Protein Kinase C Epsilon Activation and Laboratory Markers of Multi-Organ Injury in Porcine Hemorrhagic Shock

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Abstract

Background: Activation of protein kinase C epsilon (PKC-ε) enhances survival in a porcine hemorrhagic shock model. However, the systemic biochemical and hematological effects, particularly the multi-organ biomarker profile, have not been fully characterized. This study aimed to analyze laboratory biomarkers of multi-organ injury and metabolic protection in hemorrhagic shock treated with PKC-ε activation, including acid-base balance, hepatic and renal function, cellular injury markers, coagulation parameters, and endocrine stress response.

Methods: Twenty-three female swine underwent controlled hemorrhage (35% of blood volume). The PKC-ε–treated group (n=15) received 3 mg/kg of a PKC-ε activator intramuscularly five minutes after the start of hemorrhage, while the control group (n=8) received no drug. We monitored animals for 7 hours post-bleeding for survival and laboratory parameters, including acid-base balance, metabolic markers, hepatic and renal function, coagulation, and blood count.

Results: PKC-ε activation was associated with improved survival (73.3% vs. 25.0%, P=0.044). In the treated group, lactate was lower, and base excess and bicarbonate were better maintained (group-by-time interaction P=0.017, 0.022, and 0.031), and glucose was higher at hours 4 and 5 (P=0.026 and P=0.047). Hepatic injury markers did not differ significantly between groups.

Conclusions: This study characterizes the biochemical response to PKC-ε activation in hemorrhagic shock, showing that the survival benefit was accompanied by better-maintained metabolic and acid-base parameters, with no detectable between-group differences in coagulation. These findings describe the biochemical profile accompanying the previously reported survival advantage and may help generate hypotheses about PKC-ε-mediated organ protection.

Keywords: Shock, Hemorrhagic; Protein Kinase C-epsilon; Military Medicine; Acidosis, Lactic

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INTRODUCTION

Traumatic injuries leading to hemorrhagic shock are the most common cause of preventable death on the battlefield and a major cause of morbidity and mortality in civilian settings [1–3]. Although treatment in recent decades has focused primarily on fluid resuscitation and blood product administration to restore blood volume [1,4], survivors frequently develop multi-organ dysfunction despite successful hemostasis [5].

Hemorrhagic shock progresses through stages of increasing severity (Class I: <15% loss; Class II: 15–30%; Class III: 30–40%; Class IV: >40% loss) [2], with progressively inadequate compensatory responses. The initial sympathetic activation - increasing heart rate, cardiac contractility, and vasoconstriction - attempts to maintain perfusion to vital organs but ultimately fails in severe hemorrhage, allowing progressive tissue hypoperfusion, metabolic failure, and lactate accumulation with intracellular acidosis [3,5,6].

Trauma-induced tissue damage occurs in two stages: ischemic damage from hypoperfusion, followed by paradoxical ischemia-reperfusion injury during resuscitation [7]. Upon reperfusion, oxygen reintroduction triggers reactive oxygen species generation, which, combined with calcium overload, activates injurious cascades: mitochondrial permeability transition pore opening, apoptotic pathways, and pro-inflammatory signaling [8]. These molecular events propagate to distant organs through circulating mediators, leading to multi-organ dysfunction syndrome.

Severe hemorrhage triggers a hemostatic paradox: local coagulation activation coincides with systemic fibrinolysis and a prothrombotic but functionally anticoagulant state due to massive cell loss and clotting factor dilution [5]. This coagulopathy, combined with metabolic acidosis and hypothermia, forms the "lethal triad" of hemorrhagic shock [3]. Simultaneously, shock triggers endocrine stress responses and organ-specific dysfunction: hepatic ischemia impairs synthetic and immune function, and renal hypoperfusion reduces glomerular filtration [9].

The protective effects of protein kinase C epsilon (PKC-ε) activation are primarily mediated through its action on mitochondria. Previous research has shown that PKC-ε activation leads to the phosphorylation and activation of aldehyde dehydrogenase-2, a critical enzyme that reduces toxic aldehyde accumulation during ischemia [10]. This signaling cascade has been reported to prevent the opening of the mitochondrial permeability transition pore, thereby preserving mitochondrial integrity, maintaining aerobic adenosine triphosphate (ATP) production, and suppressing apoptotic pathways during reperfusion [11].

We recently reported that PKC-ε activation improves survival and hemodynamic stability in a porcine hemorrhagic shock model with enhanced mitochondrial function [12]. That study focused on survival, hemodynamics, and mitochondrial integrity. The present analysis provides a comprehensive characterization of systemic laboratory biomarkers of multi-organ dysfunction in the same cohort, offering a complete biochemical profile of PKC-ε-mediated organ protection.

This study aims to characterize the systemic laboratory biomarker profile underlying the survival advantage of PKC-ε activation, with emphasis on acid-base balance, hepatic and renal function, cellular injury markers, coagulation parameters, and endocrine stress response across the 7-hour monitoring period.

METHODS

Animal Model and Ethical Approvals

The study was conducted as a prospective, non-randomized, unblinded preclinical trial at the Hadassah Medical Center's Department of Experimental Surgery and the Institute for Research in Military Medicine. The study was performed according to the Guide for the Care and Use of Laboratory Animals, was approved by the ethics committees of the Ministry of Defense and the Hebrew University Faculty of Medicine (NIH approval number OPRR-A01-5011), and is reported in accordance with the ARRIVE guidelines. We used 23 female domestic pigs (Sus scrofa domesticus), approximately 3 months old, weighing 41–50 kg, from the Laboratory Animal Farm, Lahav, Israel. Females were selected for technical

convenience during urinary catheter insertion. No sample-size calculation was performed; the number of animals was limited to the minimum required for an initial feasibility study [12]. Two additional animals that received the peptide without bleeding, for safety assessment, were not included in the analysis.

Anesthesia and Catheterization Protocol

Animals fasted for 12 hours prior to the experiment. Anesthesia began with primary sedation using xylazine and ketamine, followed by intravenous induction with diazepam, propofol, and tramadol. We maintained anesthesia with isoflurane (2%) in 100% oxygen under mechanical ventilation. For hemodynamic monitoring and sample collection, we catheterized the left carotid artery (for invasive arterial blood pressure and heart rate monitoring), the left femoral artery (for inducing hemorrhage and blood sampling), and the right jugular vein (for inserting a Swan-Ganz catheter and monitoring venous gases).

Hemorrhagic Shock Model and Intervention

Hemorrhagic shock was induced through controlled blood withdrawal from the femoral artery until 35% of the estimated total blood volume (65 mL/kg) was lost, by withdrawal of 50 mL every 90 seconds (bleeding duration 15–60 minutes, median 40), with the bleeding rate adjusted to maintain a mean arterial pressure above 30 mmHg. After the hemorrhage, a minimal fluid volume (100 mL Lactated Ringer's solution) was administered without returning blood products. The animals were divided into two groups: a control group (n=8), which underwent shock without medication, and a PKC-ε–treated group (n=15), which received a 3 mg/kg intramuscular dose of a PKC-ε activator (peptide sequence HDAPIGYD-GG-YGRKKRRQRRR, developed at Stanford University; manufactured by Ontores Biotechnologies, Hangzhou, China), supplied as a powder dissolved in 2 mL of water for injection and injected into the right gluteus maximus muscle five minutes after the start of hemorrhage. Control animals received no injection or placebo.

Data Collection and Follow-up

Continuous monitoring was performed for 7 hours following the end of the hemorrhage. No thermal support was provided. We collected blood samples at baseline, immediately post-bleeding, and hourly throughout the follow-up period. Evaluated parameters included biochemistry (liver and kidney function, electrolytes), complete blood count, coagulation profiles, and blood gases (pH, lactate, base excess [BE]).

Statistical Analysis

Data analysis was performed using SPSS Statistics for Windows, version 27.0 (IBM Corp., Armonk, NY, USA). Normality was assessed with the Kolmogorov–Smirnov test, and all tests were two-tailed. Cumulative survival was compared using the Kaplan–Meier model and the log-rank test. Laboratory parameters over time were compared using repeated-measures analysis of variance (ANOVA) to examine the interaction between time and group (PKC-ε–treated vs control), with Greenhouse–Geisser and Huynh–Feldt corrections for within-group effects. Baseline and post-bleeding values (Table 1) are reported for all 23 animals; the longitudinal analysis (Table 2) is restricted to the 19 animals alive at hour 5. Figures show mean ± standard error for all animals alive at each time point. Differences at specific time points were assessed using Student's t tests for normally distributed variables, and Mann-Whitney U tests otherwise, and associations were assessed using the Pearson product-moment correlation coefficient (r), with $P < 0.05$ considered statistically significant.

RESULTS

Parent Study Outcomes and Baseline Characteristics

This study presents a detailed biomarker sub-analysis of the hemorrhagic shock cohort reported by Simchoni et al. [12]. As previously reported, PKC-ε activator treatment was associated with significantly improved 7-hour survival (73.3% vs. 25.0%, P = 0.044 by log-rank test). Baseline and hemodynamic characteristics were comparable between the 15 PKC-ε–treated and 8 control animals, with complete details available in the parent study publication. Deaths occurred at 1:41, 3:08, 3:58 and 5:38 hours in the PKC-ε–treated group and at

0:23, 5:07, 5:16, 5:19, 5:48 and 6:23 hours in the control group, leaving 12 and 7 animals, respectively, at hour 5.

Baseline Biomarker Characteristics (Table 1)

Detailed analysis of baseline biochemical parameters revealed that the PKC-ε–treated and control groups were largely well balanced. Among all measured biochemical parameters, statistically significant baseline differences were observed in four variables: urea, partial thromboplastin time (PTT), O2%, and partial pressure of oxygen (PO2) (Table 1). At the post-bleeding point (0:00 hours), among the available data, no significant differences were detected between the two groups except for urea and platelets (PLT) (Table 1). However, since these differences existed at the start of follow-up, caution must be exercised when interpreting changes in urea and PLT over time, and the ability to attribute late-stage variations solely to the PKC-ε activator is limited.

Detailed Analysis of Biochemical and Physiological Parameters (Table 2)

Data were collected for approximately 45 parameters at nine consecutive time points (baseline, post-bleeding, 1:00, 2:00, 3:00, 4:00, 5:00, 6:00, and 7:00 hours). Given the large volume of data, the main parameters are reported here; the full data set is available from the corresponding author on reasonable request. For the primary longitudinal analysis, repeated-measures ANOVA was performed up to the 5th hour of follow-up. This time point was chosen because it offered the best balance between a relatively large number of surviving animals (n=19 overall; 12 in the PKC-ε–treated group and 7 in the control group) and a window long enough to demonstrate the acute effects of severe hemorrhagic shock.

Acid-Base Balance and Arterial Blood Gas Parameters (Figure 1)

Individual animal data showed that BE values in the control group were lower overall and followed a downward trajectory compared with the PKC-ε-treated group. At the group level, control group values declined continuously after the second hour, whereas the PKC-ε–treated group showed no such decline. The repeated measures model showed no significant isolated effect over time (Within time P = 0.172). However, there was a statistically significant group-by-time interaction (Time*Group P = 0.022), indicating that while both groups rose initially up to hour 2:00, the control group then entered a severe downward trend while the PKC-ε–treated group stabilized. For lactate, initial post-bleeding values were similar in both groups. Subsequently, the control group displayed a sharp, severe increase in blood lactate, whereas the PKC-ε–treated group exhibited a much more stable or downward trend. The repeated measures model up to hour 5:00 showed a statistically significant isolated change over time (Within time P = 0.017) and a significant group-by-time interaction (Time*Group P = 0.017), as the control curve continued its steep upward climb after the second hour. Initial bicarbonate ($HCO3^-$) levels were similar between the groups. Over time, the control group demonstrated a severe drop in bicarbonate levels, while the PKC-ε–treated group maintained stable levels or even showed a mild increase. The repeated measures model showed no significant isolated effect over time (Within-time P = 0.243) but demonstrated a statistically significant group-by-time interaction (Time*Group P = 0.031). Initial pH values were closely matched. Beginning two hours into the follow-up, the control group curve displayed a clear downward trend (increased systemic acidity), while the PKC-ε–treated group displayed a stable to slightly increasing pH trend. The absolute pH fluctuation was small. The repeated measures model up to hour 5:00 did not yield a statistically significant interaction (Time*Group P = 0.162) or an isolated effect over time (Within time P = 0.162). Between groups, pH differed significantly at hour 4:00 (P = 0.032) but not at hour 5:00 (P = 0.091).

Blood gases (PO2, partial pressure of carbon dioxide [PCO2], O2%) showed no statistically significant longitudinal changes over time, and no significant differences were detected between the PKC-ε–treated and control groups up to the 5^{th} hour of follow-up.

Cellular Damage and Organ Function (Figure 2)

In the control group, mean AST, ALT, and LDH rose from hour 4 onward, whereas values in the PKC-ε–treated group remained close to post-bleeding levels (Figure 2). No significant group-by-time interaction (Table 2) or

	Baseline			Post-bleeding (0:00)		
Parameter (unit)	**PKC-ε (n=15)**	**Control (n=8)**	**P**	**PKC-ε (n=15)**	**Control (n=8)**	**P**
Acid–base and blood gases						
Lactate† (mmol/L)	2.62 (2.30–3.22)	2.96 (2.72–3.14)	0.40	4.37 (3.65–4.66)	4.15 (3.70–5.80)	0.64
pH	7.47 ± 0.03	7.46 ± 0.03	0.49	7.43 ± 0.04	7.42 ± 0.03	0.66
Base excess (mmol/L)	4.9 ± 2.1	4.6 ± 2.3	0.78	3.7 ± 2.8	2.3 ± 2.0	0.23
HCO_3^- (mmol/L)	28.7 ± 2.3	28.8 ± 2.4	0.93	28.8 ± 3.4	27.1 ± 2.1	0.21
PCO_2 (mmHg)	40.4 ± 4.6	41.1 ± 3.2	0.68	44.4 ± 6.8	42.6 ± 3.4	0.50
PO_2† (mmHg)	176 (156–215)	217 (207–342)	**0.019**	171 (151–209)	177 (162–210)	0.59
O_2 saturation† (%)	99.6 (99.4–99.8)	99.8 (99.8–100.0)	**0.023**	99.6 (99.2–99.8)	99.6 (99.5–99.7)	0.73
Cellular injury and organ function						
AST† (U/L)	19 (17–29)	21 (18–28)	0.68	17 (14–25)	17 (15–24)	0.93
ALT† (U/L)	47 (38–58)	40 (38–52)	0.43	42 (34–50)	35 (34–44)	0.47
LDH† (U/L)	870 (818–980)	919 (873–966)	0.99	782 (713–856)	802 (777–874)	0.43
CPK† (U/L)	532 (498–643)	750 (589–1513)	0.087	495 (440–606)	670 (519–1788)	0.17
Creatinine† (µmol/L)	103 (97–113)	95 (90–101)	0.13	137 (129–153)	137 (128–144)	0.73
Urea† (mmol/L)	2.9 (2.5–3.5)	2.1 (1.8–2.5)	**0.016**	3.6 (3.0–4.2)	2.8 (2.4–3.0)	**0.047**
Coagulation						
Fibrinogen (mg/dL)	491 ± 63	493 ± 47	0.83	451 ± 53	449 ± 40	0.94
INR	0.85 ± 0.05	0.85 ± 0.06	0.95	0.84 ± 0.06	0.82 ± 0.04	0.57
PT (%)	138 ± 17	140 ± 22	0.80	143 ± 22	149 ± 16	0.52
PTT† (sec)	19.9 (18.9–21.3)	23.9 (22.0–25.3)	**<0.001**	18.7 (16.6–22.3)	20.3 (16.6–22.8)	0.66
Biochemistry and electrolytes						
Glucose (mmol/L)	7.1 ± 1.6	7.3 ± 2.0	0.80	10.4 ± 2.3	10.3 ± 2.8	0.95
Sodium† (mmol/L)	141 (140–142)	141 (140–143)	0.93	140 (138–141)	139 (139–141)	0.98
Potassium (mmol/L)	4.6 ± 0.5	4.9 ± 0.7	0.23	5.3 ± 0.6	5.8 ± 1.0	0.11
Calcium† (mmol/L)	2.64 (2.58–2.74)	2.61 (2.48–2.73)	0.50	2.54 (2.44–2.67)	2.57 (2.37–2.60)	0.47
Phosphorus (mmol/L)	2.71 ± 0.19	2.70 ± 0.17	0.93	2.99 ± 0.27	3.05 ± 0.19	0.60
Blood count						
Hemoglobin† (g/dL)	9.7 (9.4–10.6)	10.7 (10.2–11.1)	0.49	9.2 (9.0–9.8)	9.6 (9.4–10.1)	0.065
Hematocrit (%)	29.6 ± 2.7	30.4 ± 2.5	0.49	27.6 ± 1.8	28.3 ± 1.6	0.33
RBC ($\times 10^{12}$/L)	6.00 ± 0.55	6.32 ± 0.48	0.18	5.55 ± 0.35	5.87 ± 0.42	0.07
WBC ($\times 10^9$/L)	21.2 ± 6.3	20.0 ± 8.8	0.71	17.6 ± 5.8	16.3 ± 6.2	0.62
Platelets ($\times 10^9$/L)	321 ± 114	432 ± 133	0.056	316 ± 93	418 ± 134	**0.049**
Endocrine and physiological						
Cortisol (nmol/L)	296 ± 112	318 ± 58	0.61	352 ± 89	423 ± 74	0.07
Temperature (°C)	37.5 ± 1.1	37.7 ± 1.7	0.64	37.7 ± 0.9	38.0 ± 1.5	0.62
Urine volume† (mL)	150 (70–200)	180 (95–200)	0.58	22 (10–35)	27 (9–53)	0.83

Table 1. Biomarker values at baseline and after bleeding, by group. Mean ± SD, or median (IQR) where marked †. P values from t-test or Mann–Whitney U test; bold, P < 0.05. All 23 animals included. AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; CPK, creatine phosphokinase; INR, international normalized ratio; PT, prothrombin time; PTT, partial thromboplastin time; RBC, red blood cells; WBC, white blood cells.

between-group difference at any time point was detected for these markers (AST at hour 5:00: P = 0.094), and variability in the control group was large (AST at hour 5:00: 153 ± 253 U/L, n = 7). CPK did not follow this pattern in either group. Alkaline phosphatase, gamma-glutamyl transferase, and total bilirubin remained stable throughout follow-up in both groups; no significant changes over time or between groups were detected.

Parameter	P (time)	P (time × group)	*Hours	Direction
Acid–base and blood gases				
Lactate	0.017	**0.017**	2, 3, 4, 5	Lower in PKC-ε
Base excess	0.172	**0.022**	1, 2, 4, 5	Higher in PKC-ε
HCO_3^-	0.243	**0.031**	3, 4, 5	Higher in PKC-ε
pH	0.162	0.162	4	Higher in PKC-ε
PCO_2 / PO_2 / O_2 saturation	≥0.27	≥0.27	—	
Cellular injury and organ function				
AST	0.058	0.087	—	
ALT	0.199	0.131	—	
LDH	0.076	0.178	—	
CPK	0.362	0.221	—	
Creatinine	<0.001	0.090	—	
Urea	<0.001	0.324	1, 2, 3	Higher in PKC-ε (baseline difference)
Coagulation				
Fibrinogen	<0.001	0.386	—	
INR	0.022	0.326	—	
PT	<0.001	0.142	—	
PTT	0.352	0.490	—	
Biochemistry and electrolytes				
Glucose	<0.001	0.076	4, 5	Higher in PKC-ε
Phosphorus	<0.001	**0.014**	5	Lower in PKC-ε
Potassium	<0.001	0.113	—	
Sodium	0.019	0.752	—	
Calcium	0.052	0.575	—	
Blood count				
Hemoglobin	<0.001	0.819	—	
Hematocrit	<0.001	0.544	—	
RBC	<0.001	0.281	1	
WBC	<0.001	0.703	—	
Platelets	<0.001	0.281	—	
Endocrine and physiological				
Cortisol	<0.001	0.120	—	
Temperature	0.008	0.052	2, 3, 4, 5	Lower in PKC-ε
Urine volume	0.055	0.172	—	

Table 2. Longitudinal comparison of biomarkers through hour 5. Repeated-measures ANOVA in animals surviving to hour 5 (PKC-ε n=12, control n=7). Bold, P < 0.05. *Hours: time points with between-group P < 0.05.

Serum creatinine levels rose substantially and consistently in both study groups. The longitudinal change over time was significant (Within-time P < 0.001), following a quadratic and linear function. Based

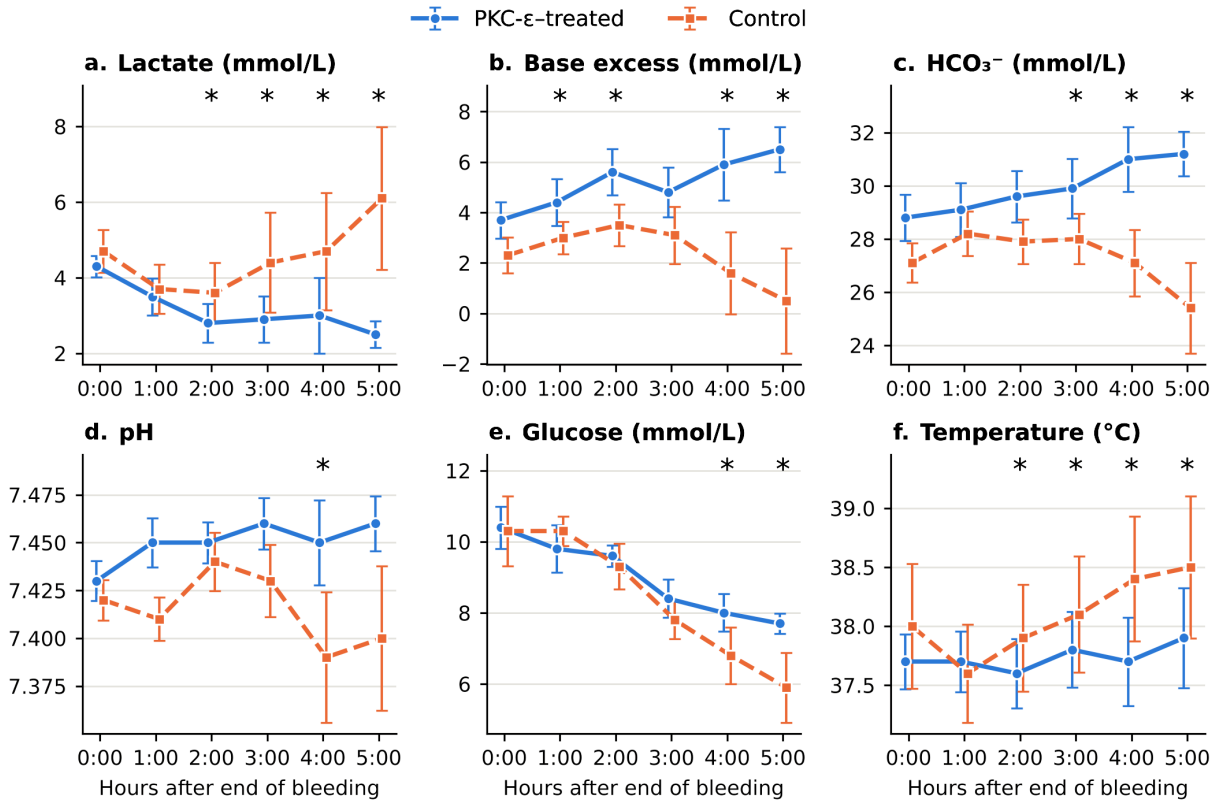


Figure 1. Metabolic and acid–base parameters after hemorrhage, by group. Mean ± standard error. *P < 0.05 between groups (Table 2). PKC-ε n=15 at 0:00 and 12 at 5:00; control n=8 at 0:00 and 7 thereafter.

on the available numbers, no significant difference was detected between the PKC-ε-treated and control curves (P = 0.28 at hour 5:00), indicating a similar rate of progressive renal impairment in both groups. For urea, both groups showed a clear upward trend over time. Although point-in-time differences were noted at multiple intervals, these differences most likely reflect the baseline differences already present after bleeding rather than a treatment effect.

Coagulation Functions

The mean fibrinogen values in the PKC-ε–treated group displayed a statistically significant upward trend over time starting one hour into follow-up (Within-time P = 0.001, following a quadratic and linear function). However, no significant difference was detected between the PKC-ε–treated and control groups at any follow-up interval up to hour 5:00. International normalized ratio (INR) values increased significantly across both study groups over the course of the monitoring period (Within-time P = 0.022, following a quadratic and linear function). No significant difference was detected between the PKC-ε-treated and control groups, and values generally remained within the normal physiological range. Longitudinal analysis of prothrombin time and PTT did not detect significant differences between the two study groups up to the 5th hour of follow-up.

Biochemistry and Electrolytes

Blood glucose levels in most animals across both groups demonstrated a continuous downward trend throughout the follow-up period. Both groups experienced a significant drop over time (within-time P < 0.001 up to the 5th hour, following a linear function). Statistically significant differences were found between the two groups at 4:00 (P = 0.026) and 5:00 (P = 0.047). The PKC-ε–treated group consistently maintained higher blood glucose levels than the control group at these later hours (Figure 1e). The group-by-time interaction was not significant (P = 0.076).

No significant point-in-time differences could be detected between the PKC-ε–treated and control groups for sodium (Na), chloride (Cl), calcium (Ca), or

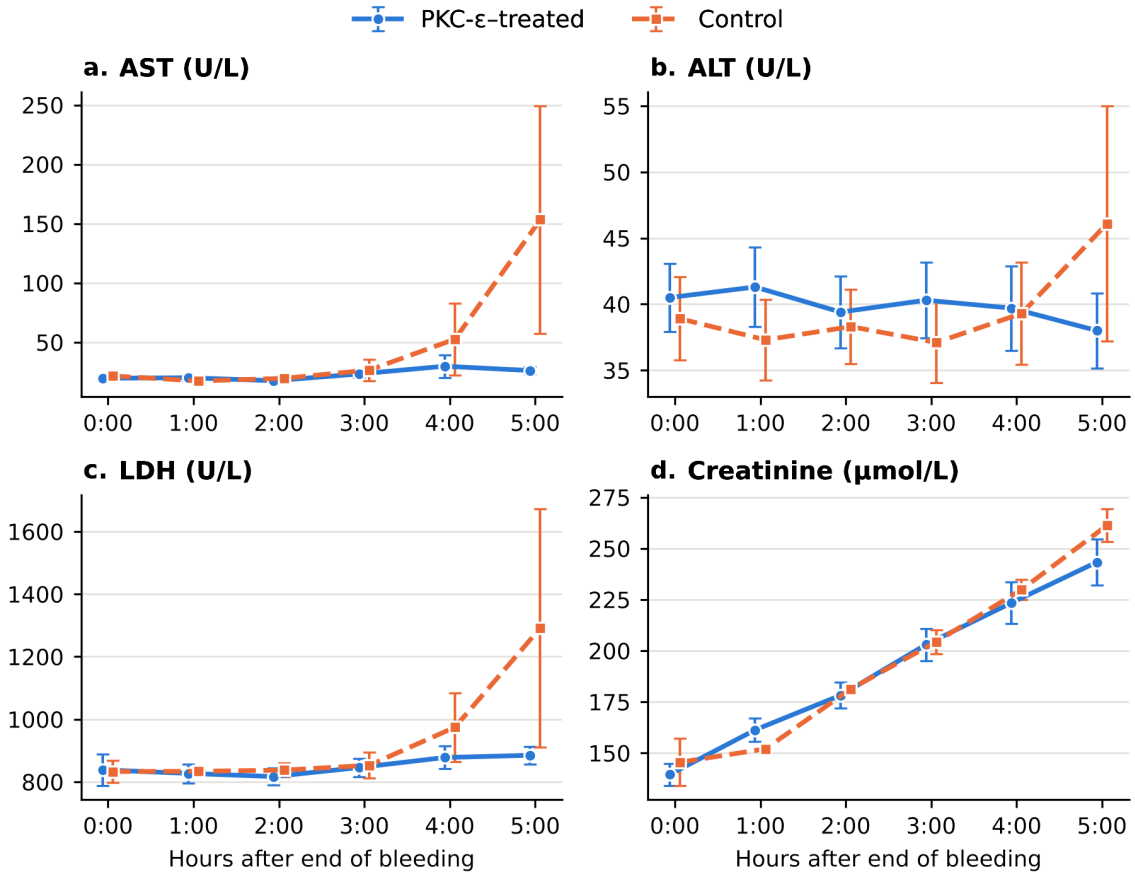


Figure 2. Markers of cellular injury and renal function after hemorrhage, by group. Mean ± standard error. AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase. No between-group difference was detected at any hour.

potassium (K) up to the 5-hour mark, or for phosphorus before hour 5:00. However, both potassium and phosphorus exhibited significant isolated increases over the timeline of the experiment across both groups ($P < 0.001$ for both). At hour 5:00, between-group testing for phosphorus was statistically significant ($P = 0.009$), indicating a higher value in the control group. The group-by-time interaction for phosphorus was also significant ($P = 0.014$).

Blood Count and Inflammatory Markers

Total leukocyte counts exhibited a significant, continuous linear upward trend over time across both groups (Within-time $P = 0.001$). No significant group-by-time interaction or between-group difference was detected. Total platelet counts demonstrated a significant downward trend over time across both groups (Within-time $P < 0.001$, following a quadratic and linear function). While point-in-time differences appeared between groups, they most likely reflect the baseline discrepancy already present after bleeding ($P = 0.049$) rather than a treatment effect. We detected no significant differences in red blood cell indices (red blood cell count [RBC], hemoglobin [HGB], hematocrit [HCT], and red cell distribution width) between the PKC-ε–treated and control groups. Significant isolated changes over time were present for HGB, RBC, and HCT ($P < 0.001$ for all): HCT trended upward, HGB values dropped sharply immediately after bleeding and recovered gradually, and absolute RBC counts increased slightly.

C-reactive protein levels remained effectively at zero throughout the entire experiment for all animals, demonstrating no change over time and no variance between groups.

Endocrine Parameters

Blood cortisol levels increased significantly over the course of the experiment across all animals (Within-time $P < 0.001$, following a quadratic and linear function). No significant difference was detected between the PKC-ε-treated and control groups at any time point. Thyroid-stimulating hormone remained at zero throughout the experiment, showing no change over time and no between-group variance.

Additional Physiological Measurements

Urine Volume: Hemorrhage caused a sharp drop in hourly urine output, which fell to a few milliliters per hour starting in the first hour of follow-up. No significant overall difference could be detected between the PKC-ε–treated and control groups under the fixed 5-hour cohort model.

Body Temperature: Rectal core temperature demonstrated a statistically significant upward trend over time across the cohort (Within time $P = 0.008$, following a linear function). Point-in-time testing revealed statistically significant differences between the two study groups at 2:00 ($P = 0.029$), 3:00 ($P = 0.036$), 4:00 ($P = 0.030$), and 5:00 ($P = 0.020$). At 1:00, the difference was not statistically significant ($P = 0.060$). At all these intervals, the core temperature of the pigs in the PKC-ε–treated group was lower than that of the control group (Figure 1f). The group-by-time interaction was not statistically significant ($P = 0.052$).

Correlation Analysis Between Parameters (Table S2)

We calculated bivariate Pearson correlation coefficients at three time points: baseline, post-bleeding, and last measurement. The following relationships were found:

Late-Stage Injury Clustering: At the final measurement (Last Measure), we identified a strong, statistically significant positive correlation ($P < 0.01$) among four parameters: lactate, LDH, AST, and ALT ($r = 0.59–0.93$); CPK and creatinine were not correlated with these markers. These markers therefore rose together, consistent with concurrent hepatic and cellular injury.

Metabolic/Acid-Base Alignment: At the last measurement, glucose, pH, $HCO3^-$ and BE were positively correlated with one another ($r = 0.46–0.92$; all $P < 0.05$); across all three time points, only the BE–$HCO3^-$ and BE–pH correlations remained significant. These four parameters declined together during systemic shock.

Lactate and Acid-Base Inverse Relationship: At the last measurement, lactate was negatively correlated with glucose ($r = −0.725$), pH ($r = −0.677$), $HCO3^-$ ($r = −0.790$), and BE ($r = −0.775$; all $P < 0.01$). This pattern is consistent with lactic acid accumulation accompanying depletion of base excess, consumption of bicarbonate buffers, and a fall in systemic pH.

Cellular Damage and Acidity Link: The tissue damage markers LDH, AST, and ALT were negatively correlated with BE, $HCO3^-$, and pH at the last measurement ($r = −0.54$ to $−0.80$; all $P < 0.01$). Markers of tissue damage were therefore closely associated with the degree of metabolic acidosis.

DISCUSSION

This comprehensive biomarker analysis characterizes the systemic biochemical response accompanying PKC-ε activation in a porcine hemorrhagic shock model. PKC-ε activation, previously demonstrated to improve survival [12], was accompanied by coordinated preservation of metabolic homeostasis while markers of hepatic, muscle, and renal injury did not differ significantly between groups.

Acid-Base Parameters and Metabolic Acidosis

The most consistent treatment-related differences emerged in acid-base parameters. Control animals demonstrated progressive metabolic acidosis with declining base excess, decreasing bicarbonate levels, and rising lactate concentrations, with sharp accumulation beginning at hour 2. PKC-ε–treated animals maintained stable acid-base parameters throughout monitoring, with significantly lower lactate levels at hours 2 to 5 (all $P \leq 0.038$). The lactate pattern in PKC-ε-treated animals - stabilization and apparent decline in later hours - suggests restoration of mitochondrial oxidative capacity and lactate clearance, consistent with previously reported hemodynamic preservation and improved survival [5,11].

The relationship between lactate accumulation and acid-base derangement is mechanistically integrated: lactate-associated tissue pH decline consumes bicarbonate buffering capacity while reducing pH and

base excess [1]. Correlation analysis showed strong inverse associations between lactate and all acid-base markers at the last measurement (pH, HCO_3^-, BE; all $P < 0.01$), reflecting a tight coupling between lactate dynamics and acid-base stability [13].

Lactate production and clearance were not measured directly; the observed lactate trajectory is consistent with reduced anaerobic production during hypoperfusion and restored clearance thereafter. This specific pattern aligns with the known protective mechanisms of PKC-ε activators, which enhance mitochondrial integrity by suppressing apoptotic cascades, preventing reactive oxygen species accumulation [13,14], inhibiting the mitochondrial permeability transition pore, and maintaining aerobic ATP production [8].

Coagulation Parameters

Fibrinogen demonstrated a statistically significant upward trend in PKC-ε–treated animals beginning at hour 1 ($P = 0.001$ within-time). However, no significant differences between groups could be detected in any other coagulation parameter, with values remaining near physiological ranges despite severe hemorrhage. The absence of differential coagulation effects is noteworthy: hemorrhagic shock typically induces a prothrombotic but functionally anticoagulant state due to massive clotting factor dilution and pathologic fibrinolysis [5,15]. PKC-ε activation neither exacerbated coagulopathy nor produced selective coagulation protection, suggesting that the survival advantage is unlikely to be explained by hemostatic modulation.

Cellular and Organ Injury Markers

Cellular injury markers (AST, ALT, LDH, CPK) showed diverging trajectories between groups that did not reach statistical significance. The control group showed continuous escalation of hepatic and muscle injury markers, while the PKC-ε–treated group remained moderate and stable. Although individual time point comparisons in the fixed 5-hour analysis did not achieve statistical significance, the direction of the trajectories is consistent with previous studies that showed PKC-ε activation protects various tissues - such as myocardium [10,11], hepatocytes, and renal tubules [16] - against ischemic and reperfusion injury.

Serum creatinine and urea increased significantly in both groups, reflecting the severe hypoperfusion state [4]. No significant difference could be detected between the PKC-ε–treated and control groups, indicating a similar acute trajectory of renal impairment in both groups. While an observational trend toward higher hourly urine output was noted in PKC-ε-treated animals - particularly in the early post-hemorrhage period compared with the near-anuria in control animals - this difference did not reach statistical significance in the fixed-cohort analysis. Although this trend might cautiously suggest preserved renal perfusion physiology, consistent with previously reported hemodynamic preservation [10], the current data remain insufficient to conclude improved renal outcomes definitively. The potential for long-term renal protection by PKC-ε activation deserves further investigation in larger cohorts with extended monitoring to determine whether these early physiological trends translate into a clinically meaningful benefit.

Integrated Metabolic-Organ Injury Axis

Correlation analysis highlights a close link between metabolic derangement and markers of cellular injury. At the last measurement, lactate was positively, and pH, BE, and HCO_3^- negatively, correlated with AST, ALT, and LDH (all $P < 0.01$), whereas CPK and creatinine were not correlated with any of the metabolic markers. This metabolic-organ injury axis reveals an integrated pathophysiology in which lactate-driven acidosis and end-organ injury are tightly coupled; consequently, interrupting metabolic decline would be expected to limit cellular injury. A plausible mechanism is that lactate-induced tissue pH decline impairs cellular enzymatic function, promotes apoptotic and necrotic pathways, and depletes cellular ATP reserves [1,12]. Conversely, maintenance of aerobic metabolism and acid-base stability preserves organ ATP availability, suppresses apoptosis, and prevents ischemia-reperfusion cascades. The correlation among AST, ALT, and LDH suggests that hepatic and cellular injury progress together during hemorrhagic shock, highlighting the need for systemic rather than organ-specific interventions.

PKC-ε activation appears to act primarily at the mitochondrial level [8,12] - the ultimate arbiter of

aerobic ATP production. By enhancing mitochondrial integrity and preventing permeability transition pore opening [12], PKC-ε preserves oxidative phosphorylation capacity, resulting in three interconnected consequences: (1) lower lactate production during hypoperfusion (fewer cells forced into anaerobic metabolism), (2) efficient lactate clearance during reperfusion (mitochondrial oxidative machinery fully functional), and (3) reduced metabolic acidosis as shown in this study. This proposed sequence - preserved mitochondrial function, stable acid-base status, less acidosis-driven cellular injury, preserved organ function, and improved survival - remains a hypothesis that the present data cannot test.

Limitations. This analysis has several limitations. The modest sample size (n=23) limits statistical power; although survival was significant, other parameters require cautious interpretation. The porcine model exhibits substantial physiologic differences from human hemorrhagic trauma, particularly in metabolic rate and coagulation physiology. Isoflurane anesthesia has independent metabolic effects; the degree to which anesthetic-pharmacologic interactions affected outcomes remains uncertain. Observation was limited to 7 hours post-hemorrhage, capturing only the acute phase; extended monitoring would clarify whether early protection sustains multi-organ recovery. The model employed minimal fluid resuscitation (100 mL Lactated Ringer's solution) rather than damage-control resuscitation with blood products, potentially limiting clinical applicability. Finally, the female-only cohort limits generalizability to both sexes. Allocation to groups was not randomized, investigators were not blinded to group allocation, and control animals received no vehicle injection. This is also a secondary analysis of a previously published cohort: the biomarker outcomes reported here were not designated primary outcomes, no sample-size calculation for them is reported, and no adjustment was made for the large number of parameters and time points tested, so the results should be read as exploratory and hypothesis-generating. The longitudinal analysis was further restricted to animals surviving to hour 5, so differential mortality between the groups may have shaped the cohorts that were compared.

CONCLUSIONS

This comprehensive biomarker analysis demonstrates that PKC-ε activation's survival benefit is accompanied by coordinated preservation of acid-base homeostasis, attenuation of lactate accumulation with evidence of metabolic recovery, while markers of cellular and hepatic injury did not differ significantly between groups. No difference in coagulation parameters was detected between the groups. The observed biomarker correlations are consistent with a metabolic-organ injury axis in which lactate-driven acidosis and end-organ injury move together; whether interrupting acidosis reduces organ injury was not tested here. These findings describe the biochemical profile accompanying the previously reported survival advantage and support further investigation of PKC-ε activation as an intervention that may act through mitochondrial preservation and coordinated metabolic-organ protection in severe hemorrhagic shock.

Disclaimer: The views expressed in the submitted article are the authors' own and not an official position of the IDF or the IMA.

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