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Importance of catecholamine signaling in the development of platelet exhaustion after traumatic injury

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Abstract

Background: Impaired *ex vivo* platelet aggregation is common in trauma patients. The mechanisms driving these impairments remain incompletely understood, but functional platelet exhaustion due to excessive *in vivo* activation is implicated. Given platelet adrenoreceptors and known catecholamine surges after injury, impaired *ex vivo* platelet aggregation in trauma patients may be linked to catecholamine-induced functional platelet exhaustion.

Objective: To determine the relationship of catecholamines with platelet-dependent hemostasis after injury and to model catecholamine-induced functional platelet exhaustion in healthy donor platelets.

Patients/Methods: Whole blood was collected from 67 trauma patients as part of a prospective cohort study. Platelet aggregometry and rotational thromboelastometry were performed, and plasma epinephrine (EPI) and norepinephrine (NE) concentrations were measured. The effect of catecholamines on healthy donor platelets was examined in a microfluidic model, with platelet aggregometry, and by flow cytometry examining surface markers of platelet activation.

Results: In trauma patients, EPI and NE were associated with impaired platelet aggregation (both $p < 0.05$), and EPI was additionally associated with decreased viscoelastic clot strength, increased fibrinolysis, and mortality (all $p < 0.05$). In healthy donors, short duration incubation with EPI enhanced platelet aggregation, platelet adhesion under flow, and increased glycoprotein

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AUTHOR CONTRIBUTION

ZAM and LZK performed study design, data analysis, data interpretation and writing of the manuscript. ZAM and ATF performed measurements of catecholamines in trauma samples performed by CJ, ZAM, and AL. Catecholamine experiments with healthy donor platelets and whole blood were performed by ZAM and ATF. Clinical data collection and interpretation was performed by BNG and JJP. RAC, AL, CMH and MAM assisted with data interpretation. All authors reviewed and provided critical revisions of the manuscript.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

SUPPORTING INFORMATION

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IIb/IIIa activation, while weaker effects were observed with NE. Compared with short incubation, longer incubation with EPI resulted in decreased platelet adhesion, platelet aggregation, and surface expression of glycoprotein IIb/IIIa.

Conclusions: These findings suggest sympathoadrenal activation in trauma patients contributes to impaired *ex vivo* platelet aggregation, which mechanistically may be explained by a functionally exhausted platelet phenotype under prolonged exposure to high plasma catecholamine levels.

Keywords

blood coagulation disorders; catecholamines; platelet activation; platelet aggregation; platelets; trauma

1 | BACKGROUND

Trauma-induced coagulopathy (TIC) exacerbates hemorrhage, promotes thrombo-inflammatory complications, and contributes to mortality in approximately one quarter of patients who are critically injured and hemorrhaging.[1-4] Impaired *ex vivo* platelet aggregation is commonly identified in patients with TIC, and is considered a central contributor to TIC-associated morbidity and mortality.[4] However, data support variable associations of impaired *ex vivo* platelet aggregation and mortality after injury,[5-7] and this *ex vivo* aggregation biology has been demonstrated even in patients who are minimally injured and not hemorrhaging,[8] and is not recovered by the transfusion of platelets.[9,10] Additionally, several investigators have identified an apparent biological discordance of increased markers of platelet activation yet impaired aggregation behavior *ex vivo* in injured patients.[5,11,12] Our knowledge of the explanatory endogenous biology and clinical relevance of these paradoxical *ex vivo* findings remains incomplete.[5,7]

Many have hypothesized that functional platelet exhaustion is one mechanism that may contribute to the impaired *ex vivo* platelet aggregation identified in injured patients. The mechanistic theory of functional platelet exhaustion is that in major trauma and hemorrhagic shock, the extensive tissue injury, vascular breach, endothelial cell damage, and associated release of platelet-activating soluble mediators may lead to a robust population of circulating activated platelets *in vivo*.[13-17] Once platelets are endogenously activated and degranulated, they will display surface markers of activation but may be unable to respond properly to *ex vivo* stimulation, thereby rendering them impaired in *ex vivo* measurements of aggregation.[4,11,18] Several specific mediators have been implicated in driving these changes in platelet activation and aggregation, including release of tissue factor, thrombin generation, microparticles, and damage-associated molecular patterns.[14-16,19-21] However, further work is needed to identify soluble mediators released after injury and their impact on specific platelet signaling mechanisms that may cause altered activation and aggregation.[11,13,16,17]

Catecholamine signaling through platelet adrenoreceptors is one pathway that may be integral in alterations in platelet aggregation after injury, but has not been well described. This pathway is of particular interest given known platelet adrenoreceptors, evidence of surges in catecholamines after injury,[22] involvement of sympathoadrenal activation in TIC

pathophysiology,[23,24] and the recent evidence suggesting a benefit to vasopressor use for adjunctive hemodynamic support during resuscitation of patients with hemorrhaging trauma.[25] Catecholamines are demonstrated to enhance *ex vivo* platelet aggregation in healthy donor blood,[26] but the platelet response to catecholamines at endogenous concentrations, with prolonged exposure, and under physiologic flow conditions akin to trauma physiology is lacking.

In this study, we aimed to characterize the relationship of plasma catecholamine concentrations with *ex vivo* platelet aggregation in whole blood samples obtained from trauma patients before resuscitation. We then examined catecholamine effects on healthy donor platelets under physiologic flow conditions and with increasing duration of catecholamine exposure across a range of concentrations observed in states of physiologic stress. Given the theory of functional platelet exhaustion, we hypothesized that increased *in vivo* plasma catecholamine concentrations in trauma patients would be associated with impaired *ex vivo* platelet aggregation. We additionally hypothesized that healthy donor platelets treated with catecholamines would enhance platelet aggregation initially, but that this effect would diminish with prolonged catecholamine exposure, recapitulating a functionally exhausted platelet phenotype.

2 | METHODS

2.1 | Trauma patient assays

2.1.1 | Inclusion/exclusion criteria and sample collection—Pre-resuscitation citrated whole blood samples were collected from trauma patients on arrival to the emergency department as part of a longitudinal study evaluating platelet biology after trauma (2017–2020).[7,27] Sixty-seven patients, not on anticoagulant or antiplatelet agents, who had matched timepoint platelet aggregometry and viscoelastic assay results with banked plasma were included. These patients encompassed a range of injury severity [injury severity score (ISS)] and degree of shock (base excess). Comprehensive demographics and clinical data were prospectively collected in parallel on all patients, including the development of multiple organ failure defined by Denver Criteria[28] and mortality at hospital discharge. The study was approved by the institutional review board at the University of California, San Francisco and informed consent was obtained from all participants.

2.1.2 | Platelet aggregometry—Platelet aggregometry was performed with the Multiplate multiple electrode aggregometer according to the manufacturer's protocol (Verum Diagnostica). Briefly, platelets were stimulated to aggregate in pre-resuscitation recalcified citrated whole blood at 37°C with adenosine diphosphate (ADP, final concentration 6.5 μM; via P2 receptors) and collagen (final concentration 3.2 μg/ml; via GpIa/IIa and GpVI receptors). Platelet aggregation was quantified as area under the aggregation curve (AUC) over 6 min with reference ranges corresponding to the 5th–95th percentiles in healthy adults of 36–101 and 24–79 AUC in response to ADP and collagen, respectively.

2.1.3 | Rotational thromboelastometry—Rotational thromboelastometry (ROTEM) was performed using pre-resuscitation recalcified citrated whole blood at 37°C with the

ROTEM delta machine (Pentapharm) and EXTEM reagent according to the manufacturer's protocol. Clotting time (CT) in seconds, clot formation time (CFT) (seconds), alpha angle (degrees), maximum clot firmness (MCF) (mm), and maximum lysis (ML%) were recorded.

2.1.4 | Plasma catecholamine measurements—Plasma samples were prepared by centrifugation from pre-resuscitation citrated whole blood collected on arrival and immediately stored at −80°C. In addition to the 67 trauma patient samples, plasma was similarly isolated from citrated whole blood from six healthy adults (four men, two women) and stored at −80°C. Epinephrine (EPI) and norepinephrine (NE) were measured in duplicate with appropriate internal standards and controls by enzyme-linked immunoassays using a commercially available kit (Abnova Corporation). The lower limits of detection (10 pg/ml for EPI and 36 pg/ml for NE) are well below mean concentrations in plasma from healthy volunteers.[29] Trauma patient plasma samples with coefficients of variation between duplicate measurements on ELISA greater than 30% were excluded from analysis ($n = 6$).

2.2 | Healthy donor assays

2.2.1 | Platelet aggregometry—Platelet aggregometry was performed as described above with the following modifications to test the effect of EPI and NE on healthy donor platelet aggregation in whole blood. Healthy donor whole blood was collected in sodium citrate and treated with the following concentrations of EPI and NE: 0, 10, 50, or 100 ng/ml for 3 min. Normal ranges for EPI and NE are 0–0.14 ng/ml and 0.10–1.7 ng/ml, respectively, in healthy donors at rest, but higher maximal concentrations were chosen to encompass the profound catecholamine elevations that have been observed in severe trauma and states of physiologic stress including cardiac arrest.[30-32] Dilute collagen (0.16 μg/ml) was added as a co-agonist and platelet aggregation was quantified over a 6-min measurement period. In a second set of experiments designed to simulate a state of functional platelet exhaustion, recalcified citrated whole blood was incubated with EPI for 0, 10, 20, or 30 min (at 0, 10, or 50 ng/ml), then dilute collagen (0.16 μg/ml) was added as a co-agonist and platelet aggregation was quantified over 6 min.

2.2.2 | Platelet isolation—Healthy donor whole blood was collected in 3.2% sodium citrate and centrifuged at 200g for 20 min. The resulting platelet rich plasma was diluted 1:1 with HEPES-buffered Tyrode's solution (HT) and prostaglandin E1 (final concentration 1 μM) was used to prevent platelet activation during isolation. Platelets were pelleted at 800g for 20 min. The supernatant was removed, and the platelet pellet was washed twice with HT buffer. Platelets were resuspended in HT with 3% bovine serum albumin (BSA, Gold Biotechnology), counted, and diluted with HT to 1×10^8 platelets/ml.

2.2.3 | Microfluidic model—A commercially available microfluidic chamber (Ibidi) containing six channels (size 0.1 mm × 1 mm × 17 mm) was coated with collagen (Corning) and then tissue factor (Extem reagent, Pentapharm). The chamber was blocked overnight with HT + 1% BSA. At the inlet, the channels were connected with polyethylene tubing to a reservoir containing washed platelets (1×10^8/L) and to a syringe pump at the outlet. Washed platelets were recalcified (to [Ca^{2+}] 3 mM) and treated with HT buffer (control), EPI, or NE at low dose (10 ng/ml) or high dose (50 ng/ml) for 3 min prior to perfusion

through the device. Platelets were perfused through the microfluidic channels for 10 min at venous shear (100 s^{-1}), and unattached platelets were cleared by perfusing the chamber with HT at approximate arterial shear (1000 s^{-1}). Phase contrast micrographs were acquired at 60× magnification. Attached platelets were counted in ten 60× fields per donor and experimental condition.

2.2.4 | Flow cytometry—Expression of the platelet activation surface receptors glycoprotein IIb/IIIa, P-selectin, and CD63 was measured by flow cytometry. Healthy donor whole blood was collected in sodium citrate and treated with EPI (50 ng/ml) or control (HT buffer only) for either 3 min or 30 min of incubation. Blood was then diluted (1:50) with HT buffer, added to tubes with or without convulxin (5 ng/ml, Santa Cruz Biotechnology) and antibodies, and incubated at 37°C for 10 min. Allophycocyanin-conjugated anti-CD41 (clone HIP-8) was used to distinguish platelets, fluorescein isothiocyanate conjugated anti-CD62p (clone AK4) to distinguish platelets expressing surface P-selectin, anti-CD41/CD61 (clone PAC-1) to distinguish platelets with glycoprotein IIb/IIIa complex, and anti-CD63 (clone H5C6) to stain platelets expressing surface CD63. Staining with fluorophore and antibody isotype matched controls was performed to determine positive staining. All antibodies and immunoglobulin isotype controls were purchased from Biolegend. Stained samples were treated with FACS Lysing Solution at a ratio of 2:1 (Becton Dickinson) for 10 min at room temperature prior to storage at 4°C for no more than 24 h. Data were collected with a BD LSRII cytometer (Becton Dickinson) and analyzed using FlowJo (FlowJo, LLC).

2.3 | Statistical analyses

Univariate analyses were performed to compare the relationships of patient characteristics with EPI and NE concentrations using the Mann–Whitney test due to non-normal distribution of EPI and NE concentrations. Based on a linear regression power estimate calculator, a sample size of at least 50 trauma patients was required to detect with 80% power a decrease in platelet aggregation of 5 units per 100 pg/ml change in EPI. Multivariable linear regression tested the association of EPI with platelet aggregation and viscoelastic measures, controlling for injury severity, degree of shock, and platelet count. We used *t*-tests to compare differences in platelet aggregation in the healthy donor studies of platelet adhesion under flow and platelet aggregometry. All data were normalized to each healthy donor's untreated control platelets tested in parallel to control for the known inter-donor variability in platelet activation and platelet aggregation. All statistical analysis was performed in Stata version 15 (StataCorps).

3 | RESULTS

3.1 | Catecholamines in trauma patients

The 67 trauma patients were young (median age 37), predominantly male (92%), and moderately injured (median ISS 9, IQR 4–22) with a mix of blunt and penetrating mechanisms (Table 1). Admission platelet counts were normal, though one quarter of patients had markedly impaired *ex vivo* platelet aggregation in response to ADP or collagen on arrival (below the 5th percentile compared with reference ranges in healthy adults) (Table 1). One-fifth of patients were transfused blood products in the first 24 h, and mortality at

discharge was 11% (Table 1). When comparing EPI and NE levels across groups by injury severity and degree of shock, there was a significant stepwise increase in EPI concentrations with increasing injury severity/shock groups (p <0 .01), and while NE was higher in trauma patients compared with healthy volunteers (p = 0.01), there was not a significant difference in NE across injury severity/shock groups (p = 0.75, Table 2). EPI and NE concentrations were significantly higher in those who had traumatic brain injury, developed multiple organ failure (Denver Criteria[28]) and in those who did not survive (Table S1).

On multivariable linear regression [controlling for injury severity (ISS), platelet count, and degree of shock (base excess)], increasing EPI concentrations were strongly associated with impaired *ex vivo* platelet aggregation in response to ADP and collagen stimulation, while NE was weakly associated with impaired *ex vivo* platelet aggregation and only in response to ADP stimulation (Table 3). Increasing EPI was also associated with notable trends of delayed viscoelastic clot initiation parameters and clot strength, and increased fibrinolysis (Table 3). In contrast, NE did not show any significant associations with viscoelastic clotting parameters on multivariable linear regression (Table 3). With respect to clinical outcomes, increasing EPI was associated with increased mortality at discharge (OR 1.2 per 100 pg/ml, p = 0.03), and with a trend toward increased likelihood of transfusions at 24 h (OR 1.1 per 100 pg/ml, p = 0.06), while NE was only significantly associated with the composite outcome of death or multiple organ failure (OR 1.3 per 100 pg/ml, p = 0.03) (Table 3).

3.2 | Effect of catecholamines on healthy donor platelets

To help us better understand the impact of catecholamines on platelet function akin to trauma physiology we used a microfluidic assay of platelet adhesion. Isolated healthy donor platelets subjected to venous flow conditions in collagen/tissue factor coated microfluidic channels showed enhanced platelet adhesion after 3 min of *ex vivo* treatment with EPI at 10 ng/ml and 50 ng/ml (1.8 and 2.5 fold, respectively) compared with untreated (both p < 0.01, Figure 1). NE resulted in more modest and non-significant increases in the number of platelet adhesion events in the microfluidic experiments (Figure 1). Qualitatively, both EPI and NE at 10 ng/ml and 50 ng/ml resulted in platelet aggregate formation and morphological changes indicative of platelet activation in the microfluidic channels under direct visualization by phase contrast microscopy (Figure 2).

We then examined the effects of dose and length of exposure of catecholamines on healthy donor platelet aggregation in whole blood. In platelet aggregometry experiments performed with health donor whole blood, increasing EPI concentrations significantly increased *ex vivo* platelet aggregation when used as a co-agonist with collagen (Figure 3). NE also increased *ex vivo* platelet aggregation, but to a lesser degree (Figure 3). However, given the inverse relationship between EPI and *ex vivo* platelet aggregation in trauma patients, we then tested whether prolonged exposure of healthy donor blood to EPI could result in a functionally exhausted platelet phenotype. Whole blood was incubated with control or EPI at 10 ng/ml and 50 ng/ml and platelet aggregometry was performed serially over time at 0, 10, 20, and 30 min. Under these conditions, EPI enhanced collaged-stimulated platelet aggregation at 0 and 10 min, with a maximal augmentation at 20 min compared with untreated control at the same timepoint (Figure 4). This was followed by a loss of the effect with a relative decrease

in aggregation by 30 min in both the 10 ng/ml and 50 ng/ml EPI groups, which were no longer significantly different from the untreated control group (Figure 4).

To further delineate the platelet activation pathways by which EPI may mediate an exhausted platelet phenotype, we performed flow cytometry for surface markers of platelet activation after exposure to EPI. This demonstrated a significant increase in glycoprotein IIb/IIIa activation after short (3 min) duration treatment with EPI, but this effect was lost with a significant relative decrease in glycoprotein IIb/IIIa activation after prolonged EPI exposure (30 min) (Figure 5). In contrast, no significant changes in P-selectin or CD63 translocation were observed after short or long duration treatment with EPI (Figure 5).

4 | DISCUSSION

In this study, we found that in trauma patients, increased *in vivo* plasma EPI concentrations were independently associated with impaired *ex vivo* platelet aggregation and viscoelastic clot strength, despite the known stimulatory actions of EPI on healthy platelets.[33-35] NE was weakly associated with impaired *ex vivo* measures of platelet aggregation and was not associated with viscoelastic clotting parameters. Consistent with prior studies, healthy donor platelet aggregation was enhanced by EPI and more weakly by NE.[26] However, we identified a differential effect of exposure time of EPI treatment on healthy platelet aggregation: the potentiation of platelet aggregation by EPI was strongest with 10 and 20 min of exposure, but significantly diminished after 30 min of exposure compared with untreated control platelets subjected to the same exposure times. These results may be explained by EPI-mediated increases in glycoprotein IIb/IIIa activation with short duration EPI treatment that is subsequently lost with prolonged exposure to EPI.

Taken together, these findings suggest that following injury, sympathoadrenal catecholamine activation of platelets leads to functional platelet exhaustion with prolonged in-vivo exposure and may contribute to findings of impaired ex-vivo platelet aggregation that is commonly identified in even minimally injured patients.[8] This mechanism may also contribute to impaired platelet aggregation in severely injured patients presenting in a shock state with TIC, though multiple mediators are known to contribute to altered platelet activation, aggregation, and morphologic structure in this setting.[13-17]

We also identified an important relationship between increasing EPI concentrations and viscoelastic evidence of impaired clot initiation, decreased clot strength, as well as increased fibrinolysis in our trauma patient samples. This provides additional evidence in support of the link between sympathoadrenal activation and TIC in trauma patients and may corroborate similar findings in prior studies.[23,24] EPI has been implicated in promoting hypocoagulability and fibrinolysis through activation of the protein C pathway and by triggering endothelial release of tissue plasminogen activator.[26,36-38] Ostrowski, Johansson, and colleagues have identified links between sympathoadrenal activation, endothelial damage, and TIC in prospective studies of trauma patients,[23,24] as well as in patients with sepsis and myocardial infarction.[39,40] They propose that there may be both adaptive and maladaptive effects of the catecholamine response to injury. While the initial catecholamine surge after injury may result in an adaptive net hemostatic

effect, the subsequent promotion of fibrinolysis and natural anticoagulation, evolutionarily theorized to ensure adequate perfusion and oxygen delivery to the microvasculature, may become dysregulated or overactivated in severe trauma.[23] It remains controversial whether overactivation of the sympathoadrenal system may exacerbate endothelial injury and further promote systemic anticoagulation through auto-heparinization in the setting of endothelial glycocalyx breakdown.[15,23] We have shown that in parallel, the catecholamine surge after injury may initially enhance platelet-dependent hemostasis, but that prolonged or extreme catecholamine elevations may become maladaptive through a mechanism of functional platelet exhaustion. The potential negative consequences of excessive sympathoadrenal activation are further highlighted by independent associations with increased mortality in this study and others.[23,41,42]

These findings should be considered carefully as they may be of additional clinical significance given the recent evidence demonstrating decreased transfusions in a randomized clinical trial of vasopressin use in hemorrhaging trauma patients,[25] leading to renewed interest in use of vasopressors for hemodynamic support in this population. Future studies of vasopressor use in trauma should consider evaluating associated effects on platelet function and coagulation and further characterizing the role of dose and duration of exposure, as well as the degree of the endogenous sympathoadrenal response.

Our microfluidic studies examining the effects of EPI and NE on isolated healthy donor platelets confirm a direct effect of catecholamines on platelets under flow conditions that results in increased qualitive hemostatic function, though the quantitative effect was stronger with EPI. Future studies examining catecholamine effects on healthy and trauma patient platelet function in microfluidic models incorporating endothelial cells are warranted to further advance our understanding of the role of sympathoadrenal activation in post-injury platelet and endothelial biology.

There are important limitations of this study that should be noted. We were not able to capture the effect of changes in catecholamines over time on platelet aggregation and coagulation in our *ex vivo* analyses of trauma patient samples given we only performed catecholamine measurements at a single timepoint (pre-resuscitation, on arrival). This was done to avoid any effect of transfusions and exogenous vasopressors which likely further complicate this biology. Furthermore, the relationship between catecholamines and platelet function *in vivo* may not be linear with threshold effects, but in our healthy donor studies we did observe a linear relationship within the broad range of concentrations tested. Both the high dose (50 ng/ml) EPI and NE concentrations tested in our *ex vivo* experiments are higher than the plasma concentrations measured in the trauma patients in this study. The higher dose was included to match the plasma catecholamine concentrations in patients with cardiac arrest, which are likely similar to the levels in the most critically injured trauma patients in profound shock who may also suffer from cardiac arrest, and who are not often able to be enrolled in research studies due to the emergent nature of their clinical condition.[32] Our microfluidic model recapitulates some critical but not all aspects of *in vivo* physiology. The lack of platelet-endothelial and other platelet-cellular interactions in our model are important to consider given the apparent role of sympathoadrenal activation in the endotheliopathy of trauma and in hypoperfusion.[23,40] The qualitative

differences in platelet morphology between control and catecholamine-treated platelets we observed by phase contrast microscopy merit further elucidation and quantification by fluorescence microscopy. Furthermore, it will be important to understand if the observed impairments in platelet aggregation with prolonged EPI exposure can also be recapitulated under experimental conditions additionally incorporating shear stress. Our flow cytometry experiments demonstrate prolonged EPI exposure decreases glycoprotein IIb/IIIa activation, which supports the hypothesis of platelet exhaustion, but additional work is needed to further delineate the biological pathways of catecholamine-induced platelet exhaustion, including the potential role of thrombin and other soluble mediators known to impact platelet function and be increased in the setting of trauma.[13-17] Lastly, future studies of vasopressors and platelet function should examine vasopressin effects, given the presence of vasopressin receptors on platelets.[43]

In summary, we identified important independent associations of catecholamines with *ex vivo* platelet impairments, hypocoagulability, and fibrinolysis in trauma patients, providing further evidence for an important role of sympathoadrenal activation in the pathophysiology of TIC. However, we have also identified that although catecholamines enhance platelet adhesion and aggregation of healthy donor platelets, this diminishes with increased duration of exposure to catecholamines suggestive of the development of functional platelet exhaustion. Further translational studies are needed to better define the potential beneficial or deleterious effects of vasopressors on platelet-dependent hemostasis in trauma patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Essentials

- Functionally exhausted platelets may drive common impairments in *ex vivo* platelet aggregation.
- The platelet-catecholamine axis was examined in trauma patients and healthy donors.
- Plasma catecholamines are associated with impaired *ex vivo* platelet aggregation after trauma.
- Catecholamine treatment of healthy platelets models a similar functional platelet exhaustion.

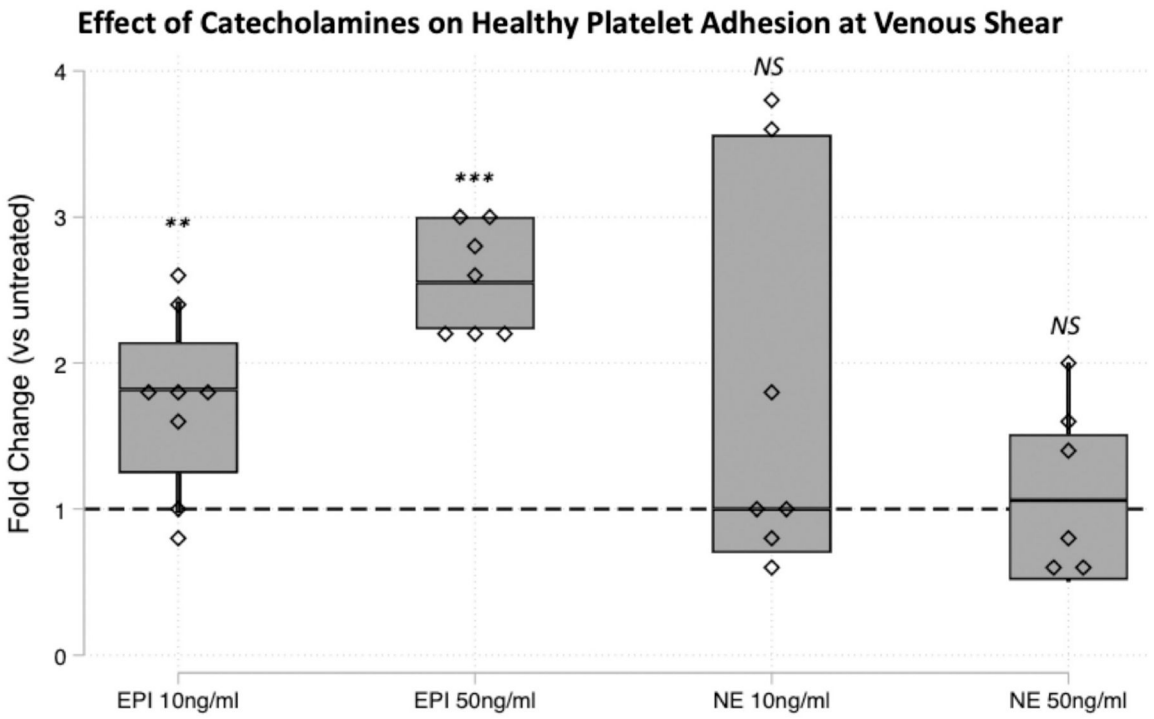

FIGURE 1. Effect of epinephrine (EPI) and norepinephrine (NE) on platelet adhesion at venous shear.

Healthy donor platelets were treated with EPI or NE at 10 ng/ml or 50 ng/ml, or untreated control, and perfused through collagen/tissue factor coated microfluidic channels at venous shear rates (100 s^{-1}). The number of adhered platelets in 10 adjacent fields of view were counted after 10 min of perfusion and the fold change (x-axis) calculated by comparing with counts of adhered platelets in treated and untreated (control) groups. EPI treatment significantly increased platelet adhesion in a dose-dependent manner, while the increase in platelet adhesion with NE treatment was not significant. $^{**}p < 0.05$, $^{***}p < 0.01$, NS, not significant for two-sided *t*-test if normalized fold change different from 1 for each group.

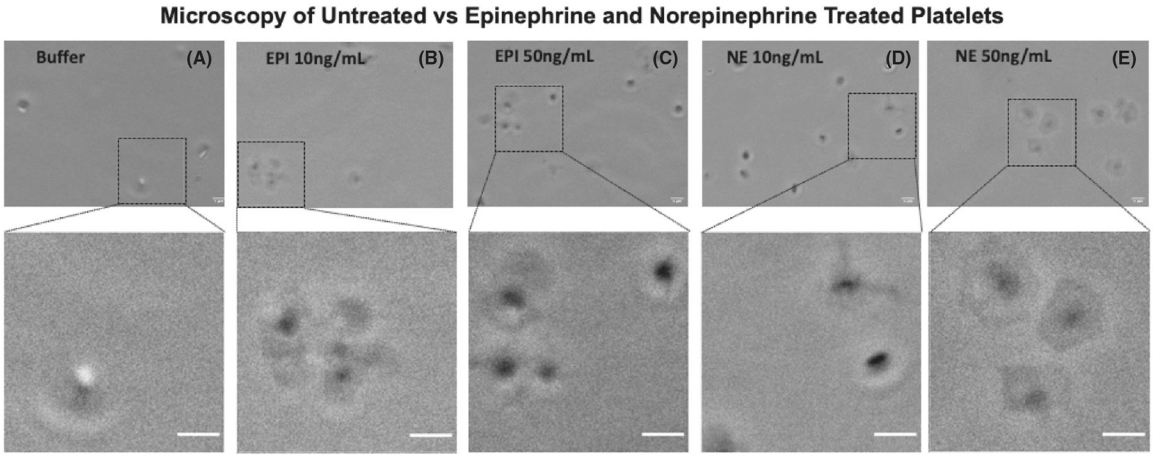

FIGURE 2. Phase contrast microscopy of catecholamine treated platelets.

Representative phase contrast microscopy images (60×) shown for healthy donor platelets perfused through collagen/tissue factor coated microfluidic channels treated with control (buffer), epinephrine (EPI) or norepinephrine (NE) (10 ng/ml or 50 ng/ml). (A) Untreated: few platelets adhered with minimal platelet spreading morphology. (B, C) 10–50 ng/ml EPI: increased numbers of adhered platelets and formation of platelet aggregates, increased density of adhered platelets in C. (D, E) 10–50 ng/ml NE: Increased number of adhered platelets compared with (A), with platelet spreading and shape change similar to (B, C), indicative of platelet activation. Brightness and contrast of images enhanced to better visualize platelets in zoomed in views. Scale bar = 5 μm.

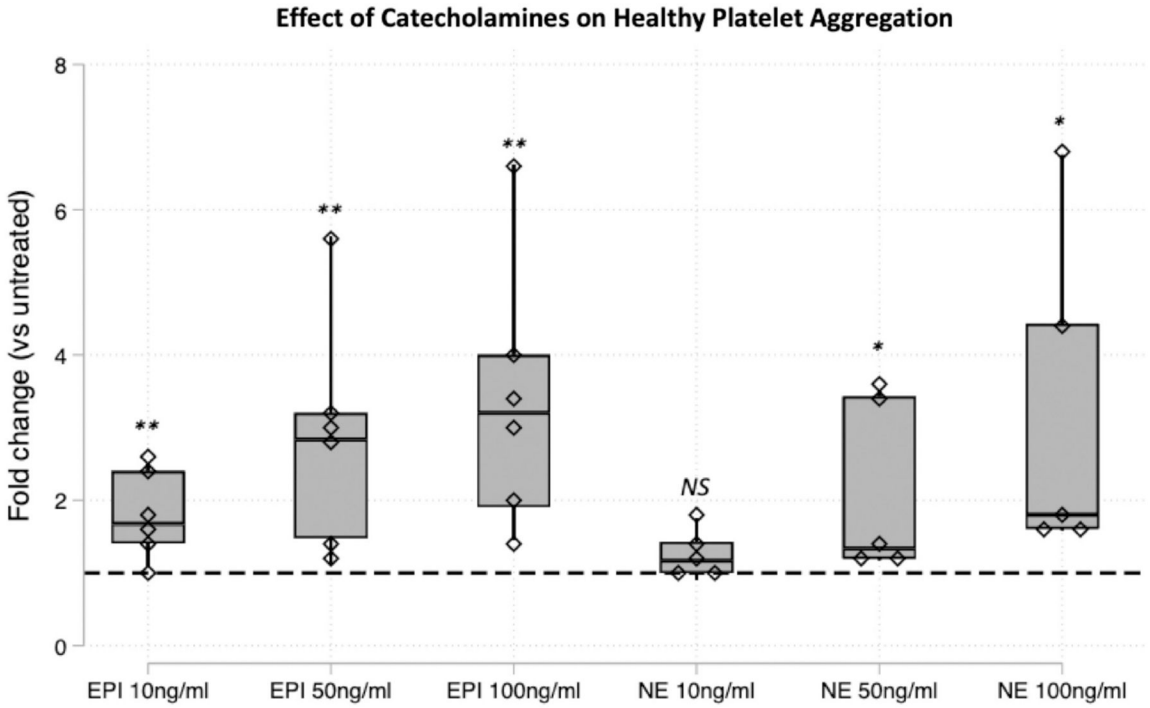

FIGURE 3. Effect of catecholamines on healthy donor platelet aggregation in whole blood.
Citrated healthy donor blood was recalcified and treated with buffer, epinephrine (EPI) or norepinephrine (NE) and dilute collagen added as co-agonist. Platelet aggregation was measured as the area under the aggregation curve after a 6-min measurement period according to the manufacturer's protocol. $^{**}p < 0.05$, $^{*}p < 0.10$, NS, not significant for two-sided *t*-test if fold change compared with untreated control group different from 1. EPI 10, 50, and 100 groups significantly increased compared with controls.

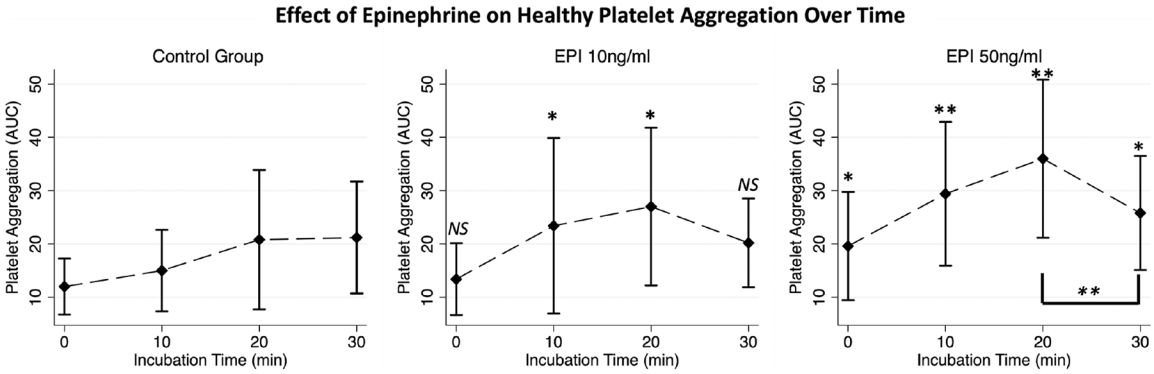

FIGURE 4. Effect of epinephrine (EPI) treatment over time on platelet aggregation in whole blood.

Citrated healthy donor blood was incubated with equal volumes of buffer (control) or EPI (10 ng/ml or 50 ng/ml) for 0, 10, 20, and 30 min. At each timepoint, platelet aggregometry was performed using dilute collagen. $N = 5$ for each time point. Standard error bars shown. $^{**}p < 0.05$, $^{*}p < 0.10$, NS, not significant, for two sided t-tests comparing each EPI treatment group with corresponding timepoint in the control group. Additional comparison for EPI 50 ng/ml at 20 versus 30 min with significant decrease at 30 compared with 20 min shown.

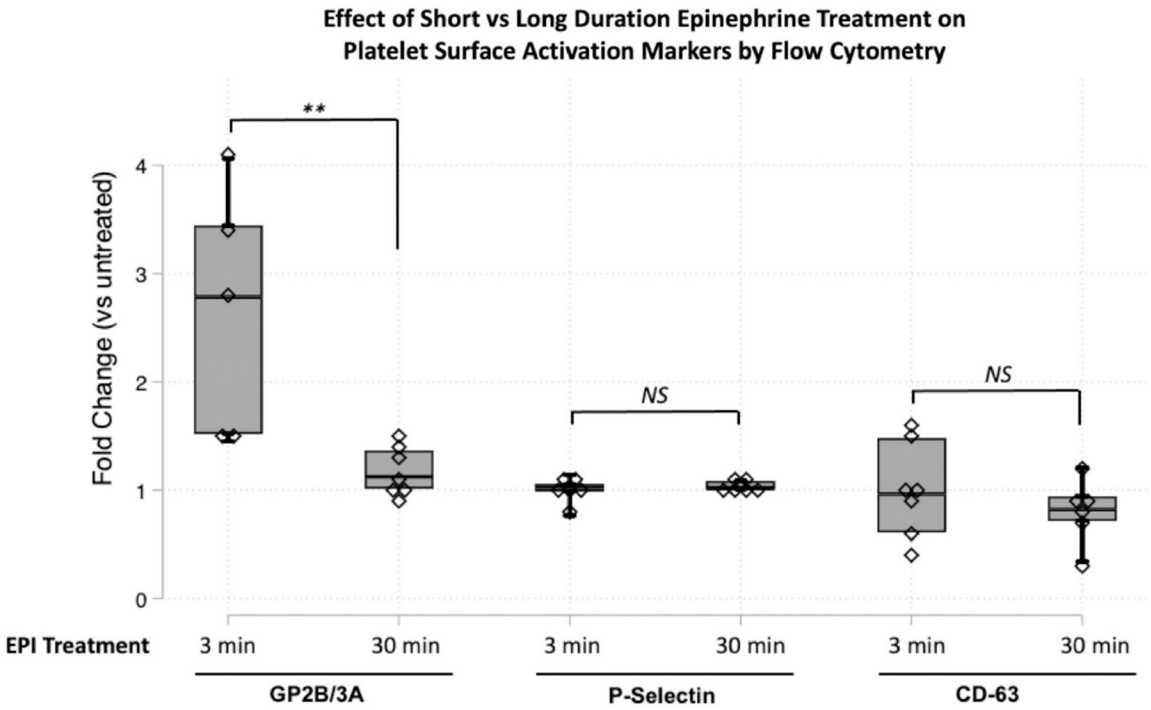

FIGURE 5. Effect of short versus long epinephrine (EPI) treatment on platelet surface activation markers by flow cytometry.

Healthy donor platelets were treated with epinephrine 50 ng/ml (EPI) for short (3 min) versus long (30 min) incubation. Platelet activation was stimulated with convulxin (collagen pathway), then flow cytometry for activated glycoprotein IIb/IIIa (GP2B/3A), P-selectin, and CD63 was performed. Data were normalized to each healthy donor's untreated/control platelet surface marker expression measured in parallel. ** $p = 0.01$, NS, not *significant*.

TABLE 1

Clinical characteristics of trauma patients

	Mean (SD) or median (IQR)
Age (years)	37 (30–52)
Injury severity score	9 (4–22)
Male	92%
Blunt mechanism	54%
Traumatic brain injury	25%
Platelet count (10^9/L)	275 (78)
INR	1.0 (1.0–1.1)
aPTT (s)	27.5 (5.6)
Hemoglobin (g/dl)	13.5 (1.8)
Base excess (meq/ml)	−2.8 (5.2)
Platelet aggregation below 5th percentile[a]	23%
Multiple organ failure	11%
Mortality at discharge	11%
Transfused platelets (24 h)	7%
Transfused any product (24 h)	21%

Abbreviations: aPTT, activated partial thromboplastin time; INR, international normalized ratio.

[a]Indicates platelet aggregation is below 5th percentile compared with healthy adults based on reference ranges obtained in healthy adults in multiple impedance aggregometry provided by manufacturer (Multiplate).

TABLE 2

Comparison of epinephrine and norepinephrine across severity of injury and degree of shock and by healthy donors versus trauma patients

Variable	ISS 0/1	ISS 2–15, BE>−6	ISS 2–15, BE≤−6	ISS 15+, BE>−6	ISS 15+, BE≤−6	*p*-value
N in each group	16	24	5	15	7	
Epinephrine (pg/ml), median (IQR)	145 (131–184)	254 (98–466)	417 (279–456)	575 (235–1238)	723 (352–3333)	<0.01
Norepinephrine (pg/ml), median (IQR)	1006 (688–1084)	972 (624–1161)	1020 (700–2745)	1110 (595–1647)	1596 (426–2724)	0.75

Variable	Healthy	Trauma patient	*p*-value
N in each group	6	67	
Epinephrine (pg/ml), median (IQR)	90 (66–114)	279 (149–621)	<0.01
Norepinephrine (pg/ml), median (IQR)	530 (346–683)	1016 (634–1388)	0.01

Abbreviations: BE, base excess (meq/ml); ISS, injury severity score.

TABLE 3

Multivariable regression results for the association of catecholamines with platelet aggregation, viscoelastic clot measures, and clinical outcomes

	Epinephrine			Norepinephrine		
Platelet aggregation	**Coefficient (per 100 pg/ml)**	**95% CI**	***p*-value**	**Coefficient (per 100 pg/ml)**	**95% CI**	***p*-value**
Platelet aggregation, ADP stimulated	−0.99	−1.65, −0.33	<0.01	−0.38	−0.76, −0.01	0.05
Platelet aggregation, collagen stimulated	−0.93	−1.88, 0.01	0.05	−0.36	−0.88, 0.16	0.17
ROTEM						
Clot formation time (s)	2.15	0.91, 3.39	<0.01	0.28	−0.46, 1.02	0.45
Alpha angle (degrees)	−0.35	−0.54, −0.16	<0.01	−0.05	−0.16, 0.07	0.42
Max clot firmness (mm)	−0.48	−0.69, −0.27	<0.01	−0.03	−0.17, 0.10	0.60
Max clot lysis (%)	0.98	0.57, 1.40	<0.01	−0.05	−0.32, 0.23	0.74

	Epinephrine			Norepinephrine		
Clinical outcomes	**Odds ratio (per 100 pg/ml)**	**95% CI**	***p*-value**	**Odds ratio (per 100 pg/ml)**	**95% CI**	***p*-value**
Transfused in 24 h	1.14	1.00, 1.30	0.06	1.06	0.96, 1.16	0.26
Death or multiple organ failure	1.20	0.98, 1.48	0.07	1.29	1.02, 1.62	0.03
Mortality at discharge	1.19	1.02, 1.40	0.03	1.03	0.96, 1.12	0.39

Note: Coefficients for platelet aggregation and ROTEM measures adjusted for injury severity score, platelet count, and base excess. Odds ratios for clinical outcomes adjusted for age, base deficit, and injury severity score. Multiple organ failure defined using Denver Criteria (see reference 31).