

Uncontrolled Hemorrhage in Insulin-dependent Diabetic Rats

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Abstract

Objectives: Diabetes mellitus (DM) is a known risk factor for higher morbidity and mortality after trauma. The authors tested the hypothesis that there is a difference in the response to uncontrolled hemorrhage between normal euglycemic rats and insulin-dependent diabetic rats.

Methods: Thirty-one adult male Sprague-Dawley rats were used in this study. Fifteen streptozocin (STZ)-injected rats became diabetic (DM+) 2 weeks after treatment. Sixteen rats served as nondiabetic controls (DM-). All rats were anesthetized with Althesin and their femoral arteries were catheterized via cutdown, allowing continuous monitoring of vital signs. Sixteen (eight DM-, eight DM+) rats underwent uncontrolled hemorrhage by 75% tail amputation. Fifteen (eight DM-, seven DM+) rats served as non-hemorrhage controls. The mean arterial pressure (MAP), lactate, and cumulative hemorrhage volume per 100 g were measured prehemorrhage and then every 15 minutes posthemorrhage for 2 hours. Data were reported as mean \pm standard deviation. Interval data were analyzed by analysis of variance (two tails, $\alpha = 0.05$).

Results: Prehemorrhage glucose was significantly higher ($p < 0.001$) in the DM+ (357.9 ± 22.2 mg/dL) versus DM- (125.7 ± 9.7 mg/dL) rats. At baseline, there was no significant difference in weight, MAP, or lactate between DM+ and DM- rats. Body-weight-adjusted mean cumulative hemorrhage volume was significantly greater ($p < 0.04$) in diabetic rats (2.52 ± 0.15 cm³/100 g body weight) than the nondiabetic rats (1.86 ± 0.25 cm³/100 g body weight).

Conclusions: Compared to nondiabetic rats, diabetic rats suffered a greater blood loss after the same uncontrolled vascular injury.

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Diabetes mellitus (DM) and hyperglycemia are associated with poor outcomes in trauma patients.¹⁻⁶ This is concerning, given that 2005 Centers for Disease Control and Prevention estimates indicate that 20.8 million Americans are living with diabetes.⁷ Therefore, a greater understanding of how

hyperglycemia relates to poor outcomes following trauma is critical for treating this patient population.

Previous work in our laboratory has shown that type 2 diabetic rats (Zucker diabetic fat rats) bleed significantly more in response to uncontrolled hemorrhage than nondiabetic controls.⁸ The mechanism causing this loss of hemostatic control is unclear.

We studied uncontrolled hemorrhage in a type 1 diabetic rodent model. We tested the null hypothesis that insulin-dependent diabetes would not affect the response to uncontrolled hemorrhage by comparing hemorrhage volume, heart rate (HR), blood pressures, glucose, and lactate posthemorrhage between diabetic (DM+) and nondiabetic (DM-) rats.

METHODS

Study Design

We studied the hemodynamic response to uncontrolled hemorrhage using a rodent model of type 1 diabetes. Diabetic rats were compared to euglycemic

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(nondiabetic) control rats. The study was approved by the Institutional Animal Care and Use Committee of the State University of New York Downstate Medical Center. We followed the guidelines for the care of laboratory animals of the American Association for Laboratory Animal Care.

Animal Subjects

Male Wistar rats were supplied by Charles River Laboratories (Raleigh, NC). Prior to surgery, the rats were housed in plastic containers and allowed free access to food and water. A temperature of 20–23°C and a 12-hour light:12-hour dark cycle was maintained in the housing area.

Study Protocol

Type 1 diabetes was induced in Wistar rats by injecting 55 mg/kg streptozocin (STZ), a pancreatic islet cell toxin, into the tail veins 2 weeks before starting the hemorrhage experiment. All animals were of similar weights before randomization to injection with STZ. Thirty-one (15 DM+ and 16 DM-) study rats were anesthetized with Althesin (Pitman-Moor LTD., Harefield, UK) prior to experimentation. Althesin sedation was administered via the intraperitoneal route. The desirable property of this agent's lack of effect on respiratory or hemodynamic function has been described in a previous study.⁸

The femoral artery was exposed by cutdown and cannulated with a 24-gauge angiocatheter. This was connected to a Transpac intravenous (Abbot Critical Care System, Chicago, IL) transducer. A Propaque No. 106 (Protocol Systems, Beaverton, OR) was used to record blood pressure.

The rats were divided into four study arms: a hemorrhage group containing a DM+ and a DM- control arm and a nonhemorrhage group also containing a DM+ and a DM- control arm. Hemorrhage rats (16 rats: eight DM+ and eight DM- controls) were randomly selected to undergo uncontrolled hemorrhage by 75% tail amputation. The tail remnant still attached to the animal was placed in a syringe barrel to record cumulative hemorrhage volume. All rats undergoing the hemorrhage protocol had a 10-minute acclimation period prior to tail amputation. Nonhemorrhage rats (15 rats: seven DM+ and eight DM-) served as controls to establish baseline effects of anesthesia and blood draws. Blood was drawn from the femoral arterial line in 0.3-mL aliquots. The drawn blood was used for serum lactate and glucose measurements. Animals surviving the 120-minute study period were euthanized at the end of the experiment.

Outcome Measures

Mortality rate and survival times were calculated from the beginning of hemorrhage. Cumulative hemorrhage volume, blood pressure, mean arterial pressure (MAP), HR, glucose, and lactate were measured 10 minutes prior to hemorrhage, 5 minutes posthemorrhage, and then every 15 minutes for 120 minutes. Lactate was measured directly from arterial blood gas samples every 15 minutes using a Model 16403-0116/40 pH/blood gas/electrolyte analyzer (Instrumentation

Laboratory, Barcelona, Spain). MAP (mm Hg) was calculated by the same technology (Propaque) that measured arterial blood pressure.

Data Analysis

Data were reported as mean \pm standard error of the mean. Student's *t*-tests were used to compare baseline data between DM- and DM+ rats. Posthemorrhage data from the control and STZ rats were compared to each other and their respective nonhemorrhage controls. One-way analysis of variance (ANOVA) with repeated measures was used to analyze continuous variables over time for statistically significant differences between DM+ and DM- groups. Cumulative hemorrhage volume was compared using the Student's *t*-test. All statistical tests were two-tailed. Serum glucose at baseline and at 2 hours of hemorrhage in the DM+ hemorrhage group was compared using paired *t*-test. When significant differences were observed, Bonferroni post hoc testing was done to isolate differences between groups. Statistical significance was defined by $p < 0.05$. Calculations were done using SPSS for Windows (release 8.01997, SPSS Inc., Chicago, IL).

A sample size analysis gave an $n = 6$ for each group assuming a two-tailed ANOVA with an alpha of 0.05, a power of 80% to detect a difference in arterial lactate of 6.0 mmol/L, and a within-group standard deviation of 3.0 mmol/L. We chose a lactate of 6 mmol/L because previous experience using this hemorrhagic shock model showed a higher mortality rate when the difference in lactate between the hemorrhage groups was greater than this. Sample size was calculated using SPSS Sample Power (version 2.0, 2003, SPSS Inc.)

RESULTS

Baseline Values

Table 1 summarizes the prehemorrhage values for body weight, blood pressure, HR, lactate, and glucose between the DM+, and DM-, hemorrhage, and nonhemorrhage control groups. All data were normally distributed. There were no significant differences between the four groups in prehemorrhage weights, blood pressures, and arterial lactates. Only baseline glucose and HR showed significant between-group differences. Glucose post hoc testing showed that both DM+ groups (hemorrhage and nonhemorrhage) had similar ($p = 1.00$) baseline values, but were both significantly ($p < 0.001$) higher than the two DM- groups. HR was significantly ($p = 0.015$) lower in both DM+ compared to DM- groups.

Effect of Diabetes on Blood Pressures Following Hemorrhage

Figure 1 compares the changes in MAP over time between the DM+ and DM- rats in both the hemorrhage and the nonhemorrhage groups. There were no significant changes in blood pressure from baseline to the end of the observation period for either the DM+ or the DM- rats in the nonhemorrhage groups resulting from anesthesia or blood sampling. Also, there was no difference in Δ MAP between the DM+ and DM- nonhemorrhage groups throughout the experiment. At

Table 1
Baseline Characteristics

Baseline	Group	n	Mean	95% CI	p-value*
Weight prehemorrhage (g)	DM- hemorrhage	8	457	391-523	0.072
	DM+ nonhemorrhage	7	361	327-396	
	DM- hemorrhage	8	363	281-446	
MAP (mm Hg)	DM+ hemorrhage	8	382	316-447	0.502
	DM- nonhemorrhage	8	124	114-135	
	DM+ nonhemorrhage	7	110	91-129	
	DM- hemorrhage	8	120	104-135	
	DM+ hemorrhage	8	117	101-134	
Lactate (mmol/L)	DM- nonhemorrhage	8	2.03	1.57-2.49	0.358
	DM+ nonhemorrhage	7	2.61	1.07-4.16	
	DM- hemorrhage	8	3.40	2.28-4.52	
	DM+ hemorrhage	8	3.18	1.15-5.20	
Glucose (mg/dL)	DM- nonhemorrhage	8	152	141-164	<0.001
	DM+ nonhemorrhage	7	372	300-444	
	DM- hemorrhage	8	99	68-130	
HR (beats/min)	DM+ hemorrhage	8	346	266-426	0.009
	DM- nonhemorrhage	8	439	417-461	
	DM+ nonhemorrhage	7	339	262-416	
	DM- hemorrhage	8	420	407-433	
	DM+ hemorrhage	8	378	318-439	

*Group comparisons by one-way ANOVA.
ANOVA = analysis of variance; DM+ = diabetic; DM- = nondiabetic; HR = heart rate.

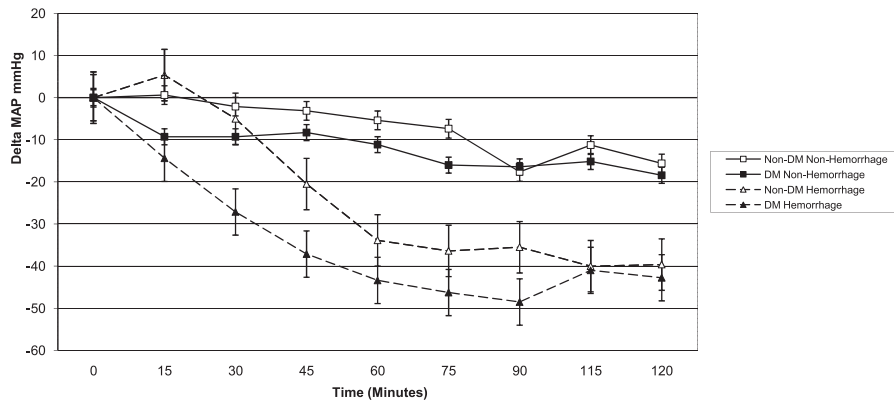


Figure 1. Change in MAP in diabetic and nondiabetic rats with and without hemorrhage. (□) DM- nonhemorrhage; (■) DM+ non-hemorrhage; (△) DM- hemorrhage; (▲) DM+ hemorrhage. DM- = nondiabetic; DM+ = diabetic; MAP = mean arterial pressure.

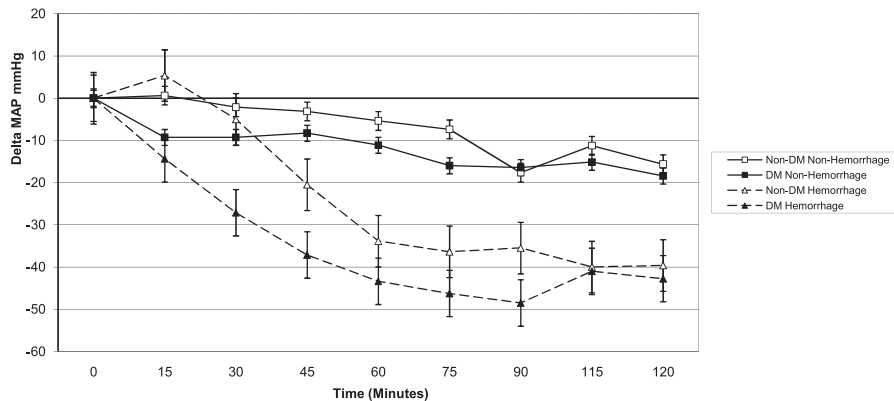


Figure 2. Change in lactate in diabetic and nondiabetic rats with and without hemorrhage. (□) DM- nonhemorrhage; (■) DM+ non-hemorrhage; (△) DM- hemorrhage; (▲) DM+ hemorrhage. DM- = nondiabetic; DM+ = diabetic.

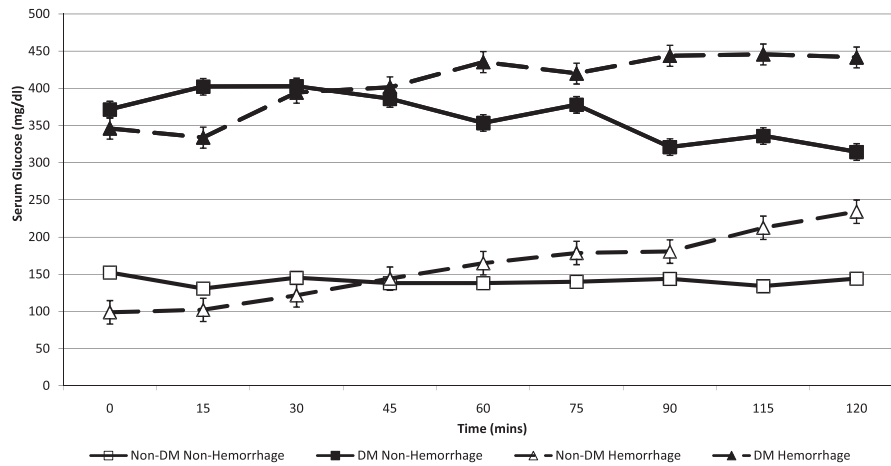


Figure 3. Serum glucose in diabetic and nondiabetic rats with and without hemorrhage. (□) DM- nonhemorrhage; (■) DM+ nonhemorrhage; (△) DM- hemorrhage; (▲) DM+ hemorrhage. DM- = nondiabetic; DM+ = diabetic.

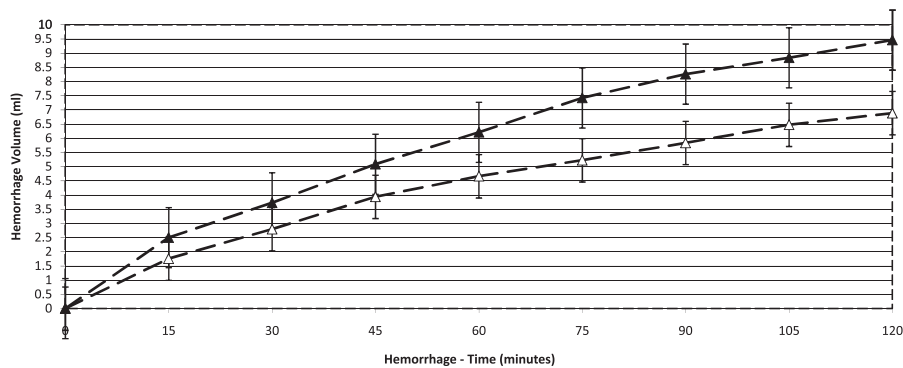


Figure 4. Time course of hemorrhage. (△) DM- hemorrhage (▲) DM+ hemorrhage. DM- = nondiabetic; DM+ = diabetic.

60 minutes, both hemorrhage groups, DM+ ($p = 0.019$) and DM- ($p = 0.020$), had significantly lower blood pressures than their nonhemorrhage counterparts, which persisted throughout the experiment. There was no statistical difference in the Δ MAP at any point between hemorrhaged DM+ and DM- rats.

Effect of Diabetes on HR Following Hemorrhage

Heart rate for the nonhemorrhage controls showed no significant changes throughout the experiment between the DM+ and DM- groups. Posthemorrhage, there was no significant increase in HR for either the DM+ or the DM- hemorrhage groups compared to their respective baselines. After hemorrhage, HR was not significantly different between the DM+ and DM- rats at any time during the experiment.

Effect of Diabetes on Arterial Lactate Following Hemorrhage

Figure 2 compares the changes in lactate between DM+ and DM- rats. For the nonhemorrhage controls of both the DM+ and the DM- groups, no significant elevations of lactate were noted as a result of anesthesia or blood sampling. There was a significantly greater increase in lactate only between the DM+ hemorrhage and DM+ nonhemorrhage groups at 45 ($p = 0.032$), 90 ($p = 0.039$), 105 ($p = 0.007$), and 120 ($p = 0.04$) minutes posthemor-

rhage. Lactate did not change significantly when comparing hemorrhage and nonhemorrhage in the DM- rats. There was also no difference in lactate between the DM+ and DM- hemorrhage groups ($p = 0.12$).

Effect of Diabetes on Blood Glucose Following Hemorrhage

Figure 3 compares blood glucose levels between DM- and DM+ rats. Blood glucose was significantly higher in DM+ hemorrhage rats than in DM- hemorrhage rats throughout the entire experiment ($p < 0.001$ at all time points). DM+ hemorrhage rats had significantly higher glucose levels ($p = 0.012$) after 2 hours of uncontrolled hemorrhage (442 ± 27 mg/dL), compared to their prehemorrhage baseline (346 ± 34 mg/dL). A stress hyperglycemic response to hemorrhage was noted in the diabetic rats, which demonstrated a significantly higher glucose in the hemorrhage compared to nonhemorrhaged DM+ group ($p = 0.01$ at 90 minutes, $p = 0.046$ at 105 minutes, and $p = 0.03$ at 120 minutes).

Effect of Diabetes on Mean Cumulative Hemorrhage Volume

Figure 4 displays the time course of hemorrhage for the DM+ and DM- groups. Hemorrhage was brisk at

the beginning and then waned as blood pressure dropped in both the DM+ and the DM- rats. The DM+ rats bled at a faster rate at all times than the DM- rats. Body-weight-adjusted hemorrhage volume was significantly greater ($p = 0.038$) in DM+ ($2.52 \pm 0.15 \text{ cm}^3/100 \text{ g}$ body weight) than the DM- rats ($1.86 \pm 0.25 \text{ cm}^3/100 \text{ g}$ body weight). All rats in both the hemorrhage and the nonhemorrhage groups survived to the completion of the 120-minute study period.

DISCUSSION

There is substantial experimental evidence that the STZ-induced diabetic rats closely mirror the physiology and pathophysiology of human patients with type 1 DM. Examples of this relationship include cardiovascular autonomic dysfunction,^{9,10} nephropathy and proteinuria,¹¹⁻¹³ coronary and cerebral macrovascular disease,¹⁴ neuropathy,¹⁵ and delayed wound healing.¹⁶

We demonstrated that rodents with STZ-induced diabetes experienced significantly greater blood loss when exposed to uncontrolled hemorrhage. This is similar to results from a previous study done in our laboratory on a type 2 non-insulin-dependent diabetes hemorrhage model.⁸ The STZ-injected rats were no different from control rats with regard to baseline MAP, weight, and lactate. As expected, initial blood glucose levels were significantly higher in the injected animals. Additionally, initial HR was significantly lower in the diabetic animals. This is consistent with previous studies that have shown lower HRs and autonomic dysfunction in STZ-induced diabetic rats.^{9,10,12,17}

The mechanism behind this decrease in hemostatic control is unclear. Khodabandehlou et al.¹⁸ showed that DM+ humans, compared to DM- humans, have significant reductions in platelet number, erythrocyte aggregation, blood and plasma viscosities, fibrinogen levels, and albumin levels. Diabetes also causes peripheral neuropathy, which results in a relative insensitivity of peripheral vessels to the vasoconstricting effects of norepinephrine.¹⁹ The model used in our experiment may be useful for further work aimed at fully understanding the hematologic and vascular effects of diabetes.

A recent, large, prospective database study showed that trauma patients with a history of DM had longer stays in the intensive care unit (ICU), more ventilator days, and increased complications.⁶ However, there was no difference in mortality or length of hospital stay. Additionally, several premorbid conditions, including DM, have also been shown to be predictors of death following blunt trauma.²⁰

Stress hyperglycemia, in response to hemorrhage, is a well described phenomenon seen in animal models^{21,22} as well as humans.^{23,24} There is evidence that this response is protective²⁵⁻²⁸ and may act by increasing serum osmolality, promoting vascular filling, and increasing perfusion to ischemic tissues.²⁹⁻³¹ In contrast, hyperglycemia at the time of admission is predictive of poor outcomes following trauma,^{3,5,32} and this is also true for nondiabetic patients admitted to the ICU.⁴ Additionally, nondiabetic, operative trauma patients admitted with hyperglycemia have

longer ICU lengths of stay, longer hospital stays, and increased mortality.^{1,33} Hyperglycemia is also associated with poor outcomes in patients with severe traumatic brain injury.³⁴

Aggressive glucose control has been shown to improve mortality in critically ill patients admitted to the surgical ICU,^{35,36} but these studies were not limited to trauma patients. A recent, quasi-prospective study showed a significant decrease in hospital length of stay, ventilator days, and mortality when critically ill trauma patients were subjected to tight glycemic control.³⁷ These results are supported by a study that found decreased mortality in trauma patients treated with a strict insulin sliding scale.² However, a recent study³⁸ showed that an aggressive computerized insulin infusion protocol for critically ill trauma patients decreased hospital length of stay and morbidity, but significantly increased mortality.

The type 1 diabetic model employed in this study may be useful in understanding one of the multitude of complex relationships between poor trauma outcomes and hyperglycemia. Our finding of a hemostatic defect related to hyperglycemia is just one piece of the puzzle, which must include investigations into other systemic effects of hyperglycemia on trauma. Future studies using this model, aimed at controlling hyperglycemia following uncontrolled hemorrhage, may lead to advances in the treatment of humans following trauma. This model may also be used to understand the cellular and molecular mechanisms behind the increased mortality seen in hyperglycemic trauma patients.

LIMITATIONS

Partial tail amputation was used for our model. Although this method allows for massive hemorrhage, this may not represent the type of bleeding seen in trauma patients. We chose this method because it allows for rapid, reproducible, and accurate measurement of blood loss in rats.

Additionally, rats were hemorrhaged 2 weeks after injection with STZ. Future study is required to determine if the effect we see on hemorrhage volume is due to the hyperglycemia present at the time of hemorrhage or chronic changes that are induced by type 1 diabetes. Although which rats were to receive STZ injection and which rats were to be hemorrhaged were chosen at random, a standardized technique, such as a random number generator, was not utilized. This may have been less efficient at eliminating bias. Additional laboratory testing such as platelet count, international normalized ratio, or prothrombin time/partial thromboplastin time was not performed. The small sample size is also a limitation of this study.

CONCLUSIONS

Hyperglycemic type 1 diabetic rodents hemorrhage a significantly greater quantity than nondiabetic, euglycemic controls in response to uncontrolled vascular injury.

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