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14. ABSTRACT

Purpose: The purpose of this project is to determine the optimal dose and timing of cilastatin therapy to determine strategies to mitigate acute kidney injury (AKI), and to determine whether or not the pragmatic administration of cilastatin prevents rhabdomyolysis induced AKI (rhAKI), hyperkalemia, and mortality caused by a combat-relevant model of severe musculoskeletal trauma.

Scope: To determine the optimal dose and timing of cilastatin administration and strategies to mitigate rhAKI, we will use the glycerol injection model of rhabdomyolysis to determine the dose response relationship and assess cilastatin's protective effect. We will test glomerular filtration rate (GFR) as the primary outcome of renal function in addition to rigorous documentation of rhabdomyolysis severity, renal inflammation, and renal histologic injury.

Major Findings: We previously found that inducible proximal tubule-specific deletion of megalin is highly protective in a mouse model of induced rhAKI. Overall findings to date from this funded work include:

1. Protection from cilastatin occurs only in the presence of renal megalin.
2. Administration of ibuprofen but not caffeine worsens rhabdomyolysis-induced AKI.
3. There is lack of harm from cilastatin administration. In pigs, blood pressure, heart rate, temperature, and hematologic parameters are not changed by cilastatin, and in mice, urine output and GFR were not altered by cilastatin administration.
4. In swine, it was discovered that hemorrhagic shock (HS) causes myoglobinemia.
5. Administered cilastatin does not exacerbate the harm in animals with combined rhabdomyolysis and HS, and may in fact be beneficial in that it ameliorates hyperkalemia
6. A single dose of cilastatin is likely not beneficial to AKI caused by severe hemorrhagic shock
7. A single dose of cilastatin IS beneficial to the AKI caused by severe muscle injury without hemorrhagic shock, in both mice and pigs.
8. A single dose of cilastatin ameliorates hyperkalemia caused by muscle injury in pigs
9. A single dose of cilastatin reduces the number of interventions required for treatment of lethal hyperkalemia in pigs
10. Administering Vitamin D (as calcitriol) with cilastatin may potentiate the beneficial effects of cilastatin, perhaps by a muscle-centric, rather than kidney-centric mechanism.
11. Treatment of crush syndrome in a combat-relevant large animal trauma model is feasible, safe and likely effective.

15. SUBJECT TERMS					
Rhabdomyolysis, acute kidney injury, rhabdomyolysis induced acute kidney injury, chronic kidney injury, megalin, proximal tubule epithelial cells, cilastatin, glomerular filtration rate, hyperkalemia, renal, myoglobinemia.					
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1. INTRODUCTION:
Rhabdomyolysis is a lethal musculoskeletal disorder. Destruction of skeletal muscle by crush trauma, blast injury, burn injury, or excessive activity causes release of muscle protein into the systemic circulation where it is toxic to the kidney. The purpose of this proposal is to test and develop an intervention exploiting novel molecular biology (discovered in the PI's lab), which prevents released muscle protein from injuring the kidney. The scope of the research is to utilize animal models to create a critical body of data supporting translation to a clinical trial for an FDA-approved medication, including effectiveness data in a combat relevant model, understanding of real-world modifiers of injury and recovery, optimal dose and timing, and submission of an investigational new drug application allowing clinical trial and use.

2. KEYWORDS:

1. Musculoskeletal	11. C57BL/6 mice
2. Rhabdomyolysis (rh)	12. Swine
3. Acute Kidney Injury (AKI)	13. Hyperkalemia
4. Chronic Kidney Injury (CKI)	14. Multitrauma
5. Megalin (Lrp2)	15. Injury (blast/burn/crush/musculoskeletal)
6. Proximal tubule epithelial cells (PTEC)	16. Renal
7. Cilastatin	17. Ibuprofen
8. Glomerular Filtration Rate	18. Caffeine
9. Myoglobin	19. Vitamin D (calcitriol)
10. Kidney	20. Myoglobinemia

3. ACCOMPLISHMENTS:

The major goals of the project are 1) to determine the optimal dose and timing of cilastatin therapy and determine strategies to mitigate real-world accelerators of AKI and 2) to determine whether pragmatic administration of cilastatin prevents RIAKI, hyperkalemia and mortality caused by combat-relevant model of severe musculoskeletal trauma.

What were the major goals of the project?

Milestones for Goal 1, Year 1 and 2:

- Obtain IACUC approval from the PVAMC for mouse experiments. Obtain ACURO approval. Recruit and hire staff. Initiate Aim 1 experiments. 100% complete.
- Complete GFR for experiment 1.1, therapeutic timing. Select timing for experiment 1.2. 100% complete.
- Complete remaining assays of 1.1. Start 1.2, optimal dose. 100% complete.
- Complete GFR analysis for 1.2 and select dose for further experiments. 100% complete
- First submission for publication. 100% complete.
- Submit yearly report. 100% complete.
- Complete Accelerant experiment, aim 1.3-1.5: 100% complete
- Complete analysis for accelerant experiment, aim 1.3-1.5: 100% complete.

Milestones for Goal 2, Year 1 and 2:

- Obtain IACUC approval from OHSU and ACURO approval. Enter FDA pre-IND consultation program. >100% complete. IACUC and ACURO approvals were achieved. Amended IACUCs and ACURO approvals received.
- Pre-IND program not available for this drug.
- Cilastatin identified as a candidate for orphan drug status (<200,000 cases/year in US)
- Have identified and contacted industry partners interested in seeking orphan status determination with us in order to pursue a clinical trial.
- Have received phase I clinical trial data (study conducted in Spain).
- Train Schreiber lab personnel in sample acquisition and storage methods for kidney tissue, blood, and urine samples. Generate experimental guidance materials and manuals. Complete FDA pre-consultation (initial) and alter Experiment 2.2 if necessary. 100% complete.
- Randomize swine to receive vehicle, cilastatin, or cilastatin+calcitriol. Initial swine experiments (Aim 2.1, first 9 animals). Initial GFR measurements in OHSU bioanalytical core. 100% complete.
- Complete interim analysis after 9 animals. Adjust 2.1 goals if necessary. Continue randomized experiments. 100% complete.

Major Tasks/Scope of Work:

Specific Aim 1	Timeline	Hutchens Lab	Schreiber Lab	% complete
Major Task 1: Determine therapeutic timing and dose of Cilastatin (Aims 1.1 and 1.2)	Months			100%*
Determine therapeutic timing of cilastatin treatment by 24h glomerular filtration rate to feed forward to subtask 2	2-6	Dr. Hutchens 72 mice		100%
Determine optimal dose of cilastatin by 24h glomerular filtration rate to feed forward to Major Task 2 and 3	7-11	Dr. Hutchens 150 mice		100%
Milestone(s) Achieved				100%
PVAMC IACUC and ACURO Approval for mouse experiments	2	Dr. Hutchens		100%
All Hutchens lab hiring completed	2	Dr. Hutchens		100%
Optimal dose and timing of cilastatin identified	11	Dr. Hutchens		100%
Major Task 2: Determine and mitigate effects of combat-relevant accelerators of kidney injury (Aims 1.3-1.6)	Months			100%**
Test effect of caffeine as an accelerator of rhAKI	13-15	Dr. Hutchens 90 mice		100%
Test effect of ibuprofen as an accelerator of rhAKI	17-20	Dr. Hutchens 90 mice		100%
Test effect of accelerator on cilastatin-induced renoprotection in rhabdomyolysis	21-23	Dr. Hutchens		100%
Test effect of vitamin D replacement	23-26	Dr. Hutchens 20 mice		20%†
Milestone(s) Achieved:				
Select most potent accelerator of renal injury	20			100%
Determine whether accelerators mitigate cilastatin effect	23			100%
Determine whether vitamin D supplementation is salutary in mice	26			20%†
Specific Aim 2				
Major Task 3: Determine whether cilastatin prevents rhAKI in a combat-relevant swine multitrauma model.				
Obtain OHSU IACUC and ACURO Approval for swine experiments	1-3	Dr. Hutchens	Dr. Schreiber	100%
Train Schreiber Lab personnel in sample acquisition for kidney tissue, blood, and urine samples. Prepare written standard work for experiment 2.1 and 2.2. Model testing.	4-6	Dr. Hutchens	Dr. Schreiber 5 pigs	100%
Randomized swine experiments to test effectiveness of cilastatin in combat-like multiple trauma – first 9 pigs (aim 2.1)	7-9	Dr. Hutchens (data analysis, assays) Dr. Andeen (pathology development)	Dr. Schreiber 9 pigs	100%
Interim analysis for pragmatic experiment, adjust experiment if necessary	10-12	Dr. Hutchens		100%
Continue randomized swine experiment to test effectiveness of cilastatin in combat-like multiple trauma.	13-21	Dr. Hutchens (data analysis, assays) Dr. Andeen (pathology)	Dr. Schreiber 39 pigs	85%
Acquire and analyze toxicology data for selected cilastatin dose (aim 2.2)	20-30	Dr. Hutchens (data analysis, assays)	Dr. Schreiber 12 pigs	15%

		Dr. Andeen (pathology)		
Milestone(s) Achieved:	9			
OHSU IACUC and ACURO Approval for swine experiments	3			100%
Interim analysis, pragmatic adjustment of experiment 2.1	12			100%
Cilastatin effectiveness primary outcome (GFR) analysis complete	22			66%
All swine experiments completed	29			70%
All swine experiment data analysis completed	33			30%
Major Task 4: Transition cilastatin to the threshold of therapeutic use.				
Enter pre-IND consultation program with FDA	1	Dr. Hutchens		n/a
Write IND for human use of cilastatin as preventive for rhabdomyolysis-induced AKI	24-30	Dr. Hutchens		n/a
Write proposed/preliminary cilastatin white paper proposing specific dose, timing, and monitoring for cilastatin in protocol for prolonged field care of crush injury. Consultation with stakeholders.	26-32	Dr. Hutchens		65%
Milestone(s) Achieved:				
Complete pre-IND consultation	6			n/a††
Submit IND	30			0%
Cilastatin field care white paper presented at MHSRS	32			0%

*Data published: Matsushita K, Mori K, Saritas T, Eiwaz MB, Funahashi Y, Nickerson MN, Hebert JF, Munhall AC, McCormick JA, Yanagita M, Hutchens MP. Cilastatin Ameliorates Rhabdomyolysis-induced AKI in Mice. J Am Soc Nephrol. 2021 Oct;32(10):2579-2594. doi: 10.1681/ASN.2020030263. Epub 2021 Aug 2. PMID: 34341182; PMCID: PMC8722809.

**Data accepted for publication: Hebert JF, Eiwaz MB, Nickerson MN, Munhall AC, Pai AA, Groat T, Andeen NK, Hutchens MP. Legal Performance-Enhancing Drugs Alter Course and Treatment of Rhabdomyolysis-Induced Acute Kidney Injury. Mil Med. Manuscript #MILMED-D-22-00791R3.

†Experiment delayed as surprising beneficial effect of calcitriol, which does not appear to be related to depletion of vitamin D, discovered in Aim 2 experiments. Post-mortality outcomes being redesigned so that we can test the hypothesis that calcitriol alters muscle cell death in rhabdomyolysis prior to starting this now mechanistic (previously hypothesis-testing) experiment.

†† Pre-IND consultation requires detailed pre-work for clinical trial. Steps detailed below

- Cilastatin identified as a candidate for orphan drug status (likely <200,000 cases/year in US)
- Industry partner identified
- Phase I data from international trial obtained
- Clinical trial partner (U Colorado COMBAT Center) identified and in active discussion.
- Adaptive and other trial designs explored to leverage existing Phase I data.

Additional findings have formed part or whole of the following list of submitted or accepted abstracts and papers:

1. *Cilastatin inhibits renal myoglobin endocytosis and acute kidney injury following rhabdomyolysis*. Abstract. American Society of Nephrologists Kidney Week, November, 2021. San Diego, CA.

2. *Rhabdomyolysis-induced acute kidney injury and treatment Are impacted by legal performance-enhancing drugs.* Abstract, Military Health Systems Research Symposium. September, Kissimmee, FL.

3. *Rhabdomyolysis-induced acute kidney injury in reproductive-age mice causes delayed pregnancy and offspring abnormalities.* Abstract, Military Health Systems Research Symposium. September 2022, Kissimmee, FL.

4. *Specific therapy for myoglobin-induced acute kidney injury in a combat-relevant, large animal polytrauma model.* Abstract, Military Health Systems Research Symposium. September, 2022, Kissimmee, FL.

5. *Applying clinical research data science methods to a collaborative translational study*. Abstract. OHSU APOM Research day. June, 2022. Portland, OR.

6. *Rhabdomyolysis-induced acute kidney injury and treatment are impacted by legal performance enhancing drugs.* Abstract. OHSU research week, May, 2022. Portland, OR.

7. *Pregnancy following recovered acute kidney injury results in placental insufficiency and perinatal mortality.* Abstract. OHSU research week, May, 2022. Portland, OR.

8. *Pregnancy following recovered acute kidney injury results in placental insufficiency and perinatal mortality.* Abstract. OHSU Department of Medicine Research Symposium, June, 2022. Portland, OR.

9. *Pregnancy after AKI results in placental insufficiency and perinatal mortality. Abstract.* DOHAD World Congress. August, 2022. Vancouver, BC.

10. *Pregnancy following resolved AKI results in growth restricted offspring in mice.* Abstract. Society for Reproductive Investigations. March, 2022. Denver, CO.

11. *Molecular mechanisms of rhabdomyolysis-induced kidney injury: from bench to bedside*. Manuscript. Kidney International Reports. September, 2022.

12. *Renally-targeted nanoparticle drug therapy for rhabdomyolysis-induced kidney injury*. Abstract. American Society of Nephrology (ASN) Kidney Week, November, 2022

13. *Harm! Foul! How acute kidney injury SHReDDs patient futures*. Manuscript. Current Opinion in Nephrology and Hypertension. March, 2023.

14. *Legal performance-enhancing drugs alter course and treatment of rhabdomyolysis-induced acute kidney injury.* Manuscript accepted for publication in Military Medicine. March, 2023.

15. *Recovered parental acute kidney injury causes pregnancy complications and adult-onset offspring renal dysfunction.* Abstract. OHSU APOM Research & Scholarship Day. June 2023.

16. *Distal nephron role in lethal crush syndrome hyperkalemia may have treatment implications.* Abstract. American Association for the Surgery of Trauma (AAST). Anaheim, CA. September 2023.

Data from this work has been included as part of the following submitted and/or pending grant proposals (grant number/status):

1. NIH TL1TR002371:OSLER/awarded
   Long-term impact of acute kidney injury: reproductive, pregnancy, and offspring health.

2. W81XWH-22-PRMRP-TTDA-GG/Administratively withdrawn
   Nanotherapy for acute and long-term sequelae of rhabdomyolysis.

3. PA-20-185/R01DK134505/not awarded
   Proximal nephron-specific drug therapy for nephrotoxic, ischemic, and crush syndrome induced acute kidney injury.

4. PA-20-185/R01DK135528/not awarded
   Role of megalin-dependent kidney injury in placental insufficiency.

5. PA-20-188/K99DK134812/not awarded
   Determining mechanisms of longitudinal reproductive and developmental impact following parental recovered acute kidney injury.

6. NRSA F32/F32DK134127/not awarded
   Long-term impact of acute kidney injury: reproductive, pregnancy, and offspring health.

7. BIRCWH K12/not awarded

Acute kidney injury impacts reproductive function.

8. Oregon Clinical & Translational Research Institute Biomedical Innovation Program (BIP) Drug Discovery/not awarded

   Novel megalin inhibitors to enhance cancer and antimicrobial chemotherapy, and treat traumatic acute kidney injury.

9. PA-20-185/R01DK132675/pending review
   Megalin targeted delivery of renal-clearable nanomedicine.

10. BX-22-001/2I01BX004288-05/Scored: not awarded
    Myocyte-to-kidney signaling in cardiorenal nephrosclerosis and hypertension.

11. Trunkey Center Research and Innovation Award/not awarded
    Kidney-targeted nanotherapeutics for crush syndrome and long-term sequelae.

12. PA-20-185/1R01DK138531-01/pending
    Proximal tubule targeted drug delivery system for Acute Kidney Injury.

13. 2023 Women's Health Research Funding Opportunity-Circle of Giving/Not awarded
    Addressing female vulnerability to kidney injury and reproductive adverse outcomes due to crush syndrome and exertional rhabdomyolysis.

14. HT9425-23-PRMRP-TTDA-GG/pending
    Nanotherapy for acute and long-term sequelae of rhabdomyolysis.

15. PA-21-271/1K99DK134812-01A1/pending
    Determining mechanisms for longitudinal reproductive and developmental impact following parental recovered acute kidney injury.

16. Oregon Clinical & Translational Research Institute KL2 Program/not awarded
    Determining mechanisms of longitudinal reproductive impact following recovered maternal acute kidney injury.

17. BX-23-001/I01BX004288/scored for funding
    Myocyte-to-kidney signaling in cardiorenal nephrosclerosis and hypertension.

18. PA-20-185/1R01DK135528-01/ not awarded
    Role of megalin dependent kidney injury in placental insufficiency.

What was accomplished under these goals?

Accomplishments below are referred to in scope of work line items above. Subitems are not present below if completed in previous years and documented on prior progress reports.

Aim 1

Goal 1: determine the optimal dose and timing of cilastatin therapy. (Aims 1.1 – 1.2)

We completed preliminary experiments and characterization of the mouse model, which led to optimizing study prior to completing this major task. During this funded year we completed recharacterizing the model and then completed experiments from subaims 1.1 and 1.2. Data analysis of secondary outcomes is ongoing; primary outcomes (GFR) are presented below. Analyses were conducted using linear mixed models; for each outcome, the most reductive and best-fit of several models was selected.

The optimal timing of cilastatin after RIAKI induction is within 30 minutes (Figure 1). For this experiment, efficacy was predesignated as mean GFR greater than twice that of vehicle treatment. This endpoint was met by 3 groups – 0h, 0.5h, and 1h delay. GFR in the 0h, and 0.5h treatment delay groups is significantly greater than that of vehicle. We interpret this data as indicating that RIAKI treatment with cilastatin is effective up to 30 minutes after injury; there may be partial efficacy with delay up to 1h after injury.

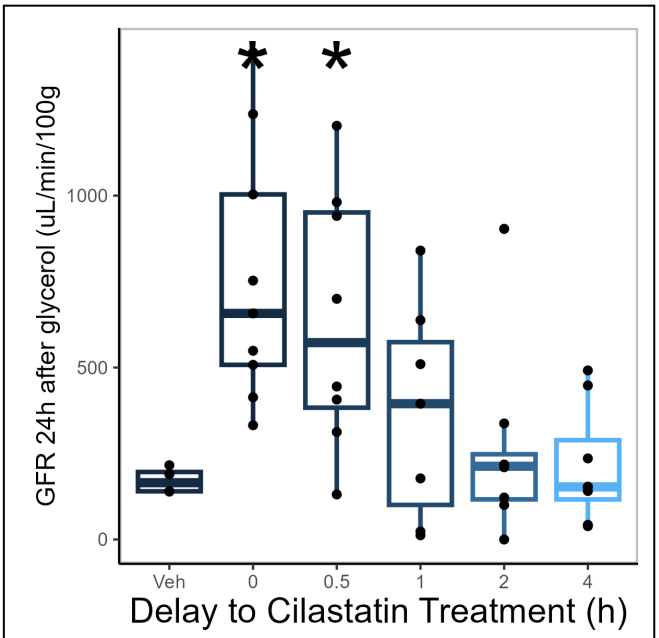

Figure 1: RIAKI treatment with cilastatin is effective up to 30 minutes after injury; there may be partial efficacy with delay up to 1h after injury. Analysis: linear mixed model (R package lmer), *p<0.05

The optimal dose of cilastatin, given 30 minutes after RIAKI induction, is 100 mg/kg (Figure 2). For this experiment, optimal was pre-defined as the lowest dose at which the efficacy endpoint (above) was reached.

The optimal dose definition is supported by measurements of urine output (UOP) for 24h after RIAKI induction. UOP is a complex outcome – it may reflect injury or recovery; recovery may increase UOP to greater than normal levels. Therefore, interpretation of this data requires correlation with GFR and other outcomes (histology, immunostaining) currently underway. The 24h UOP after RIAKI induction is the same for all cilastatin doses 0-100 mg/kg. Thereafter, as dose increases, UOP tends to decrease. 24h UOP is significantly reduced (relative to vehicle) by administration of 400 mg/kg cilastatin. These results are highlighted in Figure 3. Therefore, we conclude that the optimal dose of cilastatin sodium for experimental RIAKI in mice is 100 mg/kg when delivered 30 minutes after injury.

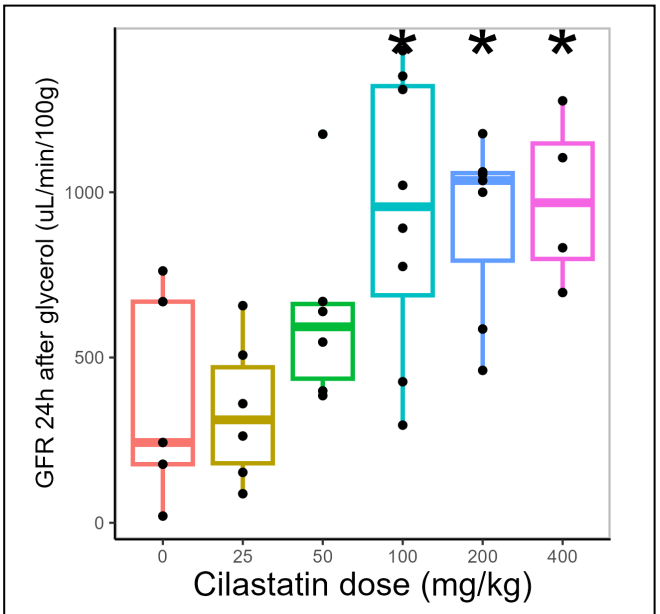

Figure 2: Administered 30 minutes after RIAKI induction, cilastatin sodium doses of 100 mg/kg and greater are effective in ameliorating GFR loss. Analysis: linear mixed methods (R package lmer), *p<0.05. The optimal dose (see text) is 100 mg/kg.

Goal 2: determine and mitigate effects of combat-relevant accelerators of kidney injury. Aims 1.3 – 1.6)

We completed these studies during the 2022-2023 funding period, presented it at MHSRS, and it is now accepted for publication in Military Medicine. As it is not currently available online, we provide access to the data here for the convenience of the reader. Acute kidney injury is understood to be exacerbated or accelerated by legal performance-enhancing drugs commonly used by warfighters. For example, 50-70% of deployed active-duty service members report using caffeine-based, performance-enhancing, or weight-loss supplements.(1, 2) Caffeine supplements cause dehydration(3) and can cause RIAKI when used in excess.(4) Similarly, ibuprofen, a non-steroidal anti-inflammatory drug (NSAID) with analgesic properties, is widely used by service members;(5) ibuprofen and other NSAIDS are well understood to cause and/or exacerbate AKI.(6) As some accelerants will be present in many injured warfighters who might be eligible for cilastatin treatment, it is critical to understand whether mitigating the effect of these drugs is necessary for cilastatin-mediated protection from myoglobin-induced kidney injury. Therefore, these investigations tested the hypothesis that caffeine and NSAIDs worsen RIAKI and interfere with both conventional and experimental cilastatin treatment.

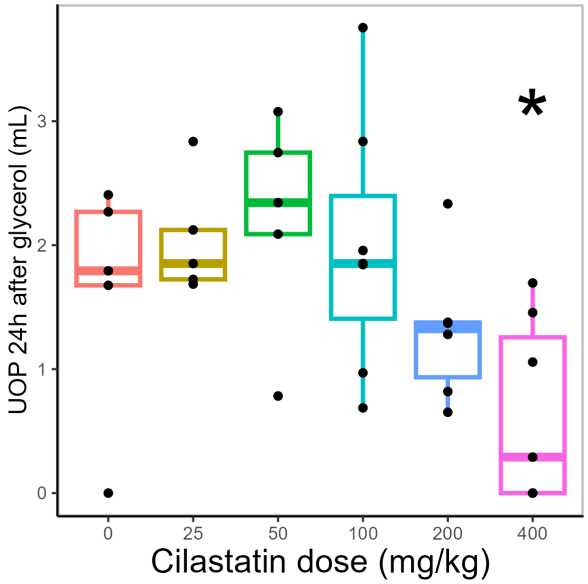

Figure 3: 24h urine output (UOP) data supports selection of 100 mg/kg as optimal after RIAKI induction, UOP is decreased by 400 mg/kg, and may be decreased by 200 mg/kg. UOP is a complex outcome and will be correlated with other data currently undergoing analysis.

Test effect of caffeine as an accelerator of rhAKI: Following glycerol injection, mice received either standard treatment alone, caffeine alone, or caffeine with standard treatment (Figure 4A). Glycerol injection and standard treatment still resulted in AKI in all mice, reducing GFR by 50% (1307±72 in sham, 717±74 µl/min/100g bodyweight in glycerol-injected mice, $p<0.001$), which was not worsened by caffeine, either with or without standard treatment (420±119 in caffeine-treated, 562±105 µl/min/100g in caffeine and PlasmaLyte-treated mice) (Figure 4B). Caffeine treatment raised blood urea nitrogen (BUN) by almost two-fold; this was not ameliorated by PlasmaLyte infusion (BUN 108±64 in caffeine-treated mice *vs.* 68±25 mg/dL in respective control, $p<0.01$, Figure 4C). Neither urine output (UOP) nor urinary myoglobin content was affected by caffeine or subsequent treatment with PlasmaLyte (Figure 4D, 4E). Periodic acid-Schiff (PAS) staining of renal cross-sections confirmed tubular injury: caffeine-treated animals demonstrated patchy tubular necrosis, attenuation of tubular cytoplasm, and tubular casts, but there were no significant quantitative or qualitative differences between treatment groups (Figure 4F). In conclusion, caffeine only minimally alters RIAKI. Therefore, a relevant and commonly-used dose of caffeine does not worsen RIAKI. GFR, urine output, and renal histology were not altered in RIAKI by caffeine administration, indicating similar renal functional and histologic injury. Caffeine did induce an increase in 24h BUN. This likely reflects the mild diuretic effect of caffeine. This hypothesis is supported by partial reversal of increased BUN in animals receiving additional fluid resuscitation. Together these findings may reinforce the importance of initial volume resuscitation in warfighters with crush injury.

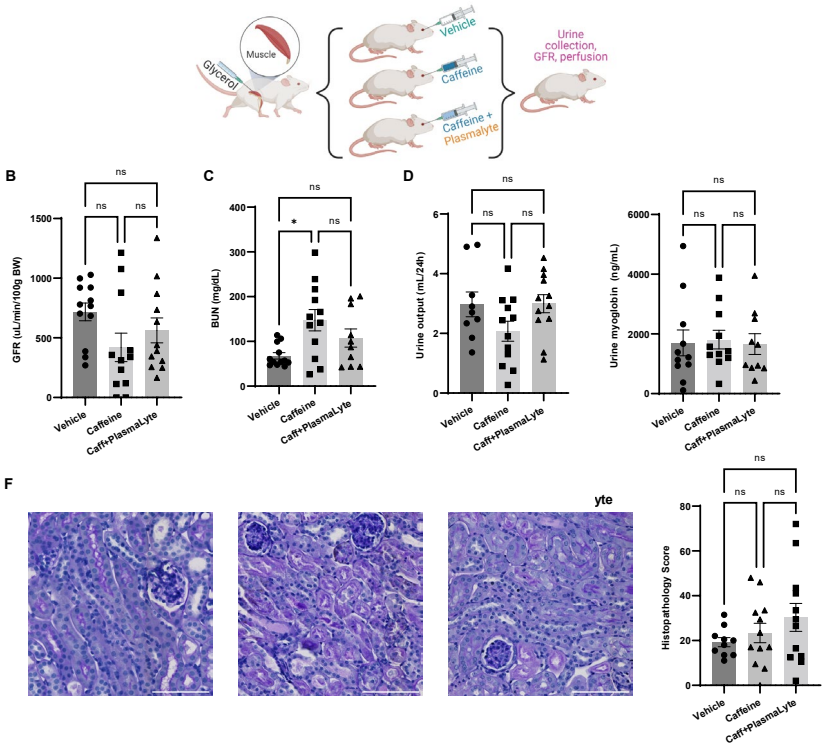

Figure 4. Caffeine increases blood urea nitrogen (BUN) but does not affect glomerular filtration rate (GFR) following rhabdomyolysis-induced acute kidney injury (RIAKI). (A) Experimental model for caffeine and resuscitative treatment following RIAKI. Caffeine did not change GFR (B), but increased BUN (C). Urine output (D) and urine myoglobin concentration (E) remained unchanged. Periodic acid-Schiff (PAS) stained sections from caffeine-injected animals showed acute tubular injury with patchy tubular necrosis, attenuation of the tubular epithelium, loss of brush border, and focal casts consistent with AKI; caffeine did not worsen this condition (F). Quantitative histopathologic scoring by a treatment-blinded renal pathologist (NA) confirmed observations. Scale bars: 100µm. Means±SEM, significance: one-way ANOVA.**: $p<0.01$. ns: not significant.

Test effect of ibuprofen as an accelerator of rhAKI: Unlike caffeine, ibuprofen administration greatly worsened RIAKI, reducing GFR by 98% and causing significant oliguria and elevation of BUN (GFR 14.3±19.5 vs 577.4±454.6 µL/min/100g in control, urine output 0.5±0.4 in IBU vs 2.7±1.7 mL/24h in control, BUN 264±201 in ibuprofen-treated mice vs 66±21 mg/dL in control, $p<0.05$ for all). Treatment with PlasmaLyte ameliorated oliguria, but did not alter GFR or BUN (Figure 5B, C), indicating that standard RIAKI treatment efficacy is mitigated in the presence of ibuprofen (PlasmaLyte treatment: GFR 169.5±190.1 µL/min/100g, $p=0.08$ compared with ibuprofen alone; BUN 189±120, $p=0.45$ compared with ibuprofen alone; urine output 2.4 mL/24h, $p=0.03$ compared with ibuprofen alone; Figure 5 B-E). There was no significant difference in urinary myoglobin content between groups. Histologically, ibuprofen-receiving animals demonstrated tubular necrosis as seen in previous RIAKI pathology, but in addition occasional, prominent protein resorption droplets in the deep cortex were observed (Figure 5F). Although PAS and KIM-1 (Figure 5G) staining both revealed severe tubular epithelial injury, there was no evidence of significant quantitative difference between treatment groups. From this data, it can be inferred that ibuprofen administered in a relevant dose at the time of injury greatly worsens RIAKI. Administration of ibuprofen in a by-weight similar dose to that used in humans at the same time as induction of rhabdomyolysis resulted in drastic worsening of AKI as indicated by GFR, BUN, and urine output 24 hours later. Moreover, standard treatment for RIAKI was less effective in the presence of ibuprofen. Histologic features of RIAKI with concomitant administration of ibuprofen suggested additional, perhaps mechanistically distinct renal injury (hyaline droplets not seen in RIAKI with vehicle). The magnitude of the increase in functional injury conferred by ibuprofen administration further suggests an additional mechanism. As NSAIDs including ibuprofen can cause AKI, this additional effect might be considered unsurprising; however, a single dose of ibuprofen would not be expected to cause this degree of AKI in isolation.(7) Therefore, this finding bears further examination. A secondary, but important, clinical implication is that muscle pain induced by exercise is often treated with NSAIDs at precisely the time at which excessive exercise may induce RIAKI.(8-11) The possibility that exercise-induced RIAKI is exacerbated by NSAID use deserves further examination as treatment with alternative analgesics might prevent or ameliorate RIAKI in this setting.

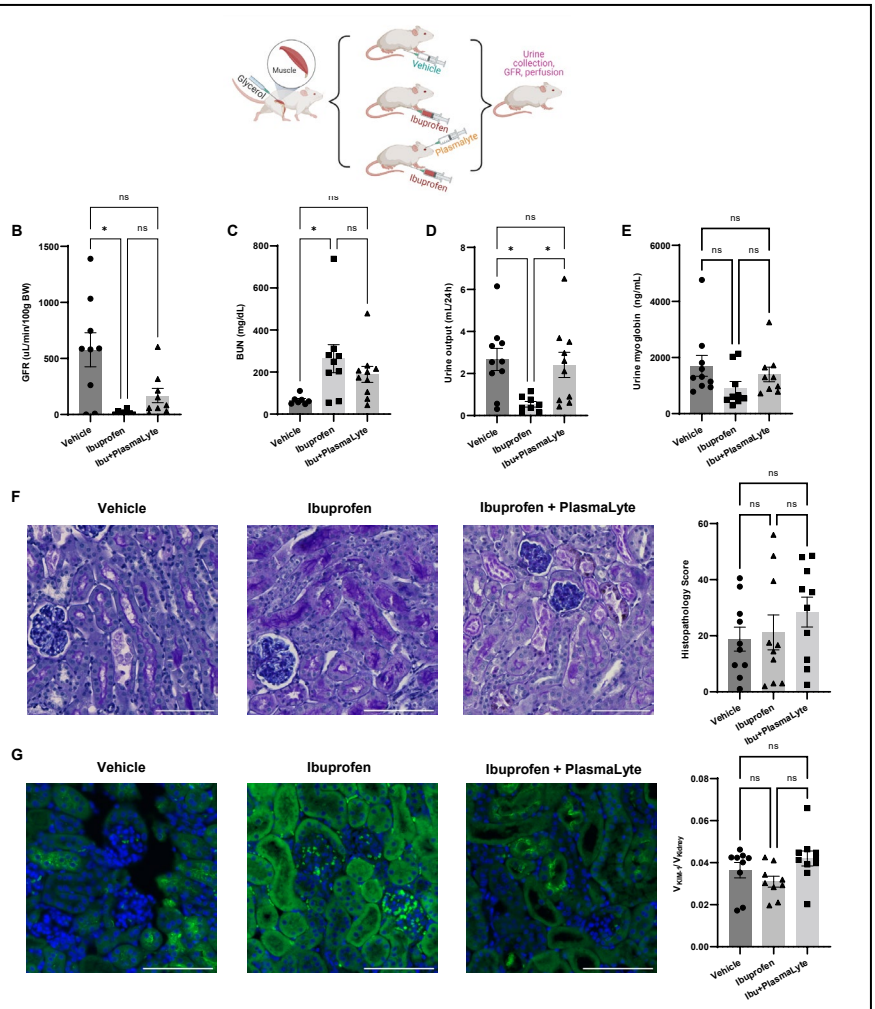

Figure 5. Ibuprofen greatly worsens RIAKI. (A) Experimental model of ibuprofen and resuscitative treatment following RIAKI. RIAKI was more severe in animals pre-treated with ibuprofen, reducing GFR (B) and increasing BUN (C). Ibuprofen worsens oliguria (D) but not urinary myoglobin (E). Damaged tubular epithelial cells were (F) PAS stained and scored and (G) KIM-1 stained and quantified by unbiased stereology. Scale bars: 100µm. Means±SEM, significance: one-way ANOVA. *: $p<0.05$. ns: not significant.

Test effect of accelerator (ibuprofen) on cilastatin-induced renoprotection in rhabdomyolysis: The efficacy of cilastatin alone and with standard fluid resuscitation treatment was tested in the compounded conditions of RIAKI with ibuprofen. 24 hours after glycerol and ibuprofen injection, mice treated with cilastatin demonstrated similar renal function to mice treated with vehicle (105.3±88.1 vs 396.2±162.0 µL/min/100g) (Figure 6B). Accordingly, both plasma urea nitrogen (BUN) and urine output (UOP) were not different between vehicle- and cilastatin-treated mice (BUN; 133.7±57.5 in vehicle vs 110±451 mg/dL in cilastatin-treated mice; UOP, 1.2 in vehicle vs 1.4 ml/24h in cilastatin-treated mice) (Figure 6C, D). Resuscitative fluid administered with cilastatin was insufficient to improve parameters to baseline. Although varying degrees of tubular injury was observed in both PAS-stained (Figure 6E) and KIM-1-stained (Figure 6F) sections, particularly with cilastatin combined with PlasmaLyte, cellular necrosis was limited and no statistical differences were observed when quantifying the effect from histological sections. These results demonstrate that cilastatin therapy does not reverse RIAKI when RIAKI is compounded by ibuprofen administration. This finding may have important translational implications, as ibuprofen is commonly used in settings in which there is high risk for RIAKI, such as fitness training, military training, and warfighting.

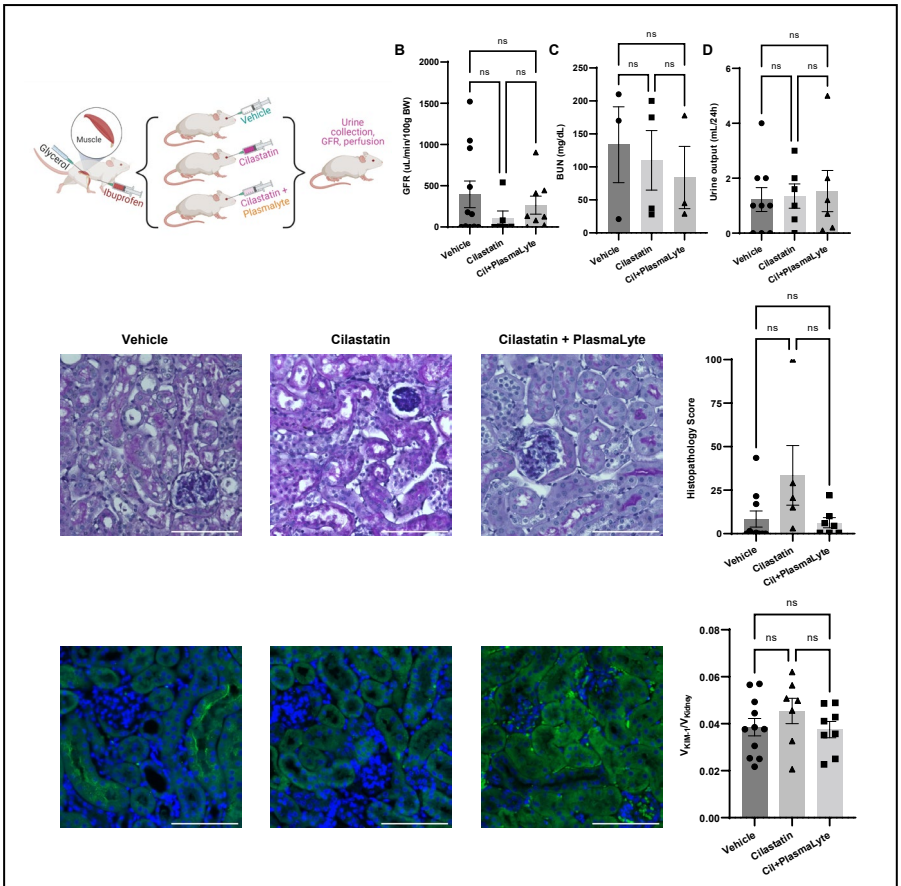

Figure 6. Resuscitative care of RIAKI is insufficient in ibuprofen-treated mice. (A) Experimental model of ibuprofen, resuscitative, and cilastatin treatment following RIAKI. RIAKI in ibuprofen-treated animals was irreversible with cilastatin with or without resuscitation, with no change in (B) GFR, (C) BUN, or (D) urinary output. Cilastatin-treated animals had tubular injury as measured by PAS (E) and KIM-1 (F) staining, consisting of epithelial attenuation with limited overt cellular necrosis. Scale bars: 100µm. Means±SEM. ns: not significant.

Test effect of vitamin D replacement: This experiment is currently under modification and has not been completed. The reason for this is that data from Aim 2 experiments in pigs indicate that addition of calcitriol to cilastatin treatment improves efficacy with respect to 48h GFR and reduces maximal plasma creatine phosphokinase (CPK). Since CPK is not renally cleared, this indicates that calcitriol likely exerts its effect by ameliorating muscle injury, or at least release of CPK. The mouse experiment was delayed by early challenges, and as a result now offers an opportunity to explore the mechanism of the effect observed in pigs. We are developing expertise, assays, and collaborations to assess injury to muscle, and will return to this experiment when outcomes are well-defined.

Aim 2

Goal 3: determine whether cilastatin prevents rhAKI in a combat-relevant swine multitrauma model.

Continue/Complete randomized swine experiment to test effectiveness of cilastatin in combat-like multiple trauma.

As of this writing (June 2023), completed experimental n (not including exclusions) is vehicle 14, cilastatin only 12, calcitriol/cilastatin 13, sham 5. The study was powered for 16 pigs in each of 3 treatment groups. We will complete this study data acquisition in September 2023 at the latest. Results below are therefore interim but reflect nearly the entire sample, and are indicative of analysis plans and methods.

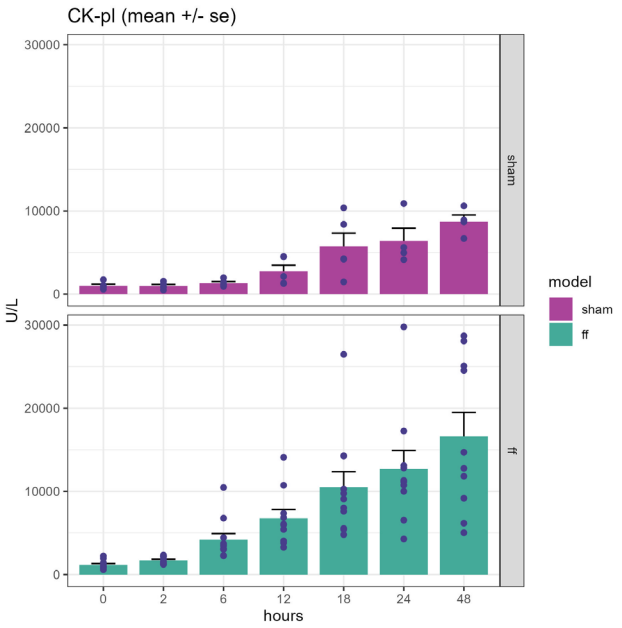

Figure 7: Injury model in pigs causes severe rhabdomyolysis. Thigh impact model ("ff") elevates mean plasma creatine kinase ("CK") above 5000IU/L by 12h (vehicle-treated animals shown). Sham animals also develop elevated plasma CK later, and less so than injured animals, likely due to immobility under general anesthesia.

Development of rhabdomyolysis in injured animals:

In these experiments after model optimization, under general anesthesia, pigs were subjected to muscle crush injury and monitored for 48h with continuous critical care treatment. Plasma creatine kinase (CK) was used to measure muscle injury, as it is not affected by renal clearance and is clinically useful to assess severity of rhabdomyolysis. Figure 7 illustrates these results. For the whole cohort, the mean baseline CK was 717±356 IU/L. The mean CK at 12h was 6,751±3,434 IU/L, and the mean CK at 24h was 12,289±6392 IU/L. Maximum CK for the cohort occurred at 32h and was 37,395±8728 IU/L. These values are consistent with human clinical values representing severe rhabdomyolysis. Values above 10,000 IU often result in the need for hemodialysis in humans. Therefore, our pig model causes severe rhabdomyolysis.

Development of hyperkalemia in injured animals:

The injury model also causes severe hyperkalemia. By protocol, plasma potassium over 5.0 mmol/L is treated with insulin, dextrose, and intravenous fluid. Therefore, maximum values reflect treatment. For the whole cohort, the maximum recorded potassium was 7.7 mmol/L, and the mean for all timepoints, including pre-injury, was 4.8±0.6 mmol/L. Figure 8 illustrates recorded potassium values in individual animals, showing results of repeated treatments. Hyperkalemia is discussed further in the section on efficacy and secondary outcomes.

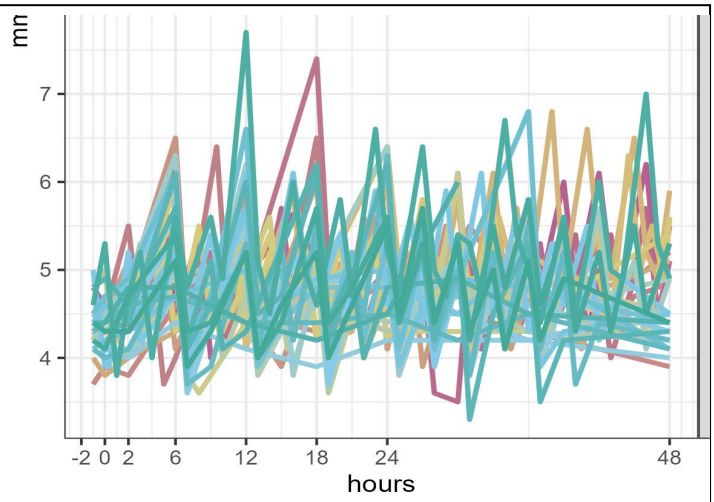

Figure 8: Injury model in pigs causes severe and repeated hyperkalemia. Thigh impact model ("ff") elevates mean plasma potassium above 5.0 mmol/L within hours of injury in nearly all animals (each line is one animal). The protocol requires treatment with insulin and dextrose for each value recorded over 5.0; the sawtooth appearance of the graph indicates episodes of hyperkalemia followed by repeated treatment.

Evidence of non-harm from treatments: Pigs in these experiments were randomized to cilastatin, cilastatin+calcitriol, or vehicle (PlasmaLyte) treatment. We assessed whether there was evidence of harm from either treatment by comparing heart rate, blood pressure, and temperature after drug and vehicle administration. Neither heart rate, mean arterial pressure, nor temperature was significantly altered by drug administration relative to vehicle (linear mixed models, p values for cilastatin and cilastatin+calcitriol versus vehicle 0.7 and 0.5 respectively for mean arterial pressure, 0.12 and 0.12 respectively for heart rate, and 0.12 and 0.13 respectively for temperature. Figure 9 illustrates these results.

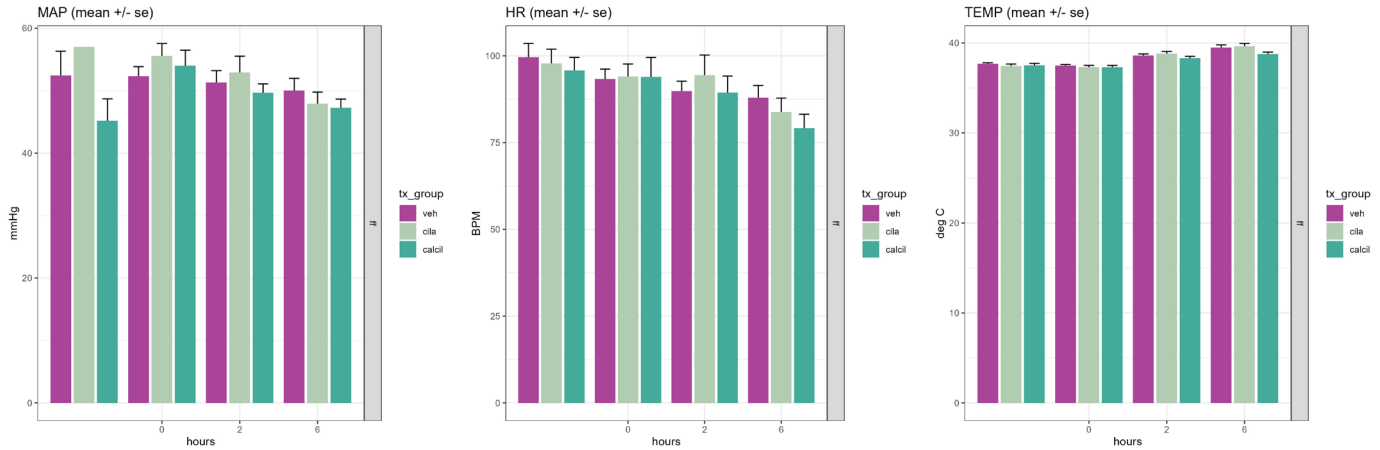

Figure 9: Mean arterial pressure, heart rate, and temperature were not affected by cilastatin or cilastatin-calcitriol administration. Analysis conducted on 48h data; data shown are baseline(time=-1h) through 6h after drug administration. See text for analysis.

Efficacy of cilastatin alone for the primary outcome: The primary outcome for drug efficacy in these experiments is 48h GFR. Statistical analysis is not complete. However, GFR is higher at every time point measured (6h after drug administration, 24h after drug administration, and 48h after drug administration). Further, during the period from 24-48 hours, most animals recover, and GFR increases. For animals treated with cilastatin, the rate of increase appears higher, suggesting improved recovery of renal function. Improved GFR is confirmed by analysis of serum creatinine and urea nitrogen (BUN), markers of renal function. Interim analysis of serum creatinine (linear mixed model) demonstrates reduced creatinine in cilastatin-treated animals ($p<0.0001$ for either cilastatin or calcitriol+cilastatin). BUN is similarly reduced by drug treatment ($p<0.0001$ for either cilastatin or calcitriol+cilastatin), and hourly and total urine output is likely greater in cilastatin-treated animals. Overall these data indicate renoprotective actions of cilastatin in a large animal trauma model. Figure 10 illustrates these results.

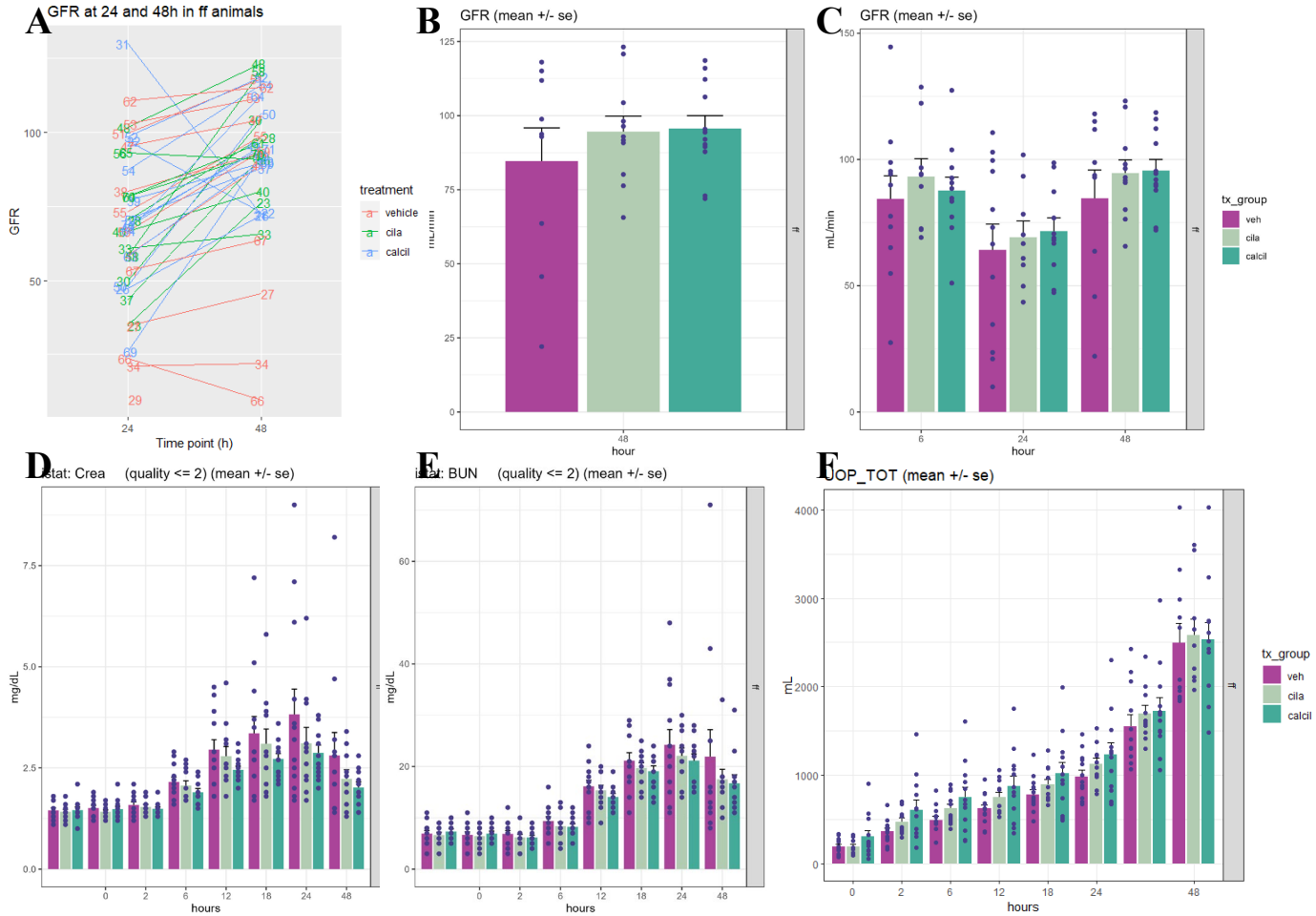

Figure 10: Indication that renal function is protected by post-injury administration of cilastatin or cilastatin plus calcitriol protects renal function. During recovery (from 24-48h), GFR rises more quickly in animals treated with cilastatin (A). At 48h, GFR appears greater in cilastatin-treated animals (B); indeed, this is likely true at all time points after injury (C). Plasma markers (creatinine and BUN, D and E) and urine output corroborate this assessment. Interim statistical analysis in text.

Efficacy of cilastatin on hyperkalemia: Hyperkalemia is the lethal component of crush syndrome and results from acute kidney injury; thus, it is essential to determine IF cilastatin ameliorates hyperkalemia. Cilastatin reduced need for hyperkalemia treatment by 50% in the first 24h ($p<0.0008$). Accordingly, Kaplan-Meier analysis demonstrates significantly longer time to required hyperkalemia intervention in animals treated with cilastatin. Figure 11 illustrates this result. Additional evaluation, including quantification of urine potassium, fractional excretion of potassium, and transtubular potassium gradient, suggest that the effect of cilastatin on GFR alone cannot account for the effect on hyperkalemia. Therefore, additional mechanistic study is underway testing the hypothesis that distal tubule potassium transport is directly affected by cilastatin administration. It is possible this investigation will yield additional treatment for hyperkalemia in crush syndrome.

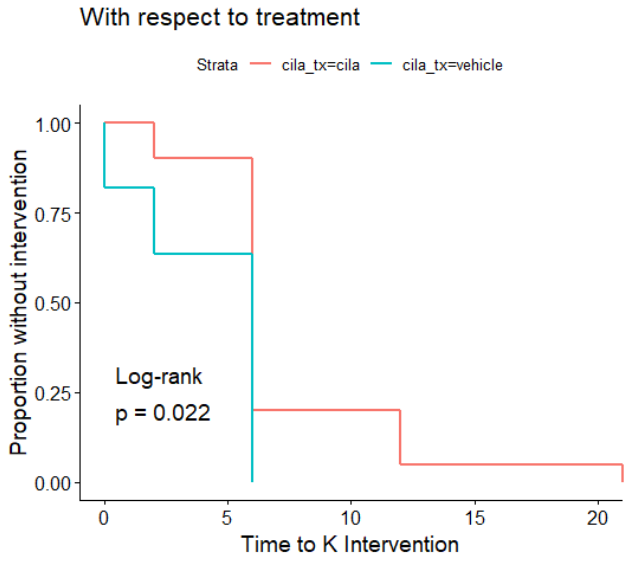

Figure 11: Cilastatin treatment, compared with vehicle, prolongs the time before hyperkalemia treatment is required. This result likely has important translational implication, since hyperkalemia is the lethal insult of crush syndrome.

Unexpected beneficial effect of calcitriol administration on plasma CK: Calcitriol is co-administered in the third arm of this study because of clinical evidence that vitamin D may mediate outcome of critical illness, particularly AKI. Cilastatin, because it inhibits megalin, may cause loss of vitamin D binding protein, and hence vitamin D in the urine. This outcome is currently being assessed. We measure plasma CK to document muscle injury in the pig trauma model. CK is too large to be filtered in the kidney, and is therefore a reliable measure of muscle destruction in rhabdomyolysis, leading to its use as a standard clinical diagnostic test. CK trends lower in animals treated with calcitriol. Statistical analysis is pending. If statistically significant, this result would have translational importance as it might lead to a method to reduce muscle injury and myoglobin release, a secondary method to reduce hyperkalemia and potentially mortality.

Quality assurance of drug preparation: Lastly, one challenge discussed in previous progress reports has been the intra-and post-pandemic availability of cilastatin. We have been required to use cilastatin sodium from several different manufacturers. Statistical analysis suggests no variation in model signal related to cilastatin manufacturer, but to assure equivalence we were able to subject samples of all cilastatin preparations to the standard dipeptidase activity assay. There is no significant difference in dipeptidase activity between the different products, providing reassurance that this will not alter study outcomes. This data is presented in figure 12.

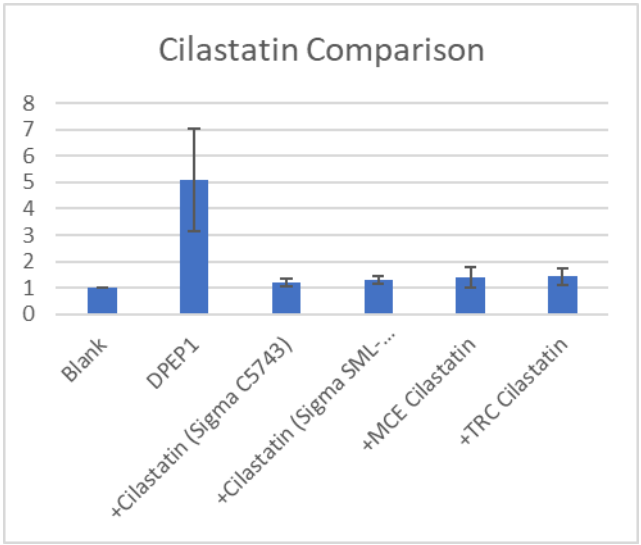

Figure 12: Cilastatin sodium preparations were subjected to dipeptidase activity assay to assess consistency of active product. There is no difference between the preparations from different manufacturers.

Discussion of goals not met:
Much progress has been made in the past year. All Aim 1 experiments are 100% complete except for our experiments to test the effect of Vitamin D supplementation in mice. This experiment was paused when preliminary data from the companion experiment in the swine model was determined to indicate there may be significant benefit to supplementing Vitamin D during rhabdomyolysis therapies; this may negate the need to determine this outcome in a smaller animal with less translational importance. After having modified the swine model in year 1 we continue to be on track to complete these experiments on time and within budget. We continue to pursue a clinical trial path for cilastatin and have identified partners. In addition, we are considering this path for several drug combination and novel preparations (see “grant applications” above) in order to ensure promulgation of the discoveries in this work. The major goal is to establish groundwork for a clinical trial; we are currently engaged in outreach to experts to ensure this goal is met. We are pleased to report that among these experts are two collaborators that were co-investigators on an international phase I trial of cilastatin and they have agreed to share their expertise and data with us.

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What opportunities for training and professional development has the project provided?

All Hutchens' lab staff are provided with 1:1 mentoring with the PI on a weekly or biweekly basis depending on the trainee's skill set and role. Mahaba Eiwaz wrote and submitted her first abstract, which has been accepted by MHSRS as a poster. She took over the research assistant duties for Aim 2 in 2022 and was taught new lab skills in Western blots, ELISAs, tissue staining and cryostat sectioning, as well as lysate preparation, brush border isolation, urine desalting, IHC, data collection and cleaning. Most of these skills were demonstrated to her by the Senior Lab Assistant, Adam Munhall, for whom teaching / team leading continues to be a goal of his professional development. Ms. Eiwaz and Mr. Munhall participate in a monthly journal club in which they are afforded the opportunity to practice creating and delivering short summaries of their projects.

All staff in the Hutchens lab participate in and attend weekly Anesthesiology and Perioperative Medicine Research Seminars. Members of the lab presented during OHSU-APOM Research Day as well as OHSU Research Week. All staff participate in professional development and training by completing the following OHSU workshops: OHSU Responsible Conduct of Research, Biosafety & Biosecurity; Working with rDNA, Infectious Agents/Toxins, Animal Care and Use/Working with the IAUCU, Information and Privacy Security Essentials, Emergency Preparedness at Work and Home, Respect at the University, COVID-19 Core Training, and Unconscious Bias Foundations. In order to meet VA requirements, lab staff also complete the following trainings: Bloodborne Pathogen Training, Fraud, Waste, and Abuse, Compliance Program Training, Biosafety and General Lab Safety, VA Information and Privacy Security. Schreiber lab members complete each of the OHSU required trainings on an annual basis. All members of both labs maintain their CITI certifications for working with animals.

In addition, select members of the lab have completed Q-fever awareness training, Laboratory Animal Occupational Health, Animal Care and Use – Working with Mice, Unconscious Bias Hiring for Managers and Supervisors, Mouse handling lab (OHSU & VA), Human Subjects Research, Good Clinical Practice, LinkedIn Learning Public Speaking Foundations, Interpersonal Communications, Emotional Intelligence, and Conflict Resolutions trainings. Dr. Hutchens and the lab manager have both completed the OHSU Mentorship Academy Intensive workshop. Ms. Groat has also participated in an additional Mentorship Academy for mentoring trainees with special accommodations.

Conferences at which lab members presented science or attended: Society for Reproductive Investigations (presented, March 2023), Military Health System Research Symposium (MHSRS) (presented August, 2022), OHSU Research Week (June 2023), APOM Research Day (June, 2023) Developmental Origins of Health & Disease (DOHAD) World Congress, (August, 2022).

The post-doctoral fellow (Dr. Jessica Hebert) on this project was awarded an Oregon Students Learn and Experience Research (OSLER) TL1 grant, which supplemented a portion of Dr. Hebert's efforts under the umbrella of the DOD project. This training grant has afforded Dr. Hebert extensive opportunities in professional development. OSLER recipients participate in the OHSU Human Investigations Program, and thus she has participated in courses on study design, biostatistics, scientific writing, and leadership skills. In the first 12 months of the OSLER grant period, Dr. Hebert conducted her proposed experiment under the mentorship of the PI. Results from her experiments were disseminated widely through presentations at OHSU, Seattle Children's Hospital Center for Developmental Biology and Regenerative Medicine, as well as national and international meetings (see Accomplishments: Disseminating Results)In addition, Dr. Hebert has presented posters describing her research supported by the DOD and OSLER grants at the American Society for Nephrology Kidney Week, the Society for Reproductive Investigation (SRI), Developmental Origins of Health & Disease (DOHAD) World Congress, and will again present at MHSRS this coming August. She has received intense training and support from her PI and the OHSU APOM division in her writing of federal level grants in her pursuit of a faculty appointment at OHSU. Dr. Hebert has submitted an F32, a K99, and a KL2. Dr. Hebert has embarked on learning the peer review process and joined the American Society of Nephrology (ASN) Career Advancement group to engage with their mentorship resources.

How were the results disseminated to communities of interest?

A. Presentations:

1. A presentation titled *Mechanisms of reproductive and developmental impact following parental acute kidney injury* was presented by J. Hebert to the Building Interdisciplinary Research Careers in Women's Health in May of 2022.
2. Our lab's work titled, *Long-term reproductive and developmental impact following parental recovered acute kidney injury*, was presented by J. Hebert at the Seattle Children's Center for Developmental Biology and Regenerative Medicine in November of 2022.
3. The PI presented his talk as part of the New York University Langone Nephrology and Health Grand Rounds titled *Plasma-borne messengers, acute kidney injury to chronic kidney disease transition* in December of 2022.
4. The PI presented his talk titled *Hyperkalemia in crush syndrome* as a guest of the OHSU Nephrology and Hypertension MEGA-lab series in January of 2023.
5. A presentation titled *The Picture of Dorian Gray('s Kidney): How Past Acute Kidney Injury Haunts the Reproductive Future* was presented by J. Hebert at the OHSU APOM Departmental Meeting in February of 2023.
6. The PI presented his talk titled *Renal megalin in acute kidney injury and subsequent cardiovascular and reproductive disease* as a guest speaker for the Harvard Medical School Molecular Imaging Seminars in May of 2023.

B. Abstracts:

Two abstracts have been accepted for poster presentations at the Military Health System Research Symposium in August, 2023.

1. *Rhabdomyolysis-induced acute kidney injury in reproductive age females causes future pregnancy complications.*
2. *Treatment of crush syndrome in mice with optimal doses and timing of cilastatin.*

The following abstract was accepted for the American Society of Nephrology (ASN) Kidney Week Conference that took place in November of 2022: *Renally-targeted nanoparticle drug therapy for rhabdomyolysis-induced acute kidney injury.*

The following abstract was accepted for the American Association for the Surgery of Trauma meeting in Anaheim, set for September, 2023: *Distal nephron role in lethal crush syndrome hyperkalemia may have treatment implications*.

Recovered parental acute kidney injury causes pregnancy complications and adult-onset offspring renal dysfunction was presented at the Society of Reproductive Investigations (SRI) in Brisbane, Queensland, Australia on March, 2023.

The following abstracts were presented at research symposiums hosted by Oregon Health & Science University:

1. *The role of myoglobin induced renal injury in reproductive disease* was presented as part of the APOM Weekly research conference in October of 2022.
2. *Specific therapy for RIAKI in a large animal model: an interim analysis* was presented as part of the APOM Weekly research conference in March, 2022.

3. Rhabdomyolysis induced acute kidney injury in reproductive age parents causes future pregnancy complications (OHSU Research Week, May 2022)

4. *The picture of Dorian Gray('s Kidney): how past acute kidney injury haunts the reproductive future* was presented at the OHSU APOM Research Conference in February of 2023.

5. *Recovered parental acute kidney injury causes pregnancy complications and adult-onset offspring renal dysfunction* (OHSU APOM Research & Scholarship Day, June, 2023).

6. *Determining optimal dose and timing of cilastatin treatment of rhabdomyolysis-induced acute kidney injury* was presented as part of the APOM Weekly research conference in June of 2023.

C. Manuscripts:

We published two manuscripts describing our findings in the past year:

1. *Harm! Foul! How acute kidney injury SHReDDs patient futures* was published in Current Opinion in Nephrology and Hypertension journal in March, 2023.

2. *The* manuscript describing our findings related to *Rhabdomyolysis-Induced Acute Kidney Injury and Treatment Are Impacted by Legal Performance-Enhancing Drugs* was published in Military Medicine in May of 2023.

What do you plan to do during the next reporting period to accomplish the goals?

In the next reporting period, the goals and objectives we plan to accomplish first are the partially completed aims from Year 1 and 2, and to commence the activities described in the milestones for Year 3.

Aim 1 Year 2 milestones to complete:
- **Months 33-36:** initiate 1.6.

Aim 1 Year 3 milestones to complete:
- **Months 25-27:** Complete experiment 1.6.
- **Months 28-30:** Complete assays and data analysis for experiments 1.3-1.6.
- **Months 31-33:** Third manuscript for publication. Cilastatin field care white paper basic science component drafted.
- **Months 34-36:** Cilastatin field care white paper presented at MHSRS. Submit final report.

Aim 2 Year 1 milestones to complete:
- **Months 25-26:** Investigate orphan drug pathway to identifying a pharmaceutical partner for RCT.
- **Months 25-27:** Initial GFR measurements in OHSU bioanalytical core.
- **Months 25-36:** Continue randomized experiments.

Aim 2 Year 2 milestones to complete:
- **Months 25-27:** Continue GFR mass spec analysis contemporaneously.
- **Months 25-31:** Continue randomized experiments in experiment 2.2. Continue GFR mass spec analysis contemporaneously.
- **Months 31-36:** Complete molecular biology, GFR, molecular biology assays, and other non-pathology data.

Aim 2 Year 3 milestones to complete:
- **Months 25-27:** Complete tissue processing from 2.1; complete pathologic analysis from 2.1
- **Months 28-30:** Complete experiment 2.2.
- **Months 31-33:** Complete experiment 2.3. Proteomics data available. Begin analysis. Cilastatin field care white paper translational (swine model) component drafted. Complete proteomics data analysis.
- **Months 34-36:** Submit final manuscripts and conference abstracts. Submit final report. Cilastatin field care white paper presented at MHSRS.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Findings from this project have been accepted for presentation at multiple conferences. Ideally, results from the project would demonstrate an impact to the general public regarding the dangers of rhabdomyolysis. In recent years the prevalence of this condition has escalated wildly. However, because we believe our results support both immediate and long-term consequences of rhabdomyolysis, we anticipate interest in investigating facile and accessible pathways to treatment in both the austere treatment environment and a clinically intense one. Below is a list of abstracts that have been accepted in the current grant reporting period.

1. Pregnancy after acute kidney injury results in placental insufficiency and perinatal mortality (DOHAD, World Congress, August 2022, Vancouver BC.)
2. Rhabdomyolysis-Induced Acute Kidney Injury and Treatment Are Impacted by Legal Performance-Enhancing Drugs (Military Health System Research Symposium [MHSRS], September 2022, Kissimmee FL).
3. Rhabdomyolysis-Induced Acute Kidney Injury in Reproductive-Age Mice Causes Delayed Pregnancy and Offspring Abnormalities (MHSRS, September 2022, Kissimmee, FL).
4. Distal Nephron Role in Lethal Crush Syndrome Hyperkalemia May Have Treatment Implications (American Association for the Surgery of Trauma, September 2023, Anaheim, CA).
5. Renally-targeted nanoparticle drug therapy for rhabdomyolysis-induced acute kidney injury. (American Society of Nephrology [ASN] Kidney Week, November 2022).
6. Recovered parental acute kidney injury causes pregnancy complications and adult-onset offspring renal dysfunction (Society of Reproductive Investigations, Brisbane, QLD Australia, March 2023).
7. Rhabdomyolysis induced acute kidney injury in reproductive age parents causes future pregnancy complications (OHSU Research Week, May 2023).
8. Recovered Parental Acute Kidney Injury Causes Pregnancy Complications and Adult-Onset Offspring Renal Dysfunction (OHSU APOM Research & Scholarship Day, June 2023).
9. Rhabdomyolysis-Induced Acute Kidney Injury in Reproductive Age Females Causes Future Pregnancy Complications (Accepted for MHSRS, August 2023).
10. Treatment of crush syndrome in mice with optimal doses and timings of cilastatin (Accepted for MHSRS, August 2023).

What was the impact on other disciplines?

The Oregon Students Learn and Experience Research (OSLER) TL1 award that was awarded to post-doctoral fellow Dr. Hebert in the first year of the grant was renewed for a second year. This enabled Dr. Hebert to continue the objectives of her training grant and further develop the science. Discoveries made as a result of Dr. Hebert's investigations during the first year of the OSLER provided preliminary data for subsequent grant proposals. In the past year Dr. Hebert resubmitted her K99, and submitted her first F32 and KL2 grants. She has the full backing of the APOM department to receive a faculty appointment. She also presented findings from this project at the Society for Reproductive Investigations conference (Brisbane, Australia March 23), and has been invited to present at The Military Health System Research Symposium (Kissimmee, August 2023). Further, findings from this project have supplemented preliminary data that has been included in four pending NIH grant submissions and one pending Peer Review Medical Research Program application.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

The two challenges most relevant to progress in years 1 and 2 of the project were COVID related supply chain issues and staffing. Supply chain issues and cost increases remain an occasional factor, but to a much lesser degree than in year 1 and have become manageable with proactive planning. We were challenged again with identifying a supply chain for our principal test drug (cilastatin) this year and were able to quickly do so; the experiment timeline was not impacted. Staffing was originally impacted by COVID restrictions, and this is no longer the case. The swine surgical team is primarily comprised of surgical residents fulfilling a research year requirement, and changeover is expected. Changeover results in training new team members and slows the timeline temporarily. This past year swine experiments were implemented by one team of surgeons and 38 swine experiments were completed, which is more than achieved in years 1 and 2 combined. The addition of a small animal surgeon and dedicating a full-time Research Assistant to bench work for swine sample analysis in year 2 has brought our project almost entirely back on schedule.

Last year we had not approached the FDA pre-IND process, however this year we are on track to enter this process prior to finishing out the NCE 4th year. The major goal is to establish groundwork for a clinical trial; we are currently engaged in outreach to experts to ensure this goal is met. In the past year we have established two new collaborations that we predict will be helpful in achieving the major goal; 1) we have identified a co-investigator of a clinical trial investigating the safety of cilastatin in healthy volunteers in Spain, which in turn 2) led us to a source for the phase I data and a resource for expediting analysis to determine the relevant dosages for a clinical trial for rhabdomyolysis in humans.

Changes that had a significant impact on expenditures

At the writing of this report we are forecast to be below our proposed spending for Year 3 and been granted a no-cost-extension to allow for completion of the project's aims in the coming year. The remaining surplus is due to carryover from the first grant year, and the general slow start due to COVID-19 precautions that impeded the commencement of our projects in year 1. Spending in the areas of travel has increased as opportunities to attend conferences in person have arisen, and we have also increased spending for publishing our study's findings.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

1. Hebert JF, Funahashi Y, Hutchens MP. *Harm! Foul! How acute kidney injury SHReDDs patient futures*. Curr Opin Nephrol Hypertens. 2023 Mar 1;32(2):165-171. Doi:10.1097/MNH.0000000000000864. Epub 2022 Dec 23. PMID: 36683541; PMCID: PMC10079264. Acknowledgement of federal support: yes.
2. Hebert JF, Eiwaz M, Nickerson M, Munhall AC, Akash A, Groat T, Andeen NK, Hutchens MP. *Legal performance-enhancing drugs alter course and treatment of rhabdomyolysis-induced acute kidney injury*. Mil Med. Accepted for publication. Manuscript #: MilMed-D-22-00791R3. Acknowledgement of federal support: yes.

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers and presentations*.*

Presentations:

1. Specific therapy for RIAKI in a large animal model: an interim analysis. (APOM Weekly Research Conference, March 2022).
2. Mechanisms of reproductive and developmental impact following parental acute kidney injury was presented by J. Hebert to the Building Interdisciplinary Research Careers in Women's Health, (May,2022).
3. The role of myoglobin induced renal injury in reproductive disease. (APOM Weekly Research Conference, October 2022).
4. Long-Term Reproductive and Developmental Impact Following Parental Recovered Acute Kidney Injury was presented by J. Hebert, by invitation from the Seattle Children's Center for Developmental Biology and Regenerative Medicine, (November, 2022).
5. The PI presented his talk, Plasma-borne messengers, acute kidney injury to chronic kidney disease transition by invitation from the New York University Langone Nephrology and Health Grand Rounds, (December, 2022).
6. The PI presented his talk, Hyperkalemia in crush syndrome as a guest of the OHSU Nephrology and Hypertension MEGAlab series, (January, 2023).
7. The Picture of Dorian Gray('s Kidney): How Past Acute Kidney Injury Haunts the Reproductive Future presented by J. Hebert at the OHSU APOM Departmental Meeting, (February, 2023).
8. The PI presented his talk, Renal megalin in acute kidney injury and subsequent cardiovascular and reproductive disease as a guest speaker for the Harvard Medical School Molecular Imaging Seminars, (May, 2023).
9. Determining optimal dose and timing of cilastatin treatment of rhabdomyolysis-induced acute kidney injury. (APOM Weekly Research Conference, June 2023).

Posters:

1. Pregnancy after acute kidney injury results in placental insufficiency and perinatal mortality (DOHAD, World Congress, August 2022, Vancouver BC.)
2. Rhabdomyolysis-Induced Acute Kidney Injury and Treatment Are Impacted by Legal Performance-Enhancing Drugs (Military Health System Research Symposium [MHSRS], September 2022, Kissimmee FL).*

3. Rhabdomyolysis-Induced Acute Kidney Injury in Reproductive-Age Mice Causes Delayed Pregnancy and Offspring Abnormalities (MHSRS, September 2022, Kissimmee FL).
4. Renally-targeted nanoparticle drug therapy for rhabdomyolysis-induced acute kidney injury. (American Society of Nephrology [ASN] Kidney Week, November 2022).
5. Distal Nephron Role in Lethal Crush Syndrome Hyperkalemia May Have Treatment Implications (American Association for the Surgery of Trauma, September 2023, Anaheim, CA).
6. Recovered parental acute kidney injury causes pregnancy complications and adult-onset offspring renal dysfunction (Society of Reproductive Investigations, Brisbane, QLD Australia, March 2023).
7. Rhabdomyolysis induced acute kidney injury in reproductive age parents causes future pregnancy complications (OHSU Research Week, May 2023).
8. Recovered Parental Acute Kidney Injury Causes Pregnancy Complications and Adult-Onset Offspring Renal Dysfunction (OHSU APOM Research & Scholarship Day, June 2023).
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- ***Website(s) or other Internet site(s)***

  Nothing to report.

- ***Technologies or techniques***

  Nothing to report.

- ***Inventions, patent applications, and/or licenses***

  Affiliate Invention application #OI2023-01699 for Cilastatin Sodium and inhibition of renal megalin to prevent chronic kidney disease. (See Appendix 3.)

- ***Other Products***

  "DATAHUB" database and analysis software, containing >10,000 lines of code in R and >125,000,000 individual datapoints, including all data acquired from pigs in this work.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	**Michael Hutchens, MD, MA**
Project Role:	Principal Investigator
Researcher Identifier:	(ORCID) 0000-0001-8583-1812
Nearest person month worked:	4
Contribution to Project:	Dr. Hutchens is the PI and responsible for every component of the project.
Funding Support:	Department of Defense

Name:	**Martin A. Schreiber, MD**
Project Role:	Co-investigator
Researcher Identifier:	(ORCID)0000-0002-4430-6779
Nearest person month worked:	2
Contribution to Project:	Dr. Schreiber is responsible for the implementation of the swine multitrauma model experiments. He will train the swine model personnel.
Funding Support:	Department of Defense

Name:	**Nicole Andeen, MD**
Project Role:	Renal Pathologist
Researcher Identifier:	(ORCID) 0000-0002-4882-6640
Nearest person month worked:	1
Contribution to Project:	Dr. Andeen applies rigorous and reproducible methods for assessment of kidney injury in the swine model. Each animal's pathology is estimated using methods described in the research narrative.
Funding Support:	Department of Defense

Name:	**Tahnee Groat, MPH**
Project Role:	Project Lead
Researcher Identifier:	(ORCID) 0000-0002-8002-7742
Nearest person month worked:	12
Contribution to Project:	Ms. Groat oversees day to day aspects of proposed work. Assures compliance with regulatory bodies, assists in the production of required reports, maintains adherence to projected milestone goals, and assists personnel in administrative and basic science procedures.
Funding Support:	Department of Defense

Name:	**Jessica Hebert, PhD**
Project Role:	Hutchens Lab Post-doctoral fellow
Researcher Identifier:	(ORCID) 0000-0003-2433-8359
Nearest person month worked:	12
Contribution to Project:	Dr. Hebert has learned and practices the rhabdomyolysis model, the glomerular filtration measurement and metabolic cage studies, and conducts all molecular biology assays as described in the research narrative. She has trained the Research Assistant 2 in these methods. She also analyzes data including the confocal microscopy imaging and quantifying injury. She will present at the Military Health Sciences Research Symposium in August.
Funding Support:	Department of Defense / TL1 OSLER

Name:	**Adam Munhall, BS**
Project Role:	Hutchens Lab Senior Research Assistant

Researcher Identifier: (ORCID) 0000-0002-1960-5486
Nearest person month worked: 12
Contribution to Project: Coordinates with Schreiber Lab Project Lead to assure proper flow of procedures and data collection. Assists with equipment set up and maintenance, drug preparation, protocol adherence, quality control, histologic preparation, molecular biology assays, and preliminary data analysis.
Funding Support: Department of Defense

Name: **Mahaba Eiwaz**
Project Role: Hutchens Lab Research Assistant II
Researcher Identifier: (ORCID) **0000-0001-6085-0304**
Nearest person month worked: 6
Contribution to Project: Ms. Eiwaz assists with lab equipment set up, take-down, cleaning, lab assay preparation and execution, assessment, data acquisition and analysis for Aims 1 and 2 of the project. Ms. Eiwaz performs model development procedures and trains new staff. Ms. Eiwaz participates in the rhabdomyolysis swine model.
Funding Support: Department of Defense

Name: **Moqing Liu, PhD**
Project Role: Schreiber Lab Sr. Research Associate
Researcher Identifier: (ORCID) 0000-0001-8385-0649
Nearest person month worked: 4
Contribution to Project: Dr. Liu oversees the intraoperative data collection of experiments in Schreiber's swine multitrauma models. She performs the quality control for samples collected and preliminary processing of these samples before distributing them to team members. Dr. Liu conducts statistical analysis on preliminary data obtained during procedures. Dr. Liu oversees laboratory assays and assists with reports.
Funding Support: Department of Defense

Name: **Karen Minoza**
Project Role: Schreiber Lab Research Associate
Researcher Identifier: (ORCID) 0009-0000-9521-8018
Nearest person month worked: 4
Contribution to Project: Dr. Minoza performs animal surgeries and monitors the animal throughout the experiment, troubleshoots and manages the animal's condition. She prepares the surgical area and instruments. She manages logistics for proposed experiments and assists Dr. Liu.
Funding Support: Department of Defense

Name: **Liujan (Helen) Liu**
Project Role: APOM Research Associate
Researcher Identifier: None Provided
Nearest person month worked: 3.6
Contribution to Project: Dr. Liu manages the APOM histology core. She oversees the processing of all mouse tissue samples for Aim 1 of this project.
Funding Support: Department of Defense

Name: **Sam Durbin**
Project Role: Schreiber Lab Surgeon
Researcher Identifier: (ORCID) 0009-0005-0618-1716

Nearest person month worked:	4
Contribution to Project:	Dr. Durbin performs all components of the swine multitrauma surgical model. She makes observations and makes decisions relevant to the procedure based on observable outcomes. Dr. Durbin works with Dr. Garay and Dr. Liu to ensure measurements are obtained as planned. Dr. Durbin participates in data analysis and the dissemination of findings.
Funding Support:	Department of Defense

Name:	**Lloyd Perrier**
Project Role:	Schreiber Lab Research Assistant
Researcher Identifier:	None provided
Nearest person month worked:	0.5
Contribution to Project:	Assists with swine model procedures by preparing and obtaining samples, model set-up and take down, animal observation and data collection.
Funding Support:	Department of Defense

Name:	**Joseph Garay**
Project Role:	Schreiber Lab Research Associate
Researcher Identifier:	(ORCID) 0000-0002-2287-3051
Nearest person month worked:	4
Contribution to Project:	Mr. Garay is the Project Lead for the swine portion of the project and thereby oversees the day to day aspects of the experiments in Schreiber's swine multitrauma models. He scheduled and managed the procedures. Mr. Garay provides consultation on measurement collection procedures. Dr. Garay has assumed responsibility for all regulatory activities for the swine ACURO and OHSU IACUC.
Funding Support:	Department of Defense

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

PI Dr. Hutchens was added as a consultant to an MRF grant at 0.25 calendar years per year for 2023.

Dr. Schreiber added an Atlantic Research Group, Inc. funded study with no overlap to this project.
Dr. Schreiber added an Octapharma AG funded study with no overlap to this project.
Dr. Schreiber added a U.S. Army MRMC funded project through a subcontract with Univ. of California, San Francisco. There is no overlap with this project.
Dr. Schreiber added a U.S. Army MRAA funded project through a subcontract with the Univ. of Pittsburgh. There is no overlap with this project.
Dr. Schreiber added a Henry M. Jackson Foundation funded study with no overlap to this project.
Dr. Schreiber added a Daxor Corporation funded study with no overlap to this project.
Dr. Schreiber has added a DoD funded clinical trial with no overlap to this project.
Dr. Schreiber has added a Medical Technology Enterprise Consortium trial with no overlap to this project.
Dr. Schreiber's previously active grant, *Mesenchymal stem cells for the prevention of acute respiratory distress syndrome after pulmonary contusion and hemorrhagic shock-BA150560*, was completed.
Dr. Schreiber's previously active grant, *Randomized trial of early hemodynamic management of patients following acute spinal cord injury – TEMPLE – W81XWH-16-1-0748*, was completed.
Dr. Schreiber's previously active grant, *Linking Investigations in Trauma and Emergency (LITES) Services*, was completed.
Dr. Schreiber's previously active grant, *Prothrombin complex concentrate for prolonged field care of war casualties (DM160342)*, was completed.
Dr. Schreiber's previously active grant, *Stem cells for the prevention of inflammatory complications of severely injured trauma patients (W81XWH-15-9-0001)*, was completed.

Dr. Schreiber's previously active grant, *Red versus white wine consumption and its effects on acute systemic inflammation and coagulation in healthy volunteers, a prospective randomized study*, was completed.

What other organizations were involved as partners?

Provide the following information for each partnership:
Organization Name: VA Portland Medical Center
Location of Organization: Portland, OR
Partner's contribution to the project:

- Facilities (e.g., project staff use the partner's facilities for project activities)

Organization Name: Oregon Students Learn and Experience Research (OSLER) TL1 Program
Location of Organization: Portland
Partner's contribution to the project:

- in-kind salary support for post-doc Dr. Jessica Hebert

Organization Name: Muruve Lab
Location of Organization: University of Calgary
Partner's contribution to the project:

- Conducted comparative analysis of study drug (cilastatin) sourced from 4 different sources for inhibitory effect of DPEP1. Results validated that there was no significant variability in cilastatin's effectiveness regardless of manufacturing source.