Plasma resuscitation restores glomerular hyaluronic acid and mitigates hemorrhage-induced glomerular dysfunction

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AUTHORS/CONTRIBUTORS
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BACKGROUND: Acute renal dysfunction following hemorrhagic shock and resuscitation carries significant morbidity and mortality. While shock-induced shedding of the glycocalyx is well described within the pulmonary and splanchnic vasculature, less is known regarding early alterations to the glycocalyx of the renal microcirculation, particularly the glomerulus. We sought to evaluate the impact of hemorrhagic shock and resuscitation modalities on glomerular glycocalyx metabolism and function. We hypothesized that fresh frozen plasma resuscitation would attenuate glomerular glycocalyx shedding and reduce glomerular barrier dysfunction.

METHODS: Male Sprague-Dawley rats were subjected to 60 minutes of hemorrhagic shock to 40% of baseline mean arterial pressure, followed by resuscitation with shed whole blood and either lactated Ringer's or fresh frozen plasma. Experimental groups included the following: (*a*) baseline, (*b*) post–hemorrhagic shock, (*c*) post–lactated Ringer's resuscitation, and (*d*) post–plasma resuscitation. Enzyme-linked immunosorbent assays and immunohistochemistry were used to evaluate alterations of syndecan-1 and hyaluronic acid within the glomerular glycocalyx. Urine protein concentration was measured as a surrogate for glomerular function, and expression of cubilin and megalin was quantified to evaluate renal tubule protein reabsorptive capacity.

RESULTS: Despite evidence of systemic glycocalyx shedding, hemorrhagic shock and resuscitation did not alter glomerular synedcan-1 expression. However, shock induced shedding of hyaluronic acid from the glomerular glycocalyx. While hyaluronic acid breakdown was exacerbated by crystalloid resuscitation, plasma utilization restored levels back to baseline. Urine protein concentration drastically increased following hemorrhagic shock and resuscitation with lactated Ringer's. By contrast, plasma administration reduced urine protein levels back to normal. Renal cortex cubilin and megalin expression did not differ among the experimental groups, suggesting that alterations in urine protein were driven by changes in glomerular function.

CONCLUSION: Plasma-based resuscitation appears to reverse shock-induced shedding of glomerular hyaluronic acid and attenuates glomerular barrier dysfunction. Differential shedding of the glomerular glycocalyx may represent a novel pathway in acute kidney injury pathophysiology. (*J Trauma Acute Care Surg.* 2025;99: 59–67. Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved.)

KEY WORDS: Hyaluronic acid; glomerulus; hemorrhage; shock; rats.

The glycocalyx is a dynamic, branching network of core proteins (including syndecan-1) and associated glycosaminoglycans (including heparan sulfate, chondroitin sulfate, and hyaluronic acid) that lines the vascular endothelium throughout the circulation. Beyond serving as a key regulator of vascular permeability, the glycocalyx is mandatory for normal microcirculatory physiology including maintenance of vascular tone, localization of key plasma proteins (e.g., anti–thrombin III, vascular endothelial growth factor), and inhibition of inappropriate inflammation and thrombosis.[1] Despite its structural complexity, systemic insults such as hemorrhage and trauma result in glycocalyx degradation ("shedding"), leading to fluid and plasma protein extravasation into the interstitium, inappropriate activation of coagulation and thrombosis, increased locoregional inflammation, and the release of glycocalyx components into the circulation.[2] Clinically, shedding of the endothelial glycocalyx is associated with increased rates of trauma-induced coagulopathy, acute respiratory distress syndrome, multisystem organ failure, in-hospital mortality, and acute kidney injury.[3]

Although the splanchnic and pulmonary microcirculations are highly susceptible to glycocalyx turnover following hemorrhage, it remains unclear whether alterations in glycocalyx metabolism occur in the kidney, particularly the glomerulus.[4–6] Furthermore, what impact glycocalyx modifications within the glomerulus have on early renal function after shock remains an area of ongoing investigation.[7] In this study, we sought to evaluate the impact of hemorrhagic shock and resuscitation modalities on the glomerular glycocalyx and hypothesized that fresh frozen plasma (when compared with lactated Ringer's) would mitigate glomerular glycocalyx shedding and reduce hemorrhage-induced glomerular barrier dysfunction.

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MATERIALS AND METHODS

Animal Model of Hemorrhagic Shock and Resuscitation

Institutional Animal Care and Use Program approval was obtained for the rodent model of hemorrhagic shock and resuscitation (protocol no. 22187). Male Sprague-Dawley rats (250–300 g) underwent acclimation for 1 week with ab libitum access to standard rat chow and water. Animals were randomized into one of four experimental groups (n = 8 per group): (*a*) baseline, (*b*) post–hemorrhagic shock, (*c*) post–lactated Ringer's resuscitation, and (*d*) post–fresh frozen plasma resuscitation. Blinding was not possible, as the performing investigator was required to know which resuscitation modality to use for each animal.

Animals were anesthetized using an intraperitoneal injection of ketamine/xylazine with subsequent redosing used to maintain an adequate surgical plane of anesthesia throughout the experimental protocol. Body temperature was maintained at 37°C ± 1°C, and all surgical interventions were performed under aseptic conditions. The left carotid artery, right jugular vein, and right femoral artery were cannulated with polyethylene tubing (PE-50) to allow for hemodynamic monitoring, blood removal, and volume resuscitation. A tracheostomy was performed to maintain airway patency, allowing animals to spontaneously breathe room air. Following cannulation, animals were monitored for 15 minutes to determine baseline mean arterial pressure (MAP), with those randomized to the baseline group undergoing euthanasia at the completion of this time interval.

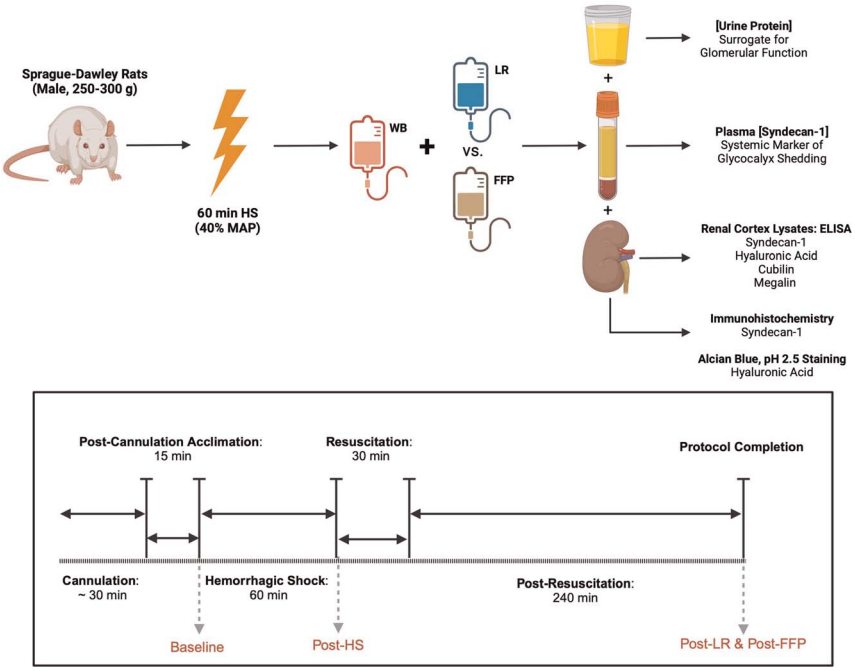

Figure 1. Experimental setup and hemorrhagic shock-resuscitation timeline. Animals in the baseline group were euthanized after 15 minutes of postcannulation acclimation. Animals in the hemorrhagic shock group were euthanized following 60 minutes of shock. Finally, animals in the resuscitation arms were euthanized after 4 hours of postresuscitation monitoring. Created in BioRender. Risinger, W. WB, whole blood; LR, lactated Ringer's; FFP, fresh frozen plasma; ELISA, enzyme linked-immunosorbent assay.

Shock was induced through a pressure-controlled hemorrhage via the femoral arterial line to 40% of the baseline MAP. The target pressure was reached within 10 minutes by an average removal of 1 mL of blood per minute. The shock state was sustained for a total of 60 minutes with additional blood withdrawn or returned as needed to maintain the target pressure.

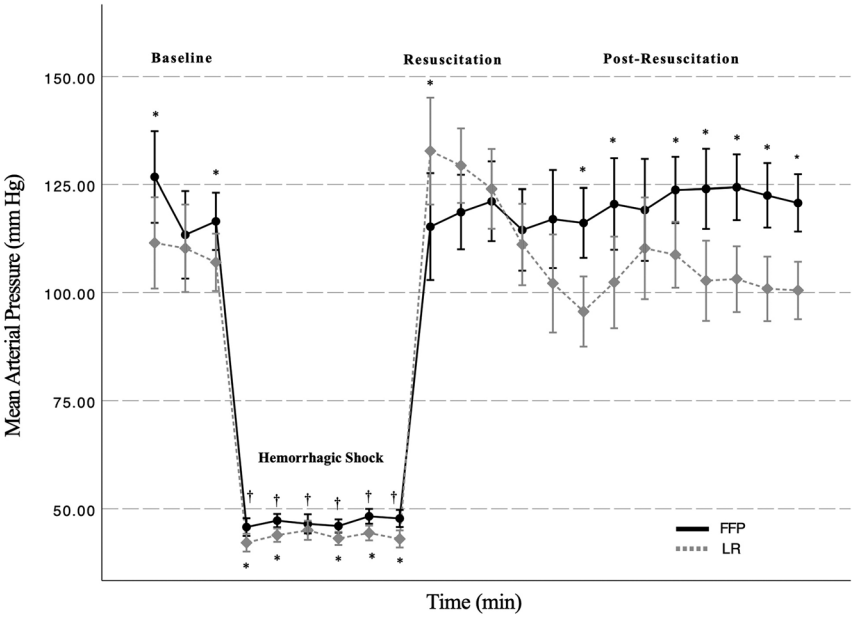

Figure 2. Mean arterial pressure during hemorrhagic shock and resuscitation. Despite randomization, animals in the FFP arm had a higher MAP at baseline. Given the pressure-controlled hemorrhage to 40% of baseline, this resulted in a higher MAP during shock for animals receiving plasma. These findings highlight the importance of pressure-controlled hemorrhage, which considers baseline hemodynamics. Importantly, within each group, there was no difference in MAP at baseline and following resuscitation, suggesting restoration of central hemodynamics with either resuscitation modality. *Between-group difference (LR vs. FFP), $p < 0.05$. †Within-group difference (both LR and FFP) between baseline/resuscitation/postresuscitation versus hemorrhagic shock, $p < 0.05$.

Shed blood was stored in a heparin-rinsed syringe and maintained at 37°C. Animals in the post–hemorrhagic shock group underwent euthanasia immediately after 1 hour of shock.

Resuscitation was performed over 30 minutes and began with the return of the entire volume of shed blood (typically 10–11 mL per animal) over the course of 5 minutes. In the remaining 25 minutes of resuscitation, animals received either lactated Ringer's (Baxter Healthcare, Deerfield, IL) at 2× the volume of shed blood or pooled Sprague-Dawley rat plasma (Innovative Research, Novi, MI) at 1× the volume of shed blood. The additional volume of crystalloid therapy was chosen based on previous work in our laboratory demonstrating the need for higher volumes of crystalloid to maintain MAP for the duration of postresuscitation monitoring. Prior to administration, plasma was thawed and filtered through a Durapore PVDF 0.22-μm membrane filter (Merck Millipore, Burlington, MA) and warmed to 37°C.

Animals were monitored for 4 hours postresuscitation prior to euthanasia, which was performed via exsanguination from the femoral arterial line after anesthetic redosing. A midline laparotomy was used to harvest the bilateral kidneys, and urine was obtained via a bladder puncture using a 23-gauge needle. Plasma for analysis was derived from whole blood obtained during exsanguination. All specimens (plasma, urine, renal tissue) were snap frozen in liquid nitrogen and stored at −80°C until analysis (Fig. 1). The animal research: reporting in vivo experiments guidelines, designed for improved reporting of animal research, were referenced during the drafting of this article[8] (Supplemental Digital Content, Supplementary Data 1, http://links.lww.com/TA/E395).

Evaluation of Glomerular Function: Urine Protein Concentration, Megalin and Cubilin Expression Within the Proximal Convoluted Tubule

Urine protein concentration was measured across the experimental groups as a surrogate for glomerular function using a standard Bradford protein assay. To account for proximal convoluted tubule protein reabsorption, quantification of megalin and cubilin (responsible for the majority of protein reabsorption from the tubule fluid) was determined using rat-specific enzyme linked immunosorbent assays (LS Bioscience, Seattle, WA).

Evaluation of Systemic Glycocalyx Shedding: Plasma Syndecan-1

Systemic shedding of the glycocalyx was evaluated through quantification of plasma syndecan-1 levels during hemorrhagic shock and resuscitation using a rat-specific enzyme-linked immunosorbent assay (LS Bioscience).

Evaluation of the Renal Cortex and Glomerular Glycocalyx: Syndecan-1 and Hyaluronic Acid

Renal cortex tissue lysates were generated using ProcartaPlex Cell Lysis Buffer (Invitrogen, Waltham, MA) and the TissueLyser LT system (Qiagen, Germantown, MD). Total protein concentration for each sample was determined using the Pierce BCA protein assay (ThermoFisher, Waltham, MA). Quantification of syndecan-1 and hyaluronic acid within the renal cortex was determined using rat-specific enzyme-linked immunosorbent assays (LS Bioscience). To investigate glomerular specific expression, sections of renal cortex were processed onto glass slides. Immunohistochemical analysis was performed using a polyclonal primary antibody for syndecan-1 (Invitrogen). Histologic identification of glomerular hyaluronic acid was performed using Alcian blue, pH 2.5 staining, which preferentially binds nonsulfated polysaccharides including hyaluronic acid.[9] Counterstaining was achieved with hematoxylin, and images were obtained using the Olympus IX51 Microscope and cellSens imaging (Olympus America, Centera Valley, PA).

Statistical and Power Analysis

Comparisons between the experimental groups was achieved using one-way analysis of variance (ANOVA), with repeated-measures ANOVA used for comparisons of MAP. Pairwise comparisons with a Holm-Sidak correction were used for post hoc testing. For all analyses, $p < 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS (IBM, Chicago, IL).

An a priori power analysis was performed using previous data from our laboratory evaluating syndecan-1 metabolism in the terminal ileum following hemorrhagic shock and resuscitation.[4] Using hypothesized means/trends consistent with our findings of syndecan metabolism in the ileum, a significance level of $\alpha = 0.05$, and a power of 0.80, the minimum sample size needed to identify a similar effect size within the renal cortex was found to be n = 32 (n = 8 per group) for one-way ANOVA.

RESULTS

MAP During Hemorrhagic Shock and Resuscitation

Mean arterial pressure was monitored throughout the experimental protocol, and comparisons were made between animals randomized to lactated Ringer's and fresh frozen plasma resuscitation to ensure similar levels of ischemic insult (Fig. 2). Animals in the FFP arm had a higher MAP at baseline, which translated into a marginally higher MAP during shock (40% of baseline), resuscitation, and postresuscitation. Within each group (LR and FFP), there was no statistical difference between the MAP at baseline and following resuscitation, implying adequate restoration of central hemodynamics with both resuscitation modalities.

Urine Protein Concentration: Surrogate for Glomerular Function

Following hemorrhagic shock, urine protein concentration (mg/mL) was significantly elevated compared with baseline (1.20 vs. 0.43, $p < 0.01$). However, animals randomized to receive lactated Ringer's demonstrated a drastic increase in urine protein concentration at 4 hours postresuscitation (1.96 vs. 0.43, $p < 0.001$). By contrast, the utilization of plasma as a resuscitation fluid lowered urine protein concentration back to baseline (0.70 vs. 0.43, $p = 0.73$) (Fig. 3*A*). The difference in urine protein concentration based on resuscitation modality was statistically significant (1.96 vs. 0.70, $p < 0.001$). To account for potential alterations of urine protein concentration because of renal tubule protein handling, expression of megalin and cubilin were compared across the experimental groups. No difference in either protein was observed after hemorrhagic shock and resuscitation (Fig. 3*B* and *C*).

Systemic Levels of Syndecan-1 Following Hemorrhagic Shock and Resuscitation

Plasma syndecan-1 concentration (μg/mL) was measured to evaluate for systemic glycocalyx shedding. Hemorrhagic

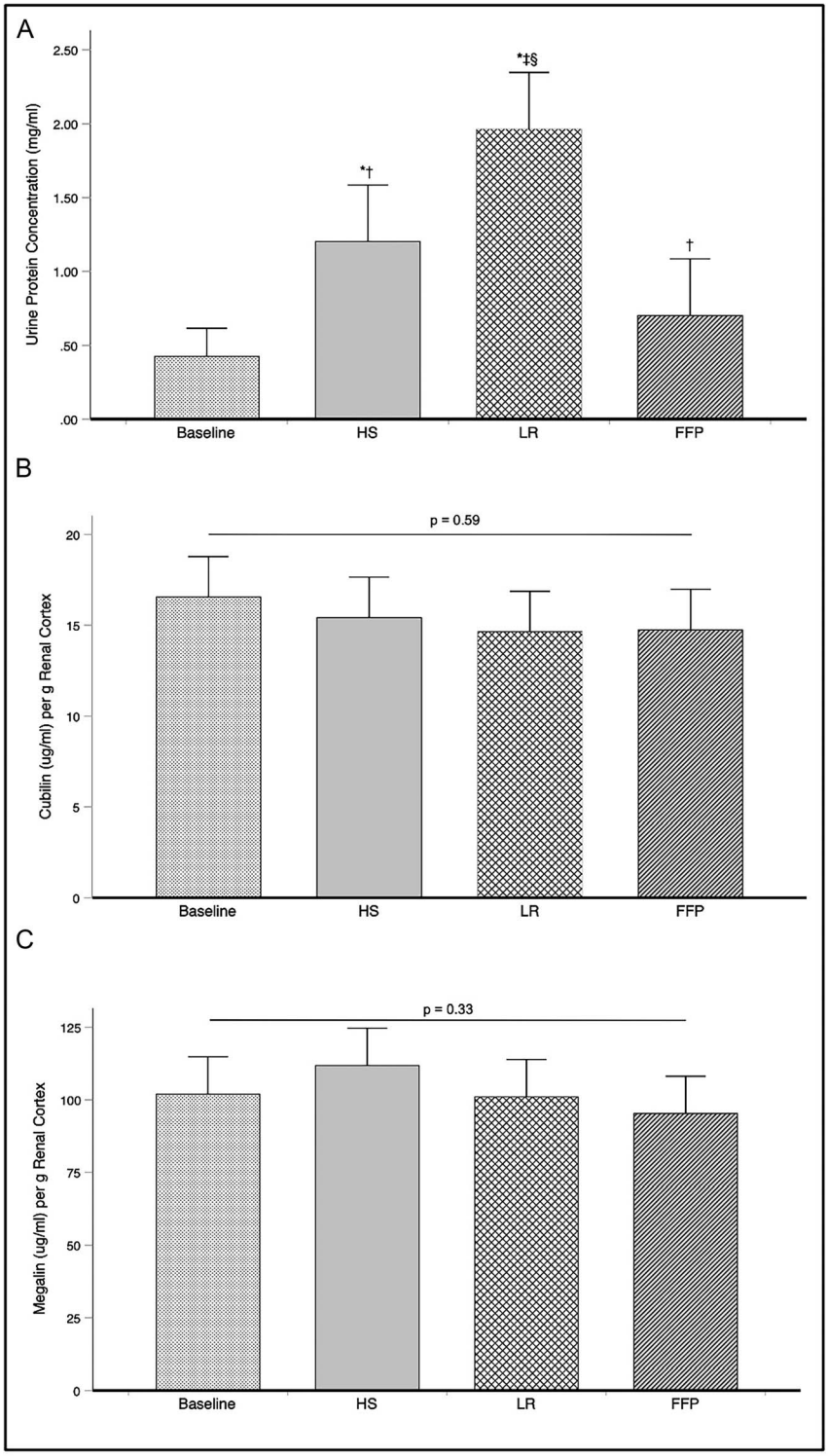

Figure 3. (*A*) Urine protein concentration following hemorrhage and resuscitation as a marker of glomerular dysfunction. Despite restoration of central hemodynamics, animals resuscitated with crystalloid exhibited ongoing elevations in urine protein concentration. By contrast, plasma restored protein levels back to baseline. Proximal convoluted tubule expression of cubilin (*B*) and megalin (*C*) demonstrated no statistically significant differences across the experimental groups. Values presented as mean ± 95% CI. *Versus baseline, §versus hemorrhagic shock, †versus lactated Ringer's, and ‡versus fresh frozen plasma ($p < 0.05$). CI, confidence interval.

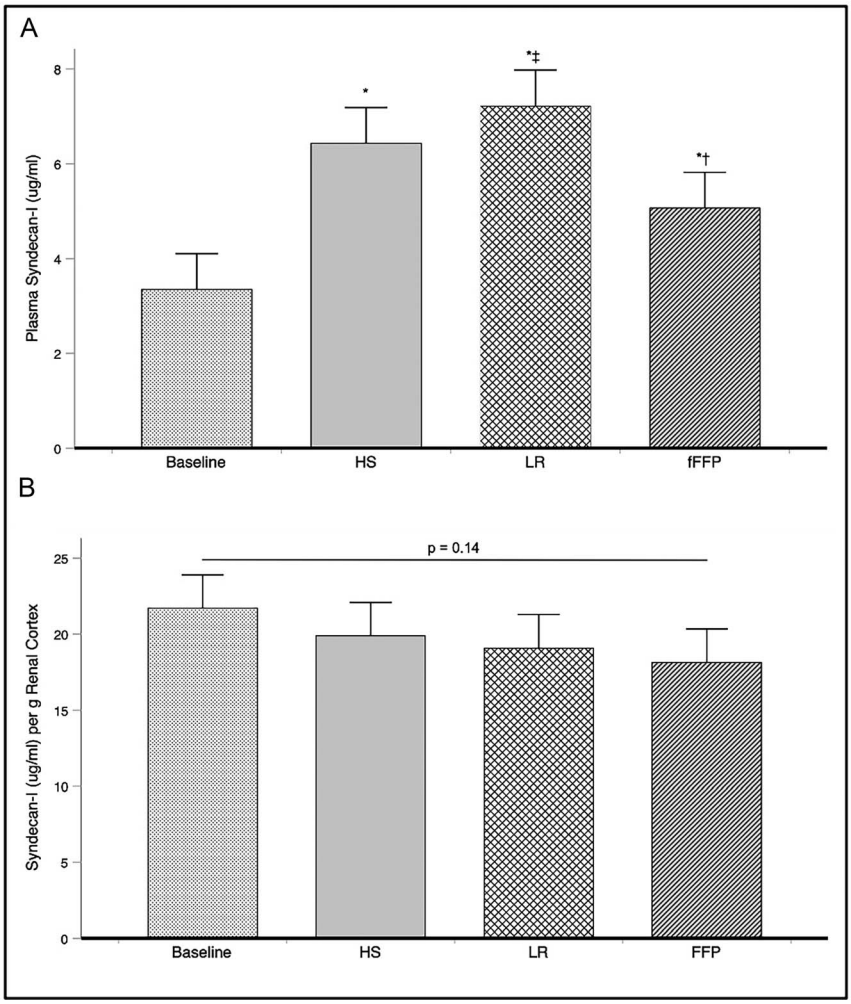

Figure 4. (*A*) Systemic shedding of the endothelial glycocalyx during hemorrhage and resuscitation based on plasma syndecan-1 concentration. (*B*) Alterations of syndecan-1 within the renal cortex during hemorrhage and resuscitation. Values presented as mean ± 95% CI. *Versus baseline, §versus hemorrhagic shock, †versus lactated Ringer's, and ‡versus fresh frozen plasma ($p < 0.05$). CI, confidence interval.

shock resulted in a statistically significant elevation in plasma syndecan-1 concentration compared with baseline (6.43 vs. 3.35, $p < 0.001$). Resuscitation with lactated Ringer's further exacerbated syndecan-1 shedding (7.22 vs. 3.35, $p < 0.001$). By contrast, administration of fresh frozen plasma attenuated syndecan-1 breakdown, with the concentration returning toward but higher than baseline (5.06 vs. 3.35, $p = 0.02$). The difference observed between resuscitation modalities was statistically significant (7.22 vs. 5.06, $p < 0.01$) (Fig. 4*A*).

Hemorrhage-Induced Alterations of Glomerular Syndecan-1 and Hyaluronic Acid

Despite evidence of systemic syndecan-1 shedding, there was not a statistically significant difference in the concentration of syndecan-1 in the renal cortex across the experimental groups (Fig. 4*B*). Furthermore, immunohistochemical staining demonstrated a preferential distribution of syndecan-1 within the renal tubules, and no differences in glomerular staining were seen following hemorrhagic shock and resuscitation with either modality (data not shown). However, the concentration of hyaluronic acid (μg/mL per g renal cortex) was significantly decreased from baseline after hemorrhagic shock and resuscitation with lactated Ringer's (281.23 vs. 378.36, $p = 0.04$). By contrast, when animals received fresh frozen plasma, hyaluronic acid levels were similar to baseline (390.77 vs. 378.36, $p = 0.98$). The difference observed between resuscitation modalities was statistically significant (390.77 vs. 281.23, $p = 0.04$) (Fig. 5*A*).

Alcian blue staining of the renal cortex demonstrated the predominant location of hyaluronic acid to be within the glomerulus (Fig. 5*B*). In parallel with the enzyme-linked immunosorbent data, glomerular hyaluronic acid exhibited decreased staining post–lactated Ringer's resuscitation. Likewise, the administration of plasma restored glomerular hyaluronic acid staining back to baseline (Fig. 6).

DISCUSSION

In this study, we investigated the effects of hemorrhagic shock and resuscitation on the glomerular glycocalyx and sought

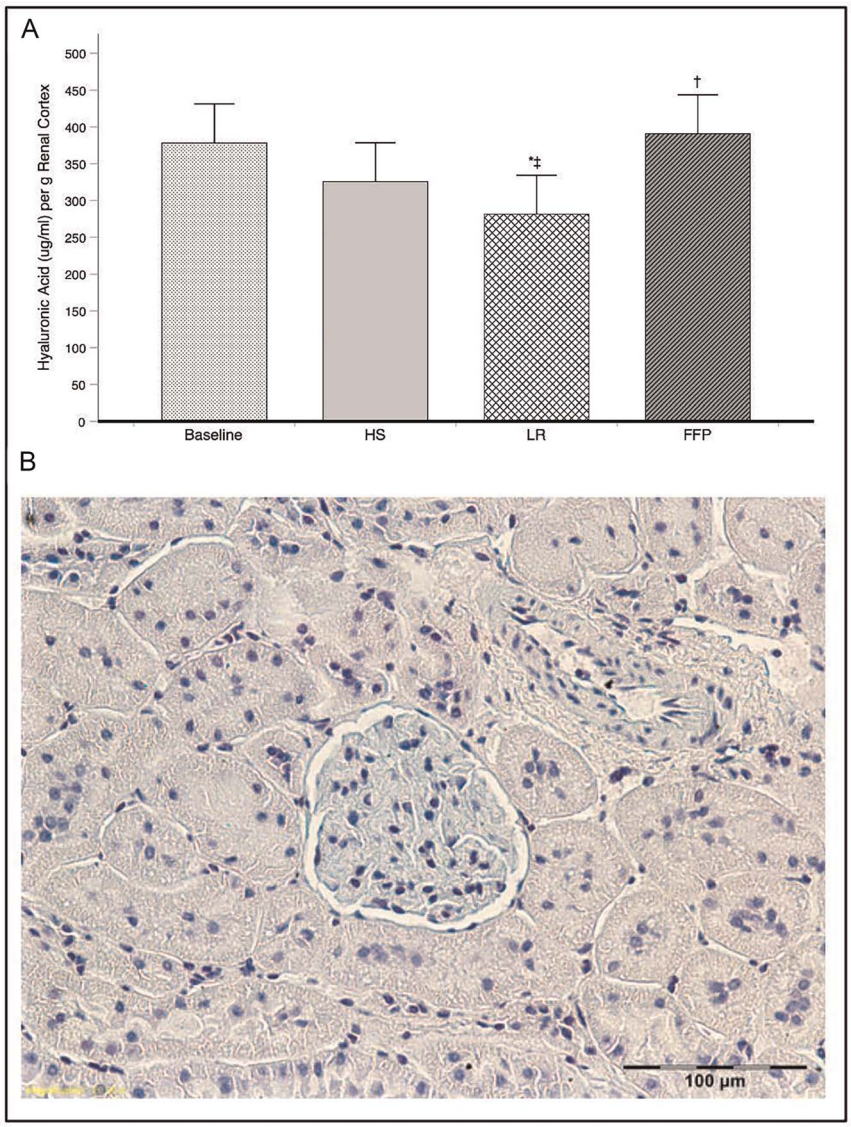

Figure 5. (*A*) Alterations of hyaluronic acid within the renal cortex following hemorrhagic shock and resuscitation. Values presented as mean ± 95% CI. (*B*) Alcian blue (pH 2.5) staining of the renal cortex demonstrates predominant localization of hyaluronic acid to the glomerulus (teal). *Versus baseline, §versus hemorrhagic shock, †versus lactated Ringer's, and ‡versus fresh frozen plasma ($p < 0.05$). CI, confidence interval.

to identify corresponding changes in glomerular function. Although shedding of syndecan-1, the prototypical marker of glycocalyx breakdown, was not observed, we demonstrated significant alterations in hyaluronic acid within the glomerular glycocalyx. In particular, resuscitation modality greatly influenced hyaluronic acid metabolism with exacerbated shedding and restoration induced by isotonic crystalloid and plasma administration, respectively. Corresponding to changes in glomerular hyaluronic acid were alterations in urine protein concentration, a surrogate for glomerular function. Ultimately, resuscitation with lactated Ringer's caused an elevation in urine protein concentration, while fresh frozen plasma restored levels back to baseline. Given the absence of alterations in proximal convoluted tubule expression of cubilin and megalin, biomolecules responsible for the reabsorption of protein from the glomerular ultrafiltrate, these results suggest that resuscitation modality has a profound impact on glomerular function. Furthermore, the restoration of glomerular hyaluronic acid may explain, in part, the improvement in glomerular function observed with plasma resuscitation.

The hallmark of glomerular dysfunction is the inappropriate loss of protein across the glomerular barrier, manifesting as proteinuria. However, attributing increased urine protein concentration to glomerular dysfunction alone is complex, as protein within the ultrafiltrate is modified by the renal tubule system and influenced by the overall tubule volume and resuscitation status. For example, the rise in urine protein concentration directly after hemorrhagic shock is likely driven by the renal physiologic response to hypovolemia, which drives sodium and water reabsorption from the ultrafiltrate, concentrating the final urine product. However, ongoing alterations in urine protein concentration after adequate

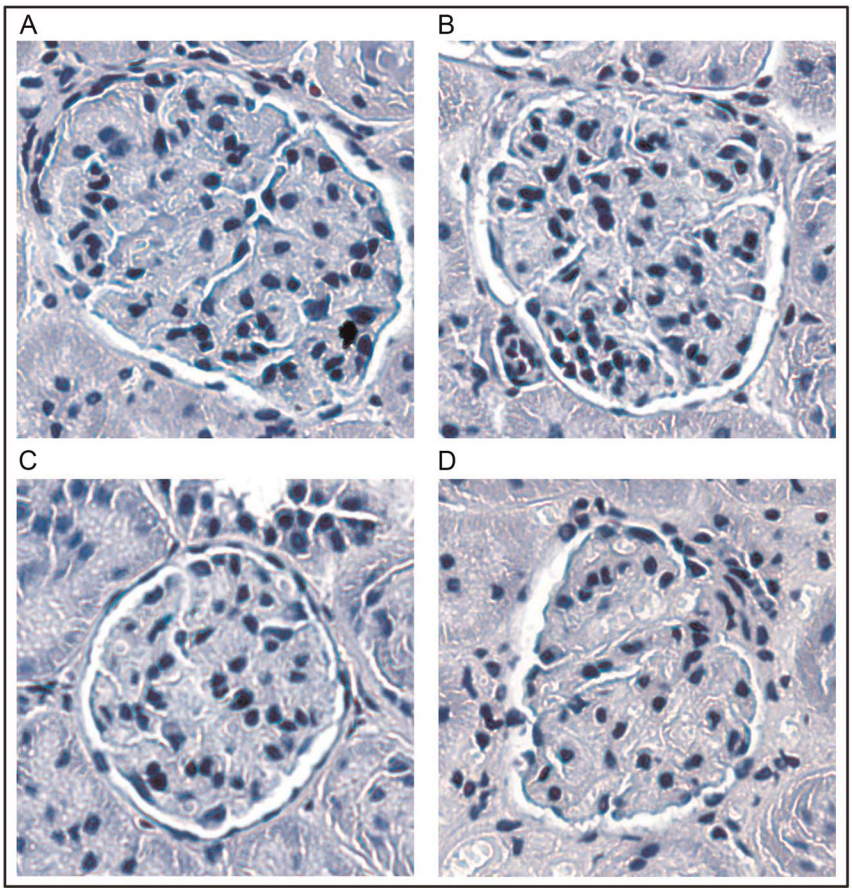

Figure 6. Glomerular hyaluronic acid by resuscitation modality visualized with Alcian blue (pH 2.5) staining. (*A*) Baseline, (*B*) post–hemorrhagic shock, (*C*) post–lactated Ringer's resuscitation, and (*D*) post–fresh frozen plasma resuscitation.

resuscitation (restoration of MAP and return of urine output) with either modality are likely related to abnormalities in glomerular filtration and/or reabsorption within the tubule system.

Under normal conditions, the majority of filtered protein is reabsorbed within the proximal convoluted tubule via receptor-mediated endocytosis, primarily by the proteins megalin and cubilin.[10,11] To evaluate tubule protein reabsorption capacity across the experimental groups, megalin and cubilin expression was quantified. Surprisingly, neither hemorrhagic shock nor resuscitation altered renal cortex levels of megalin and cubilin from baseline, suggesting preserved protein reabsorption within the proximal convoluted tubule. As a result, the difference in urine protein concentration observed between animals resuscitated with lactated Ringer's and plasma is likely due primarily to differences in protein flux across the glomerulus. Given the observed differences in hyaluronic acid between the resuscitation modalities, acute changes within the glomerular glycocalyx are likely involved in the observed alterations in glomerular function.

Although previous studies evaluating the glomerular barrier have not involved hemorrhagic shock, experimentally induced enzymatic degradation of the glomerular glycocalyx, including hyaluronic acid, has been shown to result in abnormal protein flux and size selectivity of the glomerular barrier.[12–14] A similar mechanism likely explains our experimental findings, as animals resuscitated with lactated Ringer's demonstrated the lowest concentration of glomerular hyaluronic acid, which in turn was associated with the highest amount of protein in the final urine product. The relationship between hyaluronic acid and urine protein concentration was reversed in animals receiving FFP. In this group, higher levels of hyaluronic acid within the glomerular glycocalyx were associated with improved glomerular protein handling and a final urine protein concentration near normal.

Systemic shedding of the endothelial glycocalyx is a known consequence of hemorrhagic shock and results in elevated concentrations of glycocalyx components within the plasma.[4] In our current model, plasma syndecan-1 demonstrated the expected rise following hemorrhage and crystalloid resuscitation. Likewise, the attenuation of systemic shedding (reduced plasma syndecan-1) was observed with FFP. Although these findings align with the expected outcome of global glycocalyx metabolism, the concentration of glomerular specific syndecan-1 did not demonstrate a statistically significant difference between experimental groups in our study. While previous studies of renal ischemia-reperfusion have demonstrated syndecan-1 shedding, models of hemorrhagic shock have surprisingly shown an increase in syndecan-1 immunofluorescence in the glomeruli after hemorrhage.[15] The etiology for the discrepancies between our findings and other studies is undoubtedly multifactorial but likely influenced

by the degree of ischemic insult (renal ischemia reperfusion via complete occlusion of the renal artery versus systemic hemorrhagic shock with ongoing but reduced blood flow) and the modality used to assess syndecan-1 levels.

Although syndecan-1 has historically served as the predominant marker used to investigate glycocalyx pathophysiology, the glycocalyx is a complex structure consisting of numerous components. Given the ambiguity of findings related to syndecan-1 metabolism in the glomerulus, we suspected that alterations in other glycocalyx components may shed light on the overall health of the glomerular glycocalyx following hemorrhagic shock and resuscitation. Preliminary data from our laboratory evaluating the urine proteome for markers of glomerular damage after hemorrhage demonstrated an increased abundance of hyaluronidase-1, an enzyme responsible for hyaluronic acid turnover from the glycocalyx (data not shown). This finding drove the inclusion of hyaluronic acid in our current study.

It remains unclear why there appeared to be a preferential shedding of hyaluronic acid as opposed to syndecan-1 from the glomerular glycocalyx. While glycocalyx degradation is often described as a uniform process with the release of all components within its structure, varying enzymes target different individual glycocalyx constituents. Therefore, it is possible that hyaluronidase-1 activity was significantly higher than that of other enzymes responsible for syndecan-1 metabolism in our model. As a result, a statistically significant difference in hyaluronic acid concentration was observed, while syndecan-1 levels were relatively preserved across the experimental groups. Moving forward, our findings suggest that evaluation of syndecan-1 alone may be inadequate in detecting alterations to the glycocalyx.

The results of this study must be interpreted considering several limitations. Although the volume of urine obtained via bladder puncture at the time of euthanasia was similar between animals (~1 mL) and no differences were observed in proximal convoluted tubule megalin and cubilin, using urine protein concentration at a single point in time as a surrogate for glomerular function is not without limitations. First, we are unable to comment on the functional status of megalin and cubilin. It is possible that increased protein in the ultrafiltrate may have caused receptor oversaturation. Likewise, although animals in both resuscitation arms were producing urine at similar rates, the total volume of urine produced by each animal was not captured during the experimental protocol. In addition, how the total amount of protein excreted in the urine varies beyond our short experimental timeline remains unknown. Finally, while Alcian blue titrated to a pH of 2.5 preferentially binds hyaluronic acid, other glycosaminoglycans are likely to exhibit some degree of dye interaction. In addition, hyaluronic acid is present not only in the glomerular glycocalyx but also in the underlying glomerular basement membrane. Both of these factors may have influenced histologic interpretations.

In conclusion, although no evidence of syndecan-1 shedding was seen, hyaluronic acid within the glomerular glycocalyx undergoes significant alterations during hemorrhagic shock and is heavily influenced by resuscitation modality. While LR exacerbates shedding, FFP attenuates degradation leading to levels of hyaluronic acid near baseline. The maintenance of hyaluronic acid within the glomerular glycocalyx likely contributes to the beneficial effects of plasma resuscitation on glomerular function observed after hemorrhagic shock. Overall, the identification of early alterations to the glomerulus represents a new angle into posttraumatic acute kidney injury pathophysiology and suggests that renal injury and subsequent clinical manifestations may be driven by more than renal tubule dysfunction.

AUTHORSHIP

W.B.R., P.J.M., B.G.H., and J.W.S. contributed in the study design. W.B.R., P.J.M., M.E.F., J.L., and Y.L. performed the experiments. W.B.R., P.J.M., and J.W.S. contributed in the data analysis. All authors contributed in the data interpretation. W.B.R. and J.W.S. contributed in the manuscript drafting. W.B.R., P.J.M., B.G.H., and J.W.S. contributed in the critical revisions.

DISCLOSURE

Conflicts of Interest: Author Disclosure forms have been supplied and are provided as Supplemental Digital Content (http://links.lww.com/TA/E396).

REFERENCES

1. Reitsma S, Slaaf DW, Vink H, van Zandvoort MA, oude Egbrink MG. The endothelial glycocalyx: composition, functions, and visualization. *Pflugers Arch*. 2007;454(3):345–359.
2. Jedlicka J, Becker BF, Chappell D. Endothelial glycocalyx. *Crit Care Clin*. 2020;36(2):217–232.
3. Qi F, Zhou H, Gu P, Tang ZH, Zhu BF, Chen JR, et al. Endothelial glycocalyx degradation is associated with early organ impairment in polytrauma patients. *BMC Emerg Med*. 2021;21(1):52.
4. Schucht JE, Matheson PJ, Harbrecht BG, Bond L, Ashkettle GR, Smith JW. Plasma resuscitation with adjunctive peritoneal resuscitation reduces ischemia-induced intestinal barrier breakdown following hemorrhagic shock. *J Trauma Acute Care Surg*. 2021;90(1):27–34.
5. Schucht JE, Matheson PJ, Harbrecht BG, Bond L, Jones S, Alkhateeb KJM, et al. Plasma resuscitation with adjunctive peritoneal resuscitation reduces ischemic intestinal injury following hemorrhagic shock. *J Trauma Acute Care Surg*. 2020;89(4):649–657.
6. Peng Z, Pati S, Potter D, Brown R, Holcomb JB, Grill R, et al. Fresh frozen plasma lessens pulmonary endothelial inflammation and hyperpermeability after hemorrhagic shock and is associated with loss of syndecan 1. *Shock*. 2013;40(3):195–202.
7. Schult L, Halbgebauer R, Karasu E, Huber-Lang M. Glomerular injury after trauma, burn, and sepsis. *J Nephrol*. 2023;36(9):2417–2429.
8. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *PLoS Biol*. 2020;18(7):e3000410.
9. S. Saraswathi JVT, Vasan NS. Histochemical localization of protein-polysaccharides in renal tissue. *J Biosci*. 1987;11:245–256.
10. Christensen EI, Birn H, Storm T, Weyer K, Nielsen R. Endocytic receptors in the renal proximal tubule. *Physiology (Bethesda)*. 2012;27(4):223–236.
11. Nielsen R, Christensen EI, Birn H. Megalin and cubilin in proximal tubule protein reabsorption: from experimental models to human disease. *Kidney Int*. 2016;89(1):58–67.
12. Dane MJ, van den Berg BM, Avramut MC, Faas FG, van der Vlag J, Rops ALMM, et al. Glomerular endothelial surface layer acts as a barrier against albumin filtration. *Am J Pathol*. 2013;182(5):1532–1540.
13. Jeansson M, Haraldsson B. Morphological and functional evidence for an important role of the endothelial cell glycocalyx in the glomerular barrier. *Am J Physiol Renal Physiol*. 2006;290(1):F111–F116.
14. Singh A, Satchell SC, Neal CR, McKenzie EA, Tooke JE, Mathieson PW. Glomerular endothelial glycocalyx constitutes a barrier to protein permeability. *J Am Soc Nephrol*. 2007;18(11):2885–2893.
15. Abdullah S, Karim M, Legendre M, Rodriguez L, Friedman J, Cotton-Betteridge A, et al. Hemorrhagic shock and resuscitation causes glycocalyx shedding and endothelial oxidative stress preferentially in the lung and intestinal vasculature. *Shock*. 2021;56(5):803–812.

