurement (Ascencia Elite XL, Bayer, Tarrytown, NY) and plasma insulin radioimmunoassay (SRI-13K, Millipore, St. Charles, MO) (Fig. 1). Blood was centrifuged at 10,000 g for 5 mins, and plasma was extracted and stored at -80°C . The cell pellet was resuspended in 20- μ L sterile heparin (100 IU/mL saline) and reinfused into the animal.

Endotoxemia was induced in spontaneously breathing mice by intra-arterial infusion of lipopolysaccharide (LPS) (1 mg/kg, *Escherichia coli* 055:B5, Sigma L4005, St. Louis, MO) over 30 mins at time 0, 24 hrs after the infusion of saline ($n = 12$) or D50 ($n = 8$) was initiated (Fig. 1). This dose of LPS is sufficiently lower than the reported LD_{50} (10–25 mg/kg IV) (information available at www.listlabs.com) to allow for the assessment of the effects of insulin and glucose manipulation on mortality. Animal physiology and behavior were monitored, and blood was collected serially for glucose and insulin measurements (Fig. 1).

To induce hyperglycemia, additional mice were treated with varying doses of diazoxide (Dzx) (2.5, 5, 10, 20, and 30 mg/kg/day intra-arterially in divided doses; $n = 8, 8, 8, 9, 10$, respectively) at the same time receiving chronic dextrose infusion and injury with LPS. Dzx, an inhibitor of pancreatic insulin secretion, was initiated 24 hrs before LPS infusion, concomitant with saline or dextrose infusion (Fig. 1) (20–22). As we observed the development of a threshold for insulin suppression and alteration in glucose metabolism at 20 mg/kg/day of Dzx, mice receiving <20 mg/kg/day were grouped together in a low-dose Dzx group, and mice receiving ≥ 20 mg/kg/day were grouped together in a high-dose Dzx group for the analysis of the effect of endogenous insulin suppression on mortality ($n = 19$ each after exclusion of five animals with hyperglycemia at $t = 0$). Control mice were given Dzx (20 mg/kg/day; $n = 17$) with D50 in the absence of LPS. Animal physiology and behavior were monitored, and blood was collected serially for glucose and insulin measurements (Fig. 1). Mice were excluded from the analysis if blood glucose exceeded 180 mg/dL at time $t = 0$.

Surviving animals were killed upon termination of the protocol (time $t = 48$ hrs) by pentobarbital overdose and exsanguination, according to American Veterinary Medical Association guidelines for euthanasia.

End Points

The primary outcome measured was animal mortality over the 48-hr period after LPS infusion. The study was designed to detect a 20% mortality in the group in which endogenous insulin was not suppressed vs. 65% mortality in the group in which insulin was suppressed. Such an analysis requires a minimum of 18 animals in each group (23). Secondary outcomes included time-dependent blood pressure, heart rate, blood glucose, and

plasma insulin responses. The relative impacts of hyperglycemia and hypoinsulinemia on survival after LPS infusion were evaluated *post hoc*.

Statistical Analysis

All data are presented as mean $\pm$ standard error. Kaplan Meier survival analysis was performed for each group. Kruskal-Wallis tests, one-way analysis of variance (ANOVA), or two-way ANOVA were applied to compare multiple groups within and between experiments where appropriate. *Post hoc* analyses, using Wilcoxon rank sum tests or Scheffé's tests, were run when the Kruskal-Wallis or ANOVA indicated significant differences ($p < .05$) between groups, respectively. The Bonferroni method was employed to adjust for multiple pairwise *post hoc* comparisons. Paired Student's *t* tests were applied to compare 8-hr physiologic variables with time $t = 0$ within groups.

RESULTS

A total of 100 animals were catheterized for the study. Seven mice were excluded for hyperglycemia at time $t = 0$ (glucose > 180 mg/dL, two each from the Dz x + D50 control and the diazoxide 2.5 mg/kg/day and 10 mg/kg/day groups and one from the Dz x 5 mg/kg/day group) based on the lower limit of the control groups in the large randomized trials in the ICU (2–4). Thus, 93 mice were included for primary analysis of 48-hr mortality. No statistical difference existed between groups in baseline weight, blood glucose, plasma insulin, or blood pressure (Table 1).

Dextrose Infusion Causes Mild Hyperglycemia and Hyperinsulinemia in Control Mice

Animals receiving saline infusion maintained normal caloric intake (Table 2) and remained euglycemic (109.2 ± 2.4 mg/dL), euinsulinemic (1.01 ± 0.6 ng/mL), and normotensive (103.2 ± 1.1 mm Hg) throughout the 48-hr experiment (Fig. 2A, ○). Dextrose infusion suppressed caloric intake by an amount similar to the total caloric content of the daily D50 infusion (Table 2). D50-infused mice exhibited a mild and sustained hyperglycemia (166.2 ± 18.7 mg/dL) (Fig. 2A, □) and an associated increase in plasma insulin (2.34 ± 0.59 ng/mL) (Fig. 2A, □). D50-infused mice remained normotensive throughout the experiment (99.2 ± 0.9 mm Hg) (Fig. 2A, □). In the absence of LPS administration, a continuous 3-day infusion of saline resulted in zero mortality whereas 20% of the D50-infused mice died (Fig. 2A).

Dzx Infusion Does Not Induce Mortality

A third control group of mice received dextrose and Dz x (20 mg/kg/day) without LPS (Fig. 2A, △). Blood glucose (196.5 ± 25.4 mg/dL) and plasma insulin (1.84 ± 0.14 ng/mL) differed from saline controls (Fig. 2A, ○, $p = .01$ and $p = .0001$, respectively, by two-way ANOVA). Blood pressure (98.9 ± 1.0 mm Hg) and mortality (26.7%) in the Dz x control mice (△) were similar to saline controls (○) and D50-infused mice in the absence of Dz x or LPS (Fig. 2A, □, $p = \text{NS}$). These data demonstrate that high-dose Dz x infusion does not reduce blood pressure or cause mortality in dextrose-infused mice.

LPS Induces Transient Hypoglycemia in Saline-Infused Mice

Low-dose LPS infusion (1 mg/kg) caused all mice to be anorectic for at least 24 hrs, with decreased caloric intake immediately post injection (Table 2). Saline-infused animals receiving LPS (Fig. 2B, ●) displayed similar circulating insulin at baseline compared with saline controls (Fig. 2A, ○). Post administration of LPS, saline-infused mice (Fig. 2B, ●) exhibited a transient period of hypoglycemia (nadir 56.1 ± 4.5 mg/dL at 8 hrs, $p < .0001$ compared with $t = 0$), reduced plasma insulin (0.97 ± 0.1 ng/dL at 8 hrs, $p = .01$ compared with $t = 0$), and hypotension (nadir 90.4 ± 1.0 mm Hg at 24 hrs). LPS infusion resulted in 16.7% mortality in saline-infused mice (Fig. 2B, ●). Animals that survived the LPS insult normalized blood glucose, plasma insulin, and blood pressure by the end of the experiment (48 hrs) (Fig. 2B, ●). These data demonstrate that intravascular LPS administration in mice results in an expected hypotensive response with mild transient hypoglycemia and a corresponding reduction in insulin production.

Exogenous Dextrose Infusion Prevents Hypoglycemia Induced by Intravenous LPS

Dextrose-infused mice exposed to LPS (Fig. 2B, ■) displayed similar blood glucose and plasma insulin concentrations at $t = 0$ compared with D50-infusion controls (Fig. 2A, □, $t = 0$). In contrast to saline infusion, dextrose infusion was associated with mildly elevated blood glucose (150.5 ± 23.8 mg/dL) post LPS (Fig. 2B, ■), which was similar to the D50 controls not receiving LPS (Fig. 2A, □, $p = \text{NS}$). Interestingly, LPS in the presence of D50 triggered an immediate and marked hyperinsulinemic response that peaked 8 hrs after LPS (5.17 ± 1.19 ng/mL, $p = .01$ compared with $t = 0$) and remained elevated for >24 hrs (Fig. 2B, ■). Dextrose and LPS-treated mice exhibited transient hypotension (nadir 86.3 ± 1.8 mm Hg) and zero mortality (Fig. 2B, ■). Again, mice normalized blood glucose, plasma insulin, and blood pressure by the end of the experiment (48 hrs) (Fig. 2B, ■).

Insufficient Insulin Production in Response to Hyperglycemia Is Associated With Increased Mortality in Endotoxemic Mice

In mice treated with varying doses of Dz in the presence of D50 infusion and LPS, blood glucose and plasma insulin concentrations at time $t = 0$ were similar to D50-infused control mice and to D50-infused mice exposed to Dz absent LPS (Fig. 2C, ▲ and ▼ compared with Fig. 2A, □ and △). Although mortality rates varied significantly across the dose range of Dz ($p < .005$ between 2.5 and 30 mg/kg/day), we only observed a nonsignificant trend for insulin suppression between the highest and lowest doses ($p = .014$ before Bonferroni correction for ten pairwise comparisons) (Table 3). However, two distinct patterns of insulin, glucose, and blood pressure regulation in response to LPS administration were evident in mice receiving Dz and glucose infusions (Table 3). Low-dose Dz (Table 3, Fig. 2C, ▼, $n = 19$) exhibited hyperinsulinemia in response to LPS administration (6.67 ± 1.2 ng/mL at 8 hrs, $p = .004$ compared with $t = 0$), whereas high-dose Dz (Table 3, Fig. 2C, ▲, $n = 19$) suppressed the increase in circulating insulin after LPS (3.44 ± 0.74 at 8 hrs, Fig. 2C, ▲, $p = .08$ compared with $t = 0$ and $p < .03$ compared with low-dose Dz, Fig. 2C, ▼, Table 3). In addition, mice receiving low-dose Dz experienced significant hyperglycemia (405.5 ± 49.2 mg/dL at 8 hrs, $p < .0001$ compared with $t = 0$), and transient hypotension (79.6 ± 1.2 mm Hg at 24 hrs) after LPS infusion. Overall, LPS caused 36.8% mortality in

mice exposed to low-dose Dzx in the presence of D50 (Fig. 2C, ▼, $p = .055$ compared with D50 + LPS mice, Fig. 2B, ■). Contrary to our expectation, insulin increased in the low-dose Dzx exposed mice to a level similar to that observed in D50-infused mice exposed to LPS in the absence of Dzx. The degree of hyperglycemia 8 hrs after LPS infusion in the low-dose Dzx group was skewed by a small subset of animals with profound hyperglycemia. Subsequent death of these mice revealed that glucose control in the surviving mice exposed to low-dose Dzx (Fig. 2C, ▼) approximated that seen in mice exposed to dextrose in the presence of LPS (Fig. 2B, ■). High-dose Dzx (20–30 mg/kg/day, Fig. 2C, ▲) resulted in profound hyperglycemia within 8 hrs of LPS infusion (554.8 ± 22.9 mg/mL at 8 hrs with 63% of mice displaying BG >600 mg/dL [the limit of the glucometer], $p = .0001$ compared with $t = 0$, Table 3) associated with the suppressed insulin response (Table 3, Fig. 2C, ▼). These mice exhibited profound hypotension and mortality of 94.7% ($p = .0002$ compared with low-dose Dzx) (Fig. 2C, Table 3). Taken together, these data suggest that both the insulin response and the degree of hyperglycemia effect mortality after sublethal doses of LPS.

Elevated Levels of Insulin Are Protective in Glucose-Matched Survivors After LPS Infusion

As death typically occurred between 12 and 24 hrs after LPS infusion, we examined the relationships between 8-hr glucose and insulin levels with mortality in D50-infused, LPS-injured mice, independent of Dzx dose (Fig. 3). In all mice for which we had complete insulin and glucose data 8 hrs after LPS ($n = 38$), increasing levels of insulin were associated with improved survival, whereas increasing hyperglycemia was associated with worse survival (Fig. 3A). It is clear that relatively normoglycemic mice (BG 180 mg/dL) survive endotoxemia whereas profoundly hyperglycemic mice (BG 600 mg/dL) die. Further inspection of the relationship between blood glucose, plasma insulin, and survival suggests that insulin may be predictive of survival. Receiver operating characteristic analysis revealed that plasma insulin levels >7.0 ng/mL 8 hrs after LPS infusion predicted survival 100% of the time, whereas insulin levels <7.0 ng/mL predicted death in 80.9% of cases, exclusive of relatively normoglycemic mice with low insulin levels and 100% survival as would be expected (area under the curve = 0.90) (Fig. 3A).

After excluding profoundly hyperglycemic (BG 600 mg/dL) and relatively normoglycemic (BG 180 mg/dL) mice, we matched nonsurvivors with survivors for degree of hyperglycemia 8 hrs after LPS (nonsurvivors 520.1 ± 40.7 mg/dL vs. survivors 457.0 ± 42.9 mg/dL, $p = .31$, $n = 7$ each). Surviving mice mounted a significantly greater insulin response to hyperglycemia (9.34 ± 1.44 ng/mL at 8 hrs) compared with nonsurvivors (3.48 ± 0.75 ng/mL, $p < .004$, Fig. 3B), indicating a protective effect of endogenous insulin over a wide pathophysiological range of hyperglycemia in this murine model of endotoxemia.

DISCUSSION

In this study, we developed a novel model of endotoxemia in which we manipulated blood glucose by exogenous administration of dextrose, and circulating endogenous insulin by blockade of pancreatic insulin secretion to determine the impact on mortality. Our data demonstrate a number of new findings that enhance our understanding of how glucose and

insulin impact on mortality in critical illness in the absence of confounding factors that have limited the interpretation of previous clinical studies. We show that in animals rendered mildly hyperglycemic by dextrose infusion, LPS administration resulted in an unexpectedly large increase in insulin secretion that was sustained for 12 to 24 hrs before returning to pre-LPS levels at 48 hrs (glucose + LPS) (Fig. 2B, ■). The large endogenous insulin response maintained mild hyperglycemia after LPS injury and was associated with zero mortality. As hypothesized, the highest LPS-induced mortality occurred when the endogenous insulin response was blocked by Dzx during dextrose infusion and was associated with profound hyperglycemia (high-dose Dzx group) (Fig. 2C, ▲). Decreasing the dose of Dzx enabled a larger endogenous insulin response, which mitigated the degree of hyperglycemia and reduced LPS-induced mortality (low-dose Dzx group) (Fig. 2C, ▼). Overall, in this model of endotoxemia, plasma insulin was positively associated and blood glucose negatively associated with survival. Importantly, at matched degrees of hyperglycemia, survivors had two- to three-fold higher circulating insulin levels than nonsurvivors, suggesting that endogenous insulin has a protective effect on critical end-organ function independent of controlling blood glucose.

Strengths and Weaknesses of Model

Based on our previous experience of long-term exogenous glucose infusion in mice (24, 25), we created a nondiabetic but vulnerable mouse model of endotoxemia in which increasing degrees of metabolic disruption were associated with higher rates of mortality. The model has several technical strengths including the use of indwelling venous and arterial catheters in unhandled mice with serial blood sampling capability and continuous monitoring of blood pressure and heart rate. Future studies can now exploit the genetic and transgenic power of this murine model to explore the underlying mechanisms through which hypoinsulinemia and hyperglycemia impact on organ-specific morbidity and overall mortality in LPS-induced critical illness.

Despite the strengths of our model, there are certain limitations warranting discussion. Dzx, in addition to suppressing insulin secretion, is known to have vasodilator properties and may have a direct action to raise blood glucose (26), although neither of these effects were evident in our control Dzx group. We preferred Dzx to models of pancreatic destruction, using alloxan or streptozotocin (14–16), despite an unforeseen variability in sensitivity between animals in its ability to constrain insulin release in response to LPS and glucose administration. However, separating groups into high-dose and low-dose Dzx, based on the physiologic response to the drug, as well as matching for the degree of hyperglycemia allows us to begin to separate out the effect of endogenous insulin secretion from that of hyperglycemia. Despite the limitations of this *post hoc* analysis, our model provides new information and extends the insights gained from earlier models utilizing agents that induce a Type 1 diabetic state.

The chronically catheterized mouse model that we have developed is technically challenging and difficult to maintain over a 7-day period and, as such, is expected to have a degree of failure. The combined mortality in all of our five control groups (○, □, ▲, ●, ■, n = 55) totaled 14.5%, which is similar to our overall historical rate of 14% failure of chronically

catheterized mice completing a 7-day infusion protocol. Thus, the mortality of 20% reported in the glucose alone group is only slightly above that expected, suggesting that the mortality is not likely to be related to the presence of hyperglycemia, but rather reflective of the expected attrition in this sophisticated, technically difficult model.

It is worth mention that we have not reported any measures of end-organ dysfunction or counterregulatory hormone responses in this study, although these measures are important when discussing endocrine responses to critical illness. As previously stated, the primary outcome measure in this study was mortality which occurred most commonly 12 hrs to 18 hrs after initiation of injury. As plasma obtained at the 8-hr time point was consumed in the course of the insulin assay, insufficient sample remained for organ function or counterregulatory hormone assessment. Time from death to organ harvest precluded any useful evaluation of tissue in the nonsurvivors, and those animals surviving to 48 hrs were functionally and physiologically normal. The complexity of the model is evident in that simply developing the model and obtaining adequate data to identify the relationships between insulin and glucose regulation and mortality required 100 animals. Quantification of the inflammatory and counterregulatory hormone responses and of premorbid organ dysfunction remains a focus on interest and provides the basis for future studies.

As with other studies, we were unable to strictly separate the actions of insulin and glucose as the two are so closely linked and tightly controlled *in vivo*. Additional insulin “rescue” experiments would provide insight into whether exogenous insulin infusion can protect against mortality in the glucose + LPS + high-dose Dz group (Fig. 2C, ▲). However, our current study was specifically designed to test the hypothesis that suppression of endogenous insulin was associated with poor outcome in LPS-induced critical illness. Our observations in different Dz dose groups and our analysis in which hyperglycemia was matched between a subset of survivors and nonsurvivors demonstrate that insulin itself may exert a protective effect against mortality, even in the presence of marked hyperglycemia. It remains possible that this effect is due to a protective benefit of other endogenous pancreatic peptide production rather than insulin per se, e.g., C-peptide. Further study is required to address this question more specifically.

LPS Leads to Increased Pancreatic Insulin Secretion in the Presence of Mild Hyperglycemia

An unexpected, and potentially important, finding of the current study was that insulin secretion from the pancreas in response to LPS administration was distinctly affected by the presence vs. the absence of exogenous glucose (Fig. 2B). As shown, in the presence of dextrose infusion, the administration of LPS caused a two- to three-fold increase in circulating insulin above the already elevated insulin that accompanied glucose infusion at $t = 0$ (Fig. 2B, ■). Interestingly, a supportive observation was recently reported in a human study in which circulating insulin and C-peptide levels increased markedly when subjects were administered LPS during a hyperglycemic clamp study (27). These human LPS data suggest that the increase in circulating insulin is a direct result of pancreatic secretion (i.e., C-peptide is markedly elevated) and not secondary to decreased degradation in peripheral tissues. The mechanisms by which glucose, or other nutrients including free fatty acids or

triglycerides, and LPS interact at the level of the pancreatic β cell to increase insulin secretion remain to be determined.

Relative Insulin Deficiency Is Associated With Mortality in Endotoxemic Mice With Hyperglycemia

When animals were maintained in a chronic euglycemic state, intra-arterial LPS induced a transient hypoglycemia as the primary metabolic response (Fig. 2B, ●). Various animal models have also demonstrated hypoglycemia in response to LPS (28–35) and, furthermore, glucose levels do not increase in normal humans exposed to intravenous LPS (27, 36). Although these observations may seem counterintuitive to what is commonly encountered in the clinical setting, where hyperglycemia is often seen in critically ill patients, clearly both humans and animals do not exhibit a primary hyperglycemic response to LPS administration in the absence of confounding or comorbid conditions. Whatever the mechanism, it is apparent that hypoinsulinemia in response to LPS is not, in the absence of hyperglycemia, able to impact on mortality.

Conversely, for animals maintained in a mild hyperglycemic state by glucose infusion, the ability to survive endotoxemia was intimately linked to the insulin response. Our experimental paradigm of manipulating the release of insulin from the pancreas with Dzx resulted in two possible scenarios. In some animals, Dzx administration resulted in insufficient blockade of insulin production (low-dose Dzx) (Fig. 2C, ▼). A hyperinsulinemic response temporized the acute hyperglycemia induced by LPS administration and prevented significant end-organ dysfunction and death in these animals. In contrast, mice receiving high-dose Dzx in the presence of exogenous glucose infusion exhibited a blunted insulin response and severe acute hyperglycemia with concomitant mortality (Fig. 2C, ▲). These data demonstrate the critical role that endogenous insulin secretion likely plays in determining whether LPS-mediated critical illness leads to death or recovery when in a hyperglycemic state.

Conclusion and Clinical Implications

Our study shows that, in endotoxemia, both hypoinsulinemia and hyperglycemia are necessary, but not sufficient, conditions to cause mortality. Although it is difficult to directly equate glucose levels in humans in response to critical illness to mice in response to endotoxemia, our data clearly demonstrate that endogenous insulin secretion was associated with survival, irrespective of the magnitude of the hyperglycemic response (Fig. 3), and that hyperglycemia alone is not sufficient to induce mortality from endotoxemia in the absence of a concomitant relative deficiency of insulin production. As such, our data suggest that, to improve mortality in sepsis, increasing insulin may have a relatively greater benefit than reducing the hyperglycemia. A major clinical implication that derives from our data is that the endocrine pancreatic response to stress may predict clinical outcome experienced by critically ill patients. Inappropriate pancreatic function may be the driving force behind the hyperglycemia commonly seen in critically ill, nondiabetic patients. Identification of such “pancreatic nonresponders” may provide important insight into those likely to experience poor outcomes from critical illness. Thus, future clinical trials in critically ill patients may benefit from measuring endogenous insulin and C-peptide levels before initiating insulin

therapy to control glucose. The convention of titrating insulin to control glucose levels may not accurately reflect the insulin requirements of the critically ill patient. Controlling the circulating levels of insulin to a degree that may have therapeutic benefits over and above the lowering of blood glucose may represent a more prudent approach to treatment, even to the extent of infusing glucose to avoid potentially detrimental hypoglycemia.

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Figure 1.

Timeline schematic of study protocol. Glucose and saline infusions start 24 hrs before lipopolysaccharide (*LPS*) injection (time $t = 0$) and continue throughout experiment. Diazoxide is administered intra-arterially twice per day starting at $t = -24$ hrs and continuing throughout the experiment. *Arrowheads* represent blood sampling for glucose and insulin determination.

Figure 2.

Diazoxide induces dose-dependent insulin suppression, associated hyperglycemia, and increased 48-hr mortality in dextrose-infused mice exposed to lipopolysaccharide (LPS). The figure depicts the blood glucose (*top row*), plasma insulin (*second row*), blood pressure (*third row*), and Kaplan Meier survival (*bottom row*) over time. *Panel A* depicts three control groups: saline (○), dextrose (D50, □), and high-dose diazoxide (Dzx, △). Saline-infused mice exhibit normal blood glucose and plasma insulin, remain normotensive, and survive the 48 hr study period (○). D50-infused mice (□) and those treated with D50 and high-dose Dzx (△) exhibit mild hyperglycemia and mild hyperinsulinemia throughout the study period in the absence of LPS. Both groups remain normotensive but experience 20% to 27% mortality (*Panel A*). *Panel B* depicts saline- (●) and D50- (■) infused mice exposed to LPS. Intra-arterial LPS precipitates transient hypoglycemia, hypoinsulinemia, and hypotension in saline-infused mice with 16.7% mortality over 48 hrs (●). Continuous intravenous D50 prevents the hypoglycemia induced by LPS and is associated with a marked increase in plasma insulin. D50-infused mice injured with LPS experience transient hypotension, but all survive the insult (■). *Panel C* depicts LPS- and D50-infused mice exposed to low-dose (2.5–10 mg/kg, ▼) or high-dose (20–30 mg/kg, ▲) Dzx. Low-dose Dzx precipitated moderate hyperglycemia with associated increase in circulating insulin in mice exposed to LPS in the presence of D50 (▼); 63% of these mice survived. High-dose Dzx suppressed insulin production in response to LPS and D50 leading to profound hyperglycemia, hypotension, and 94.7% mortality (▲). Baseline values were not different from controls across all groups. #*p* < .05 compared with saline control by two-way analysis.

of variance (ANOVA); $\dagger p < .05$ compared with saline + LPS by two-way ANOVA; $*p < .05$ compared with time 0 by paired Student's t test; $\ddagger p < .03$ compared with high-dose DzX by Wilcoxon rank sum test; $\S\S p = .0002$ compared with low-dose DzX by Kaplan-Meier survival analysis. Time course data were not plotted as representative of a given group after mortality exceeded 50%.

Figure 3.

Relative insulin deficiency predicts mortality in a mouse model of systemic endotoxemia. Surviving mice (○) display increased circulating insulin and reduced blood glucose compared with nonsurvivors (●). Independent of blood glucose, a circulating insulin level >7 ng/mL predicts survival in 100% of animals (*Panel A*). Matching for the degree of hyperglycemia 8 hrs post lipopolysaccharide after excluding relatively normoglycemic (blood glucose <180 mg/dL) and profoundly hyperglycemic (>600 mg/dL) mice, survivors exhibited higher circulating insulin levels compared with nonsurvivors (*Panel B*, n = 7 each). Blood glucose data >600 mg/dL are plotted together as these fall in a range above the upper limit of detection of the glucometer.

Table 1

Unfasted baseline characteristics (after exclusion of hyperglycemic mice at time t = 0)

Group	N	Weight (g)	Glucose (mg/dL)	Insulin (ng/mL)	MAP (mm Hg)
Saline	10	23.6 ± 0.8	94.0 ± 7.7	1.00 ± 0.22	102.3 ± 0.7
Dextrose	10	24.5 ± 0.5	104.6 ± 5.3	1.05 ± 0.29	106.3 ± 4.4
Dextrose + diazoxide 20 mg/kg/day	15	25.2 ± 0.5	102.5 ± 4.8	0.89 ± 0.17	107.2 ± 2.6
Saline + LPS	12	25.2 ± 0.5	100.1 ± 5.1	1.43 ± 0.23	97.6 ± 1.5
Dextrose + LPS	8	25.9 ± 0.6	103.6 ± 7.1	1.15 ± 0.28	101.4 ± 4.0
Dextrose + diazoxide + LPS					
2.5 mg/kg/day	6	22.2 ± 0.5	97.5 ± 4.1	1.17 ± 0.24	105.6 ± 2.1
5 mg/kg/day	7	24.8 ± 0.9	109.0 ± 10.3	1.68 ± 0.35	106.0 ± 2.0
10 mg/kg/day	6	24.0 ± 0.6	100.0 ± 8.3	1.12 ± 0.24	97.1 ± 3.1
20 mg/kg/day	9	23.7 ± 0.4	104.4 ± 5.5	1.37 ± 0.34	101.1 ± 0.9
30 mg/kg/day	10	25.2 ± 0.4	111.9 ± 5.3	1.25 ± 0.26	105.7 ± 2.4

MAP, mean arterial pressure; LPS, lipopolysaccharide.

Data presented as mean ± standard error of the mean immediately after recovery period. No statistical difference between groups in baseline weight, blood glucose, plasma insulin, or blood pressure.

Table 2

Time course of caloric intake

Group	Day of Experiment					
	-3	-2	-1	0	1	2
Saline	2.34 ± 0.18	5.52 ± 0.90	8.64 ± 0.49	6.75 ± 0.92	8.73 ± 1.40	7.92 ± 1.55
D50 ^a	0.98 ± 0.37	4.73 ± 1.07	6.06 ± 0.95	3.77 ± 0.66	1.66 ± 0.64	1.58 ± 0.51
D50 + Dz ^a 20 mg/kg/day	2.20 ± 0.22	4.89 ± 0.84	9.45 ± 1.54	2.43 ± 0.57	1.25 ± 0.33	1.28 ± 0.81
Saline + LPS	1.63 ± 0.34	4.87 ± 0.96	7.45 ± 0.97	9.69 ± 0.79	1.12 ± 0.39	7.17 ± 1.31
D50 + LPS ^a	1.20 ± 0.49	9.58 ± 2.54	11.26 ± 1.45	7.72 ± 1.10	1.15 ± 0.36	2.65 ± 0.76
D50 + Dz + LPS ^a						
2.5 mg/kg/day	2.11 ± 0.73	5.84 ± 1.90	7.63 ± 1.04	4.50 ± 1.34	0.96 ± 0.24	2.85 ± 0.80
5 mg/kg/day	2.14 ± 0.43	7.62 ± 2.17	10.07 ± 1.62	4.80 ± 1.30	0.39 ± 0.20	0.60 ± 0.32
10 mg/kg/day	2.44 ± 0.60	5.24 ± 1.64	8.05 ± 1.02	3.19 ± 0.90	0.66 ± 0.31	3.00 ± 0.11
20 mg/kg/day ^b	1.86 ± 0.25	8.15 ± 0.72	12.12 ± 1.55	2.92 ± 0.85		
30 mg/kg/day ^b	2.22 ± 0.50	6.18 ± 1.21	9.97 ± 0.81	3.80 ± 0.23		
Calories from D50 infusion ^a				4.08	4.08	4.08

D50, dextrose; Dz, diazoxide; LPS, lipopolysaccharide.

^aCalories from dextrose infusion, 4.08 kcal/day on days 0, 1, and 2;^bcaloric intake not reported after mortality exceeded 50%. Data presented as mean ± standard error of the mean measured in kcal/day.

Table 3

Diazoxide dose response after LPS administration in the presence of glucose

Diazoxide Dose	N	Glucose ^a (mg/dL)	Insulin ^d (ng/mL)	MAP ^d (mm Hg)	Mortality ^a (48 hr)
2.5 mg/kg/day	6	319 ± 88	7.3 ± 1.6	90.8 ± 2.1	0
5 mg/kg/day	7	535 ± 46	8.1 ± 3.3	93 ± 2.7	0.57
10 mg/kg/day	6	373 ± 90	4.4 ± 1.6	87 ± 7.9	0.50
20 mg/kg/day	9	556 ± 31	4.8 ± 1.2	79.8 ± 4.8	0.89
30 mg/kg/day	10	553 ± 35	2.1 ± 0.7	62.3 ± 3.9	1
Low dose ^d	19	406 ± 49 ^b	6.7 ± 1.2 ^b	90.2 ± 2.5	0.37 ^c
High dose ^d	19	555 ± 23	3.4 ± 0.7	74.0 ± 4.4	0.95

LPS, lipopolysaccharide; MAP, mean arterial pressure.

^a $p < .05$ for diazoxide dose by Kruskal-Wallis test or Kaplan-Meier survival analysis;^b $p < .05$ compared with high dose diazoxide by Wilcoxon rank sum test;^c $p = .0002$ compared with high dose diazoxide by Kaplan-Meier survival analysis;^d low dose includes 2.5–10 mg/kg/day diazoxide and high dose includes 20–30 mg/kg/day diazoxide. Data presented 8 hrs after LPS administration as mean ± standard error of the mean.