COMPARATIVE EVALUATION OF EXPERIMENTAL INJURY MODELS FOR ASSESSING SYSTEMIC LACTATE RESPONSE

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COMPARATIVE EVALUATION OF EXPERIMENTAL INJURY MODELS FOR ASSESSING SYSTEMIC LACTATE RESPONSE (Abstract): Experimental models of tissue injury may differ in their ability to obtain a measurable systemic metabolic response. The **aim** of our study was to compare systemic lactate dynamics across three trauma models-muscular, vascular, and bone trauma-and to assess their suitability for investigating circulating lactate as a marker of metabolic response. **Materials and methods:** Male Wistar rats were allocated to three groups: skeletal muscle ischemia-reperfusion, vascular trauma, and tibial bone injury (n = 11/group). Blood lactate was measured at three time points (T1-T3). Lactate peak, final concentration, clearance, and temporal slope were calculated and compared between models. Non-parametric statistical methods were applied because of deviations from normality. **Results:** The three models generated distinct lactate trajectories. In the muscular trauma group, lactate changed from 2.19 ± 0.67 mmol/L at T1 to 1.44 ± 0.84 mmol/L at T2 and increased to 3.33 ± 1.15 mmol/L at T3 (Friedman $\chi^2 = 14.73$, $p < 0.001$). This model showed negative lactate clearance (−59.0 ± 40.3%) and a positive temporal slope (+0.57 ± 0.46). Conversely, vascular and bone trauma resulted in lower final lactate concentrations (0.98 ± 0.56 and 1.14 ± 0.18 mmol/L), positive clearance (+22.8 ± 27.0% and +27.6 ± 12.5%), and negative temporal slopes. **Conclusions:** Systemic lactate kinetics depend on injury type. Muscular trauma produced the most pronounced and sustained response, characterized by late lactate elevation and negative clearance, whereas vascular and bone trauma showed progressive lactate reduction. These findings support careful model selection when circulating lactate is used to investigate systemic metabolic responses to tissue injury. **Keywords:** LACTATE, EXPERIMENTAL MODEL, ISCHEMIA-REPERFUSION, SKELETAL MUSCLE, TISSUE INJURY.

INTRODUCTION

Lactate is widely used as a marker of metabolic disturbance in acute and critical conditions, including trauma. Although elevated circulating lactate has traditionally been associated with impaired tissue oxygenation and anaerobic metabolism, its biological significance is more complex. Blood lactate concentration reflects the balance between production, utilization, and clearance and may increase through several mechanisms other than systemic hypoxia (1, 2). Lactate is also increasingly recognized as an active metabolic substrate involved in intercellular and inter-organ energy transfer rather than simply as an end

product of anaerobic glycolysis (3).

In trauma, circulating lactate has been investigated both as an indicator of metabolic stress and as a prognostic marker. Clinical studies have shown associations between elevated lactate and injury severity or adverse outcomes, while systematic reviews support its value as an additional marker in the assessment of trauma patients (4, 5). However, an isolated lactate value provides only a limited representation of an evolving metabolic process. Serial measurements and lactate clearance may better characterize changes occurring over time (6, 7, 8).

Experimental models offer the advantage of investigating these responses under controlled conditions. Nevertheless, the systemic metabolic response may depend on the type of tissue affected and on the mechanism of trauma. Skeletal muscle represents a large and metabolically active compartment, and experimental ischemic muscle injury may generate substantial local and systemic metabolic alterations (9, 10). Conversely, vascular and bone trauma involve different tissue compartments and injury mechanisms and may therefore produce different circulating lactate patterns.

Direct comparison of different experimental trauma models may consequently be useful when selecting a model for studies in which systemic lactate is the principal outcome. Such comparisons also contribute to a more accurate interpretation of experimental findings, since biomarker responses cannot necessarily be extrapolated from one type of trauma to another. Careful characterization and transparent reporting of experimental models are essential for reproducibility and appropriate interpretation of animal studies (11).

The aim of the present study was to compare systemic lactate dynamics across three experimental trauma models-muscular, vascular, and bone trauma-and to evaluate their relative suitability for studying circulating lactate as a marker of metabolic response. We hypothesized that the three models would generate different lactate profiles and that these differences could identify the model producing the most distinct systemic metabolic response

MATERIALS AND METHODS

Experimental animals and study design

Adult male Wistar rats, approximately 12 weeks of age, were used in this experimental study. Animals were allocated to three experimental trauma models according to the predominantly affected tissue: *muscular trauma* (n = 11), *vascular trauma* (n = 11), and *bone trauma* (n = 11). A control group (n = 6) and corresponding sham-operated groups (n = 3 for each trauma model) were also included.

All experimental procedures were performed under general anesthesia. Anesthesia was induced with 5% isoflurane and maintained with 1.8-2.5% isoflurane throughout the intervention. Analgesia was administered according to the established experimental protocol.

Experimental trauma models

Three standardized experimental models were used to reproduce distinct types of peripheral trauma (fig.1).

In the *muscular trauma model*, a localized ligature was applied to the hindlimb skeletal muscle, producing controlled muscular ischemia. The ligature was maintained for 3 hours and was subsequently released to allow reperfusion.

In the *vascular trauma model*, the femoral vascular structures were surgically exposed and temporarily clamped, resulting in interruption of regional blood flow. After a 3-hour period, the clamp was removed and reperfusion was initiated.

In the *bone trauma model*, a standardized experimental tibial fracture was produced, generating predominantly osseous injury. The model was designed to reproduce direct skeletal trauma and its associated systemic metabolic response.

Thus, the three models represented distinct mechanisms of peripheral trauma. Sham-operated animals underwent the corresponding surgical exposure and manipulation without induction of the target trauma.

Blood sampling and lactate assessment

Serial blood samples were collected at three predefined time points. T1 was obtained 3 hours after trauma induction, immediately before reperfusion. T2 was collected approximately 15 minutes after reperfusion, and T3 was obtained 2 hours after reperfusion. At each time point, blood gas and metabolic parameters were measured, including lactate, partial pressure of oxygen (PO_2), glucose, hematocrit (Hct), and calculated oxygen saturation (SO_2c).

For the present study, circulating lactate was considered the primary outcome. Lactate concentrations were evaluated at T1, T2, and T3. Additional parameters were derived to characterize the metabolic response associated with each experimental trauma model, including peak lactate concentration, final lactate concentration, lactate clearance, and temporal lactate slope.

Lactate clearance was expressed as the percentage change between the initial and final measurements. Positive clearance indicated a decrease in circulating lactate during the observation period, whereas negative clearance reflected an increase relative to the initial concentration. The temporal slope was used as a descriptive measure of the overall direction of lactate change across the three sampling points.

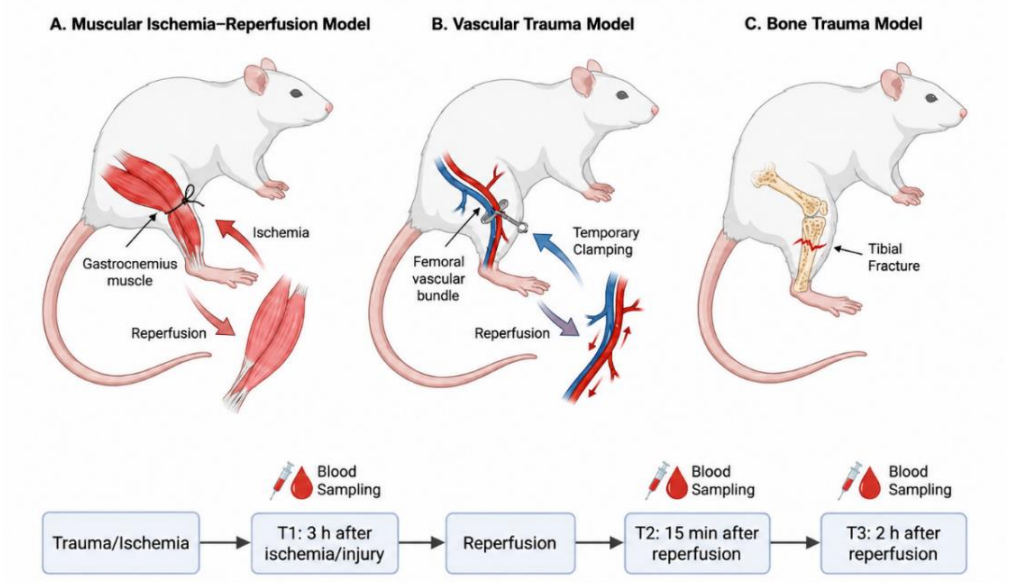

Fig. 1. Experimental trauma models used in the study. Schematic representation of the three experimental models: (A) muscular trauma induced by localized skeletal muscle ligation; (B) vascular trauma induced by temporary femoral vascular clamping; and (C) bone trauma induced by experimental tibial fracture (Created with Biorender.com)

The primary analysis focused on the comparison of lactate kinetics among the muscular, vascular, and bone trauma models.

Statistical analysis

Data distribution was assessed using the Shapiro-Wilk test. Because several variables showed deviations from normality, non-parametric statistical methods were used. Serial changes within each experimental group were evaluated using the Friedman test for comparisons across T1, T2, and T3. Selected paired comparisons were performed using the Wilcoxon signed-rank test.

Between-group comparisons were performed using the Mann-Whitney U test, with adjustment for multiple comparisons where appropriate. Spearman's rank correlation coefficient was used to assess associations between continuous variables when relevant.

Continuous variables are presented as mean ± standard deviation. A two-sided p value < 0.05 was considered statistically significant.

Ethical considerations

All procedures were conducted in accordance with institutional and national regulations governing the care and use of laboratory animals. The experimental protocol was approved by the Ethics Committee of Grigore T. Popa University of Medicine and Pharmacy, Iași, Romania (Approval No. 505/21 December 2024).

RESULTS

Serial lactate dynamics

The three experimental trauma models showed different temporal lactate profiles (fig. 2). In the *muscular trauma group*, mean lactate concentration was 2.19 ± 0.67 mmol/L at T1, decreased to 1.44 ± 0.84 mmol/L at T2, and subsequently increased to 3.33 ± 1.15 mmol/L at T3. The overall change across the three sampling points was statistically significant (Friedman χ^2 = 14.73, p < 0.001).

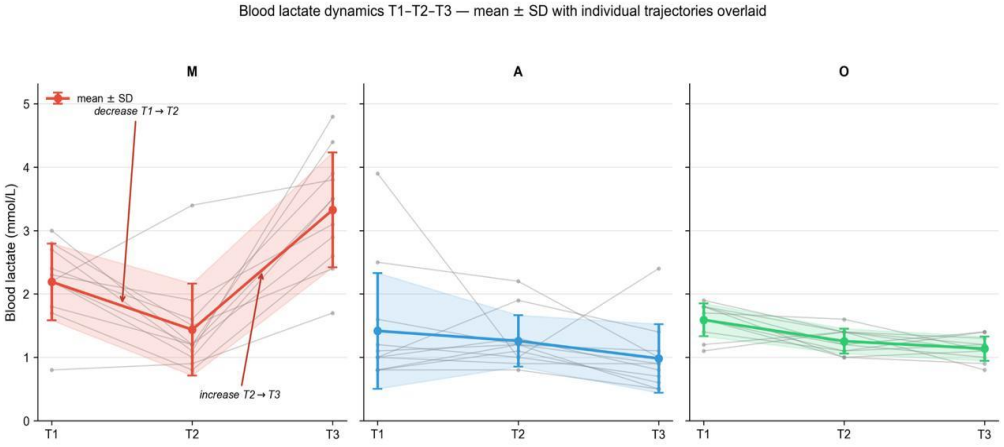

Fig. 2. Systemic lactate dynamics across the three experimental trauma models. Serial blood lactate concentrations at T1 (3 h after trauma induction, immediately before reperfusion), T2 (15 min after reperfusion), and T3 (2 h after reperfusion) in the muscular, vascular, and bone trauma groups. Values are presented as mean ± standard deviation.

In contrast, the *vascular trauma* and *bone trauma* groups showed a different pattern, with lower final lactate concentrations at T3. Final lactate was 0.98 ± 0.56 mmol/L in the vascular trauma group and 1.14 ± 0.18 mmol/L in the bone trauma group, compared with 3.33 ± 1.15 mmol/L in the muscular trauma group.

These findings indicate a late increase in circulating lactate after muscular trauma, whereas lactate tended to decrease over time in the vascular and bone trauma models.

Lactate-derived parameters

The differences between trauma models were also apparent when lactate-derived kinetic parameters were examined. The muscular trauma group had the highest peak lactate concentration, 3.37 ± 0.87 mmol/L, compared with 1.58 ± 0.86 mmol/L in the vascular trauma group and 1.64 ± 0.18 mmol/L in the bone trauma group (tab. I).

TABLE I.

Lactate kinetic parameters according to experimental trauma model. Data are presented as mean ± standard deviation.

Parameter	Muscular trauma	Vascular trauma	Bone trauma
Peak lactate (mmol/L)	3.37 ± 0.87	1.58 ± 0.86	1.64 ± 0.18
Final lactate (mmol/L)	3.33 ± 1.15	0.98 ± 0.56	1.14 ± 0.18
Lactate clearance (%)	−59.0 ± 40.3	+22.8 ± 27.0	+27.6 ± 12.5
Temporal slope	+0.57 ± 0.46	−0.22 ± 0.26	−0.22 ± 0.10

Lactate clearance was negative in the muscular trauma group (−59.0 ± 40.3%), indicating an increase in lactate between the initial and final assessments. In contrast, clearance was positive in both the vascular (+22.8 ± 27.0%) and bone trauma (+27.6 ± 12.5%) groups. The same divergence was observed for the temporal slope: +0.57 ± 0.46 in the muscular trauma group, compared with −0.22 ± 0.26 in the vascular trauma group and −0.22 ± 0.10 in the bone trauma group.

The metabolic response observed after muscular trauma was also evaluated in relation to the corresponding sham group. Lactate concentrations were higher in the muscular trauma group at all three sampling points: 2.19 versus 0.93 mmol/L at T1 (p = 0.042), 1.44 versus 0.83 mmol/L at T2 (p = 0.034), and 3.33 versus 0.83 mmol/L at T3 (p = 0.013). The largest difference was observed at the final sampling point. Muscular trauma was characterized by a distinct late lactate rise, negative clearance, and a positive temporal slope, whereas vascular and bone trauma showed lower final lactate concentrations together with positive clearance and declining temporal trends.

DISCUSSION

The present study showed that systemic lactate kinetics differed according to the experimental trauma model. Muscular trauma generated the most distinct temporal profile, with a late increase in circulating lactate, negative clearance, and a positive temporal slope. In contrast, vascular and bone trauma were characterized by lower final lactate concentrations, positive clearance, and declining temporal trends. These observations reinforce the concept that circulating lactate reflects a dynamic balance between production, utilization, and elimination rather than serving as a

specific indicator of tissue hypoxia alone (1, 2, 3).

The more marked response associated with muscular trauma is biologically plausible. Skeletal muscle constitutes a large metabolically active tissue compartment, and ischemic injury followed by reperfusion can produce substantial metabolic alterations. Experimental studies have demonstrated that skeletal muscle damage is strongly influenced by the duration of ischemia and reperfusion (9). More recently, Gallo de Carvalho *et al.* demonstrated substantial skeletal muscle injury after relatively short periods of ischemia and reperfusion in a rat model, supporting the sensitivity of muscular tissue to this type of experimental injury (10). The present results are consistent with this susceptibility, although our study was designed to compare systemic lactate patterns rather than to establish the cellular mechanisms responsible for lactate elevation.

An important finding was the biphasic lactate pattern observed in the muscular trauma group, with an initial decrease after reperfusion followed by a subsequent increase. Consequently, interpretation based only on the early post-reperfusion value would have provided a substantially different picture from that obtained through serial assessment. This observation supports the growing emphasis on lactate kinetics rather than isolated measurements. Serial lactate measurements and changes in lactate clearance can provide additional information about the direction of metabolic evolution over time (6, 7, 8). Clinical trauma studies similarly indicate that failure of elevated lactate to decline is associated with unfavorable outcomes, illustrating the biological relevance of the temporal pattern itself (12, 13).

The lower final lactate concentrations and positive clearance observed in the vascular and bone trauma models further emphasize that experimental model selection can influence the systemic biomarker response. Clinical evidence confirms the usefulness of lactate in trauma assessment, but also demonstrates substantial heterogeneity related to sampling time, injury characteristics, and patient context (4, 5). In an experimental setting, these sources of variability can be reduced, allowing the influence of the trauma model itself to be examined more directly. Our findings therefore suggest that muscular trauma may be particularly informative when systemic lactate kinetics constitute the primary experimental outcome.

The study has several limitations. The number of animals in each group was limited, and lactate was assessed over a relatively short observation period. Furthermore, the three models differed not only in the predominant tissue affected but also in the mechanism by which trauma was induced. The results should therefore be interpreted as a comparative characterization of these specific experimental models rather than as evidence of universal tissue-specific lactate behavior. Nevertheless, the clearly different lactate trajectories indicate that the choice of experimental trauma model should be considered when designing studies focused on systemic metabolic biomarkers.

CONCLUSIONS

Systemic lactate kinetics differed among the three experimental trauma models. Muscular trauma produced a distinct late increase in circulating lactate, together with negative clearance and a positive temporal slope, whereas vascular and bone trauma showed declining lactate trends and

positive clearance. These findings highlight the influence of experimental model selection on systemic lactate responses and support the muscular trauma model as an informative approach for further investigation of post-traumatic lactate dynamics.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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