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Critical Illness Myopathy and Trajectory of Recovery in Acute Kidney Injury Requiring Continuous Renal Replacement Therapy: A Prospective Observational Trial Protocol

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Abstract

Introduction: Acute kidney injury requiring renal replacement therapy (AKI-RRT) is common in the intensive care unit (ICU) and is associated with significant morbidity and mortality. Continuous RRT (CRRT) non-selectively removes large amounts of amino acids from plasma, lowering serum amino acid concentrations and potentially depleting total-body amino acid stores. Therefore, the morbidity and mortality associated with AKI-RRT may be partly mediated through accelerated skeletal muscle atrophy and resulting muscle weakness. However, the impact of AKI-RRT on skeletal muscle mass and function during and following critical illness remains unknown. We hypothesize that patients with AKI-RRT have higher degrees of acute muscle loss than patients without AKI-RRT and that AKI-RRT survivors are less likely to recover muscle mass and function when compared to other ICU survivors.

Methods and Analysis: This protocol describes a prospective, multicenter, observational trial assessing skeletal muscle size, quality, and function in ICU patients with AKI-RRT. We will perform musculoskeletal ultrasound to longitudinally evaluate rectus femoris size and quality at baseline (within 48 hours of CRRT initiation), day 3, day 7 or at ICU discharge, at hospital discharge, and 1-3 months post-discharge. Additional skeletal muscle and physical function tests will be performed at hospital discharge and post-discharge follow-up. We will analyze the effect of AKI-RRT by comparing the findings in enrolled subjects to historical controls of critically ill patients without AKI-RRT using multivariable modeling.

Ethics and Dissemination: We anticipate our study will reveal that AKI-RRT is associated with greater degrees of muscle loss and dysfunction along with impaired post-discharge recovery of physical function. These findings could impact the in-hospital and post-discharge treatment plan for these patients to include focused attention on muscle strength and function. We intend to disseminate findings to participants, healthcare professionals, the public, and other relevant groups via conference presentation and publication without any publication restrictions.

Strengths and limitations of this study

- This study has several notable strengths including the study design based on multidisciplinary collaboration, the multicenter patient representation, the collection of longitudinal in-hospital and outpatient measures and outcomes, and the wide range of skeletal muscle and physical function tests being performed.
- Due to the pilot nature of this study, we will recruit critically ill patients with acute kidney injury (AKI) requiring continuous renal replacement therapy (CRRT) to collect a broad array of study measures and compare them with available datasets of recent historical controls of critically ill patients without AKI requiring RRT. While the use of these control groups enhances the feasibility of the project, it also represents a limitation.
- The high morbidity and mortality inherent to the study population may introduce competing risk of death and selection bias when assessing the outpatient outcomes.
- Differentiating the exact contributions of AKI, CRRT, and underlying acute illness may not be possible in this observational study due to several confounding variables that could be better addressed with an interventional trial design in the future. Nonetheless, our pilot study will generate critical data for the design and sample size estimations of such future trials.

Introduction

Background and Rationale: Acute kidney injury (AKI) complicates approximately 20% of all hospital admissions and up to 50% of all intensive care unit (ICU) admissions.[1 2] Moreover, 13.5% of critically ill patients develop AKI requiring renal replacement therapy (AKI-RRT).[1] AKI is associated with poor short- and long-term prognoses. Even stage 1 AKI – defined by as little as a 0.3 mg/dL increase or 50% rise in serum creatinine above baseline[3] – is associated with up to a 10-fold increase in the odds of in-hospital mortality.[4] Similarly, AKI-RRT has an in-hospital mortality rate >50%, making it one of the deadliest conditions encountered in the hospital.[5] After discharge, AKI survivors are at increased risk of developing chronic kidney disease (CKD), end-stage kidney disease, cardiovascular disease, and death.[6] Other studies suggest that AKI predisposes to disparate sources of morbidity including infection[7-10], bone fracture[11], stroke[12], GI hemorrhage[13], and dementia.[14] While muscle wasting is well-described in patients with CKD[15 16], the contribution of AKI-RRT to muscle wasting in critically ill patients has not been previously studied.

Acute skeletal muscle wasting occurs in up to 65% of patients admitted to the ICU.[17] Critical illness myopathy (CIM), defined as a deficit in muscle size and strength that develops as a result of an ICU admission, is associated with high rates of short- and long-term mortality and morbidity, including decreased quality of life (QoL) due to persistent functional mobility impairments and inability to perform simple activities of daily living.[18-21] AKI of any stage is known to alter tissue utilization of amino acids, making it plausible that AKI exacerbates CIM.[15 22] Studies have demonstrated that plasma amino acid levels are reduced and multiple non-essential amino acids become conditionally essential in the setting of AKI.[23-25] In addition, AKI leads to a state of increased amino acid oxidation but reduced amino acid transport into muscle.[15] RRT exacerbates this issue through non-selective removal of amino acids from plasma.[15] Amino acids are small and easily filtered during RRT, and, as a result, daily losses of amino acids in effluent can be immense at up to 18 grams daily with CRRT.[26 27]

The gold standards for assessing CIM are muscle biopsy or electrodiagnostic testing.[28] Furthermore, the measurement of psoas muscle area on a single cross-sectional computed tomography

(CT) image at the level of the L3 vertebra has been suggested as a standard clinical measure for skeletal muscle quantification.[29] However, biopsy, electrodiagnostic testing, and CT imaging present challenges which limit their clinical application. Musculoskeletal ultrasound (MSKUS), a relatively inexpensive, noninvasive alternative, has gained significant traction over the last decade for assessing muscle in ICU patients.[30] Studies have demonstrated that MSKUS has excellent inter-rater reliability and high clinical utility and have suggested that MSKUS has strong construct validity.[31-35] Recent data suggest that MSKUS can be reliably performed at the bedside in the ICU.[36]

The primary objective of this study is to characterize longitudinal measures of muscle size [rectus femoris (RF) muscle cross-sectional area (CSA) and muscle thickness (mT)] and quality [echo intensity (EI)] in critically ill adults with AKI requiring CRRT during their ICU stay and in post-discharge follow-up among survivors. This trial protocol was designed using the elements of the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) checklist for cohort studies[9] whenever applicable.

Objectives:

Aim 1. To characterize changes in RF muscle mass and quality at baseline and days 3 and 7 following study enrollment in critically ill adults with AKI requiring CRRT and to compare these measurements with those of historical ICU controls without AKI-RRT.

Hypothesis 1: RF muscle mass and muscle quality will be lower at 7 days in patients with AKI-RRT compared to the corresponding inpatient measurements of historical ICU controls without AKI-RRT.

Aim 2. To characterize changes in RF muscle mass and quality at hospital discharge and within 3 months post-discharge in survivors of AKI requiring CRRT in the ICU and to compare these measurements with those of historical ICU controls without AKI-RRT.

Hypothesis 2: The muscle mass and functional parameters of survivors of AKI-RRT obtained within 3 months of hospital discharge will be worse than the corresponding measurements from

historical controls of ICU survivors without AKI-RRT obtained within a similar post-discharge timeframe.

Aim 3. To examine if changes in plasma or effluent amino acid levels correlate with skeletal muscle loss during CRRT or with skeletal muscle function at 1-3 months post discharge.

Hypothesis 3: The concentrations of amino acids in blood and effluent during CRRT will correlate with MSKUS parameters of muscle mass and will be associated with muscle function following discharge.

Methods: Participants, Interventions, and Outcomes

Trial Design: This is a prospective multicenter observational study to evaluate longitudinal inpatient and outpatient measures of muscle mass and function in critically ill adults with AKI requiring CRRT and to compare these measurements with those of historical ICU controls without AKI-RRT. The study will have two phases, an ICU phase and a recovery phase for subjects who survive to discharge.

Study Setting: This study will be conducted at the adult ICUs at the academic medical centers of the University of Kentucky, University of Iowa, and University of New Mexico. Following discharge, survivors will return for outpatient evaluation of skeletal muscle and physical function.

Eligibility Criteria: To be included in the study, patients are required to be ≥18 years old and have AKI-RRT with enrollment within 48 hours of CRRT initiation. Exclusion criteria include: (1) ICU admission for >7 days; (2) RRT of any kind at any time before ICU admission; (3) CKD with estimated glomerular filtration rate <20 mL/min/1.73 m^2 as calculated by the 2021 CKD-EPI equation[37]; (4) underlying muscle disorders or muscle atrophy such as quadriplegia or hemiplegia, stroke with residual motor deficits, end-stage liver disease, active alcohol use disorder, active malignancy (other than non-melanoma skin cancer) within 1 year, burns, or other baseline neuromuscular disease; (5) pregnancy; (6) concomitant use of other extracorporeal support devices such as ventricular assisted devices or extracorporeal membrane oxygenation; or (7) anticipated inability to engage in weight-bearing testing after discharge (e.g., trauma

or orthopedic surgery). For outpatient testing, patients will be ineligible if they remain on RRT in the week prior to the research appointment.

Control Population*:* Given the pilot nature of this study, we will use recent historical controls defined as critically ill adults without AKI-RRT in whom similar measurements of muscle size, quality, and function were collected. Specifically, we have previously collected data on 41 ICU patients, of which 36 did not have AKI-RRT and will serve as the control group for the ICU phase of this study, and have published the results of MSKUS performed in the ICU and functional assessments performed at both ICU discharge and hospital discharge.[38] The controls for the recovery phase will come from an ongoing prospective observational study being performed at the University of Kentucky, which will include outpatient functional assessments performed on 200 ICU survivors (NCT05537298). See **Table 1** for a summary of the demographic and clinical characteristics of the control cohorts which have been published thus far.[21 38 39]

Primary Outcome (ICU phase): The primary outcome is the change in RF CSA, mT, and EI measured by MSKUS at baseline, assessed within 48 hours of CRRT initiation, to ICU days 3 and 7 of study enrollment (or at ICU discharge, if sooner). Operational and standardization procedures have been previously published.[35 38] In brief, patients are positioned supine with the lower extremity in neutral alignment. RF ultrasound images are acquired two-thirds of the distance from the anterior superior iliac spine to the superior border of the patella of the right lower extremity at all timepoints. Sonographers will use a linear probe (5-15 Hz) with the same machine for all timepoints and a minimal-to-no-compression technique. Sonographers will obtain three images per assessment to reduce variations in EI. Ultrasound images will be assessed for CSA, mT, and EI at baseline within 48 hours of CRRT initiation, at days 3 and 7 from enrollment (or at ICU discharge, if sooner). A representative ultrasound image and the techniques for landmarking and probe pressure are provided in **Figure 1.**

Secondary Outcomes (ICU Phase): Patients will have blood collected at baseline (within 48 hours of CRRT initiation), at study days 3 and 7 (or ICU discharge, if sooner). Creatinine and cystatin C will be measured at each timepoint. Additionally, 5-mL blood samples and CRRT effluent samples at each

Table 1. Demographic and clinical characteristics of the cohorts being used as our historical controls for this study.

Cohort Characteristic	Control cohort for ICU phase*[38]	Control cohort #1 for recovery phase†[21]	Control cohort #2 for recovery phase[39]
ICU or admission type	Medical 73%; cardiothoracic 27%	Medical 100%	Mixed
Years of enrollment	2018 to 2019	2018 to 2019	2018 to 2021
N	41*	12†	59
Primary inclusion	Acute respiratory failure or sepsis and anticipated ICU stay >3 days	Survivors of acute respiratory failure requiring >48 hours of mechanical ventilation	Survivors of sepsis and/or acute respiratory failure
Timing of initial assessment	Within 48 hours of admission; median 1.1 days [IQR 0.7-1.4]	N/A	N/A
Timing of outpatient assessment	N/A	4-8 weeks after hospital discharge	3 months after hospital discharge
Age, median years [IQR]	61 [55-68]	58 [45.5-65]	56 [48-64]
Female, no. (%)	18 (44%)	4 (33%)	31 (53%)
No. (%) mechanically ventilated	30 (73%)	12 (100%)	43 (73%)
Duration of mechanical ventilation, median days [IQR]	3.4 [1-7.7]	6.45 [2.8-11.95]	8 [5-14]
No. (%) requiring RRT	5 (12%)*	NR†	0
Disease severity (SOFA) scores	Mean 8.1 [SD ±4.8]	Median 8 [IQR 4.5-10.25]	Median 10 [IQR 8-12]
ICU LOS, median days [IQR]	8 [4]	7.4 [4.3-18.6]	10 [6-15]
Hospital LOS, median days [IQR]	11.2 [8-19]	16.7 [9.4-28.7]	15 [11-22]
Hospital mortality	12%	N/A	N/A

Presented here are only previously published data. The two control cohorts for the recovery phase represented in the table are only a subset of the 200 patients in our ongoing registry that will serve as the control for the recovery phase. *Data for this entire cohort are presented as published, including data from the 5 patients treated with RRT who will be excluded from the control group in our analysis. †Though not reported in the published manuscript, RRT status is available for this cohort and any patients with AKI-RRT will be excluded from the control group in our analysis. Abbreviations: ICU, intensive care unit; IQR, interquartile range; LOS, length of stay; N/A, not applicable; NR, not reported; RRT, renal replacement therapy; SD, standard deviation; SOFA, Sequential Organ Failure Assessment.

timepoint will be sent for gas chromatography-mass spectrometry evaluating a panel of analytes including amino acids, carbohydrates, and fatty acids. Remaining samples will be stored for future analysis, though no genetic analysis will be conducted now or in the future.

Patients will be scored at the same timepoints as above using the ICU Mobility Scale, an 11-point scale ranging from 0-10 which involves the clinician scoring the patient's maximum level of mobility in the prior 24-hour period.[40 41]

Outcomes (Recovery Phase): Survivors will participate in skeletal muscle and physical function testing at hospital discharge and during the outpatient visit at 1-3 months post-discharge. Measurements will include MSKUS to determine RF CSA, mT, and EI. We will also conduct an array of standardized and validated tests including:

- **Medical Research Council Sum-score (MRC-ss):** MRC-ss is a measure of global peripheral muscle strength that is the current clinical standard for diagnosing ICU-acquired weakness (ICU-AW).[42] Muscle strength is assessed by physical exam and rated on an ordinal scale (0-5) at six bilateral muscle groups: shoulder abductors, elbow flexors, wrist extensors, hip flexors, knee extensors, and ankle dorsiflexors. A score <48 is considered indicative of ICU-AW, with a score <36 indicative of severe weakness with the inability to act against resistance.[43]
- **Muscle strength using hand-held dynamometry for knee extension:** Maximal isometric knee extensor strength will be measured as peak force production and rate of force development following previously published standardized positions (**Figure 2**).[44]
- **Muscle strength using hand-grip dynamometry:** Hand-grip dynamometers will be utilized to measure maximum isometric strength of the hand and forearm muscles at previously published standardized positions.[42 43] The patient will undergo three repetitions with both the right and left hand, alternating between hands.

- **Short Physical Performance Battery (SPPB):** Physical function and physical frailty will be measured using the SPPB, a performance-based composite test with a total of 12 points including components of balance (side-by-side stand, semi-tandem stand, and full-tandem stand), chair-to-stand test, and 4-meter habitual gait speed.[45 46]
- **Timed Up and Go (TUG) Test:** The TUG assesses the time (in seconds) for a subject to stand on command from a seated position, walk 3 meters, turn around, walk back to the chair, and sit down. The purpose of TUG is to assess mobility, physical function, and fall risk. TUG has been validated in and recommended for patients with critical illness.[47-49]
- **Six-minute walk test (6-MWT):** The 6-MWT assesses the distance a subject can walk in six minutes, providing a global representation of physical function and cardiopulmonary endurance.[50 51] Meta-analysis provides benchmark data for survivors of critical illness.[52]
- **Quality of Life testing using EuroQol Group 5-dimension 5-level (EQ-5D-5L) questionnaire:** The EQ-5D is a standardized measure of health status developed by the Euro-Qol Group to provide an assessment of health for clinical and economic appraisal.[53] It consists of two sections: the descriptive system and the visual analogue scale (EQ VAS). The descriptive system assesses five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. The EQ VAS records the respondent's self-rated health on a 20-cm vertical visual analogue scale with endpoints labeled the "best health you can imagine" and the "worse health you can imagine." This information can be used as a quantitative measure of health as judged by individual respondents.[51 54 55]
- **Clinical Frailty Scale (CFS):** The CFS is designed for clinical use and has been widely adopted as a judgement-based tool to screen for frailty and to broadly stratify degrees of fitness and frailty.[56 57] It is not a questionnaire, but a way to summarize information from a clinical encounter to roughly quantify an individual's overall health status. While CFS has traditionally been used

specifically in older patients, recent data have demonstrated its utility in an ICU population demographically similar to our target population.[58]

- **Functional Assessment of Chronic Illness Therapy Fatigue (FACIT-F) Questionnaire:** The FACIT-F scale is a 13-item measure that assesses self-reported fatigue and its impact upon daily activities and function.[59 60]
- **36-Item Short Form Health Survey Physical Function Scale (SF-36 Physical Function):** The SF-36 is a 36-item patient-reported survey of health commonly used to evaluate adult patients which contains 8 domains, including a physical function scale based on 10 of the 36 items which has been shown to have high reliability.[61]
- **Additional Events:** Finally, we will document the occurrence of the following events: return to driving, return to work or hobby, hospital readmission, and need for emergency department care.

Assessor Training: This multisite study is an interdisciplinary collaboration with expertise in critical care, nephrology, muscle biology, and physical function. Physical therapist-scientists and exercise physiologists with established expertise in MSKUS and functional testing serve as coinvestigators at each site. Three two-hour sessions of teleconference training will be performed to promote standardization of ultrasound and outcome assessments. In addition, novice sonographers were instructed to perform and practice a minimum of 10 acquisitions of RF muscle images from healthy individuals before study initiation. To better establish inter-rater reliability of image acquisition, the first 5 patients at each site will have ultrasound studies conducted by two team members. The first sonographer will obtain images and leave the room, and the second will enter and repeat the test. After a 10-minute wash-out period, the team members will sequentially repeat the ultrasound measurements, which will allow us to establish both inter- and intra-observer variability. Finally, images will be blinded, coded (rater 1 or rater 2; site location) and sent securely to the University of Kentucky to be reviewed by an expert sonographer (KPM, who has > 6 years of MSKUS experience) to ensure cross-site standardization of measurements.[35] All images will be analyzed for muscle CSA, mT, and EI by the same blinded expert sonographer. The images from the first 5 patients obtained by the two sonographers at each site will be examined with intra-

class correlation coefficient (ICC). Sites with ICC <0.7 will receive additional training to improve reliability at each site. ICC values will be disseminated with our final results.

Participant Timeline: Patients enrolled in the study will participate in up to two phases, an ICU phase and – for those who survive their critical illness – a recovery phase. The schedule of assessments for both phases is delineated in **Table 2**.

Table 2. Planned evaluations at each study timepoint.

Parameter	Timepoint				
	ICU Phase			Recovery Phase	
	Within 48 hours of CRRT start	3 ± 1 days from enrollment	Study day 7 (or ICU discharge)	Hospital Discharge	Outpatient 1-3 months
Rectus Femoris US	X	X	X	X	X
Amino Acids (Blood)	X	X	X		
Amino Acids (Effluent)	X	X	X		
Cystatin C	X	X	X		
ICU Mobility Scale	X	X	X		
Muscle Strength - MRC-ss				X	X
Muscle Strength - HGD				X	X
Muscle Strength - HHD				X	X
SPPB				X	X
TUG test					X
6-MWT					X
EQ-5D-5L					X
Clinical Frailty Scale					X
FACIT-fatigue					X
SF-36 Physical Function					X
Return to driving					X
Return to work or hobby					X
Readmission					X
ED visit					X

Abbreviations: 6-MWT, six-minute walk test; ED, emergency department; EQ-5D-5L, EuroQol Group 5-dimension 5-level version; FACIT-F, Functional Assessment of Chronic Illness Therapy-Fatigue; HGD, hand-grip dynamometry; HHD, hand-held dynamometry; ICU, intensive care unit; MRC-ss, Medical Research Council sum-score; SF-36, 36-Item Short Form Health Survey; SPPB, Short Physical Performance Battery; TUG, Timed Up and Go; US, ultrasound.

Sample Size: Our previous study reported a decrease in muscle RF CSA at ICU day 7 of 18.5%.[38] To detect an absolute difference in percent decrease in muscle size of 6% at 7 days (24.5% change at 7 days in AKI-RRT group vs. 18.5% change in controls), 61 patients per group are needed assuming an alpha of 0.05 and a power of 80%. This will require inclusion of 20 AKI-RRT patients per site that provide all ICU datapoints. Our previous study also reported that RF CSA was 2.47 ± 0.88 cm^2 at ICU day 7, down from a baseline on ICU day 1 of 2.99 cm^2.[38] To detect a full return to baseline in the outpatient setting with an alpha of 0.05 and a power of 80%, 22 patients are required in each group. To detect a 75% recovery to baseline from day 7 values, 40 patients are required. We anticipate a 40-50% in-hospital mortality rate which would leave 31 to 37 patients alive at discharge. Assuming 22 patients are needed, this provides room for attrition or loss to follow-up of 30-40%.

Recruitment: Patients will be recruited in the multidisciplinary adult ICUs of the three sites involved in the study. Patients will be identified through communication with the nephrology consult services at each site, who will independently make decisions regarding indications for and timing of initiation of RRT. The enrollment will occur for a full year following institutional review board (IRB) approval at each site, with the funding dates ranging from August 1, 2022, to July 31, 2023, at the University of Kentucky and University of Iowa and from October 1, 2022, to September 30, 2023, at the University of New Mexico.

Patient and Public Involvement: No formal patient advisory committee was established and there was no patient or public involvement in the design or planning of the study. However, to inform future study design, we will conduct a brief open-ended post-study survey following the outpatient visit at 1-3 months to help discern which of the patient-reported outcome measures and functional assessments performed appear most valuable to the study subjects (**Supplementary material 1**).

Methods: Data collection, management, and analysis

Data Collection Methods: Ultrasound images will be transferred through secure link to the data coordinating site (University of Kentucky). One expert sonographer with clinical and research experience (KPM) will analyze the images for CSA, mT, and EI. All muscle analyses will be performed blinded with

all images coded by a different research coordinator. The blinded assessor will be unaware of the patient identification, the investigator obtaining the images, or the timepoint of the MSKUS. Blood and effluent fluid will be collected at the defined timepoints. Plasma samples will be collected in EDTA tubes, centrifuged at 1,000 g for 10 minutes at 4 °C. Following extraction of supernatant and transfer into storage tubes, samples will be stored at -80 °C. The specimens will be shipped to the biospecimen site (University of Iowa) where we will perform the metabolomic analysis on all samples in one batch. Patient data including demographics, data related to acute illness and comorbidity, and test results will be recorded using standardized case report forms and then uploaded to REDCap.

Data Management: Data will be stored using REDCap software at each site. REDCap is a secure web application for building and managing online databases.[62] REDCap is HIPAA compliant and is specifically geared to support online and offline data capture for research studies and operations.

Statistical Methods: We will summarize descriptive statistics at each timepoint using frequencies and proportions for categorical variables and means and standard deviations or medians and interquartile ranges, as appropriate, for continuous variables. Binary outcome variables include the diagnosis of ICU-acquired weakness (defined as MRC-ss <48/60) and the additional recovery phase events (i.e., return to driving, return to work or hobby, hospital readmission, and need for emergency department care). Continuous variables include MSKUS parameters, strength testing, and physical function testing. Ordinal variables include scores on the EQ-5D-5L, FACIT-F sub-question, and CFS.

- **ICU Phase:** The primary outcome in the ICU phase of the study, corresponding to Aim 1, will be the change in RF CSA, mT, and EI from baseline to day 7 (or ICU discharge) within AKI-RRT patients and in comparison to historical ICU controls without AKI-RRT. Repeated measures analysis will be used with muscle parameters as fixed effects. Of note, in the cohort to be used as the historical control for the ICU phase, MSKUS was performed on ICU days 1, 3, 5, and 7 and muscle strength assessment (by MRC-ss, hand-held dynamometry, and hand-grip dynamometry) was performed at ICU and hospital discharge.[38] For AKI-RRT patients, time 0 is study enrollment (which must be within 48 hours of CRRT initiation). To account for the resulting differences in

timing of assessments, time from ICU admission to MSKUS and muscle strength assessments will be included as covariables in our analyses. We will develop multivariable models for within-group comparisons (in AKI-RRT cases) and between-group comparisons (AKI-RRT cases vs. controls). Additional variables entered in the analyses will include demographic variables including age and sex, Charlson comorbidity score, illness severity as measured by sequential organ failure assessment (SOFA) score[63], and ICU variables (including mechanical ventilation, trauma, sepsis, corticosteroid use, use of paralytic agents, and ICU type). We will conduct univariable analysis to determine if any of these variables are significantly associated with the primary outcomes. To avoid overfitting, only significant variables will be added in the subsequent adjusted model. We will use paired t-test to examine within-group differences and Analysis of Variance (ANOVA) for between-group differences of MSKUS parameters and other continuous measures. Binary outcomes will be compared by chi-square test. Bonferroni adjustment will be utilized for multiple comparisons.

- **Recovery Phase**: The primary outcomes in the recovery phase of the study, corresponding to Aim 2, will include both (1) the detailed characterization of longitudinal changes in muscle mass and quality and functional status in AKI-RRT survivors, including comparison of post-discharge recovery of RF size and quality assessed by MSKUS and of muscle strength in AKI-RRT survivors as measured within 1-3 months of discharge compared to in-hospital baselines, and (2) comparison of MSKUS parameters and of muscle strength assessed at 1-3 months after discharge in AKI-RRT survivors with the same parameters in historical controls of ICU survivors without AKI-RRT. The assessments of muscle strength at this phase will include MRC-ss, hand-held dynamometry for knee extension, and hand-grip dynamometry. Using the same methods and covariables as outlined for the ICU phase analysis, we will generate multivariable models for within-group and between-group comparisons of MSKUS parameters and muscle strength, with the exception that lengths of ICU and hospital stay and time from hospital discharge to outpatient assessment will be included as covariables in this phase. For the SPPB, TUG, 6-MWT, EQ-5D-

5L, CFS, FACIT-F, SF-36, and additional recovery phase events, differences between the AKI-RRT survivors and the historical ICU survivor controls without AKI-RRT will be compared using t-test, Wilcoxon rank sum test, Mann-Whitney U test, and chi-square test, as appropriate.

- **Metabolomic analysis**: The metabolomic analysis will have no control group. The primary outcome of the metabolomic analysis, corresponding to Aim 3, will be the correlation between changes in plasma and effluent amino acid levels measured during CRRT treatment in the ICU phase and both the MSKUS parameters obtained throughout the study and muscle strength measured in AKI-RRT survivors at hospital discharge and 1-3 months post-discharge. The primary analysis will be performed using a mixed effects model with ANOVA using Sidak's multiple comparison test for paired samples to compare baseline to subsequent samples. Correlations with muscle changes based on MSKUS measurements at days 3 and 7 and correlations with muscle mass, strength, and function at hospital discharge and at 1-3 months will be assessed with Pearson correlation test for continuous variables and Spearman Rho test for non-parametric data.

Ethics and Dissemination

Research Ethics Approval: This protocol (IRB #71153) was approved by the University of Kentucky Office of Research Integrity Medical IRB, which served as the single IRB for this multisite study according to National Institutes of Health single IRB Policy.[64]

Consent: Given the expectation that most AKI-RRT patients will be mechanically ventilated, consent will be obtained prior to enrollment from a legally authorized representative if necessary. Consent will be obtained by the local research team, which may include the site principal investigator, coinvestigators, or research assistants. Patients will be identified based on discussion with nephrology consult teams regarding patients about to be initiated on CRRT. The patient or the patient's legally authorized representative will undergo detailed consent and will be given a copy of the signed consent form.

Confidentiality: Confidentiality of the data obtained from enrolled participants will be achieved by storing the data using REDCap data management to reduce the risk of accidental loss of confidentiality. Each patient will be assigned a unique research ID, which will be used to identify the REDCap record and the biospecimens in storage for each patient. Once all data are collected, the records will be de-identified by removing any identifying information including medical record numbers, names, and dates of birth and hospital admission.

Data Access: The final de-identified dataset will be made fully accessible upon reasonable request once the results are published.

Dissemination Policy: We intend to disseminate results to participants, healthcare professionals, the public, and other relevant groups via conference presentation and publication and without any publication restrictions. The final manuscript will be drafted by the primary investigators. We plan to grant public access to the full protocol, participant-level dataset, and statistical code upon reasonable request.

Author Contributions:

Conceptualization: JPT, BRG, JAN, KPM

Methodology: JPT, BRG, CAP, FGS, NJ, BMJ, YY, NG, HPI, LG, JAN, KPM

Resources: JPT, BRG, CAP, FGS, NJ, BMJ, YY, NG, HPI, JAN, KPM

Writing (Original Draft): JPT, BRG, JAN, KPM

Review and Editing: JPT, BRG, CAP, FGS, NJ, BMJ, YY, NG, HPI, LG, JAN, KPM

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Figure Legends

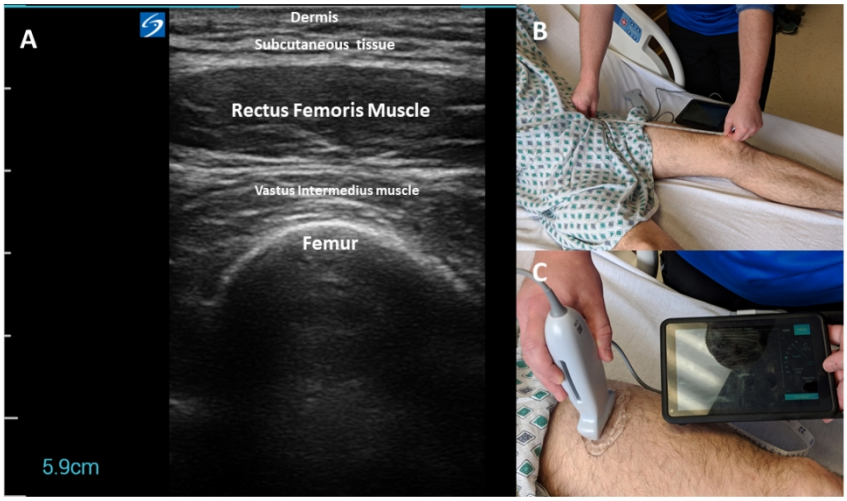
Figure 1: Representative images of ultrasound acquisition techniques and the obtained image of the rectus femoris muscle.

Caption: Panel A is a representative ultrasound image with anatomical structures labeled for the quadriceps muscle. Panel B demonstrates the technique to locate the anatomical landmarks for rectus femoris ultrasound (two-thirds of the distance from anterior superior iliac spine to the superior border of the patella). Panel C depicts the-minimal-to-no-compression technique using the ultrasound probe with adequate ultrasound transmission gel to obtain images.

** These images were staged by the authors to demonstrate appropriate technique and were not taken from a patient encounter.*

Figure 2: Representative image of the performance of hand-held dynamometry to measure isometric knee extensor strength with a subject in the supine position with a towel roll keeping the knee in 20-30 degrees of flexion.

** This image was staged by the authors to demonstrate appropriate technique and was not taken from a patient encounter.*



338x190mm (96 x 96 DPI)

338x190mm (96 x 96 DPI)

End-of-study feedback survey for research study, “The Impact of Renal Replacement Therapy on the Development of Critical Illness Muscle Wasting”

Patient subject no.:_____

Thank you for participating in our study about the effects of acute kidney injury in the intensive care unit on muscle loss and muscle weakness. We ask that you to please fill out this brief survey to help us determine what kind of research would be best in the future.

1. Of all the tests we completed, including the different surveys you took and the different types of functional tests we performed, which one do you think was the most important or valuable to you as a survivor of acute kidney injury? Multiple answers are okay.

2. Is there anything else about muscle strength and physical function that you think we should test or measure in future research in patients like yourself?

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5-6
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6, 11-12, 23
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6-7, 11, 23
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-10, 12-14
Bias	9	Describe any efforts to address potential sources of bias	10-11; 12-14
Study size	10	Explain how the study size was arrived at	11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	12-14
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	12-14
		(*b*) Describe any methods used to examine subgroups and interactions	12-14
		(*c*) Explain how missing data were addressed	N/A
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	N/A
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	N/A
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	Report numbers of outcome events or summary measures over time	N/A

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	N/A
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	N/A
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	3
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	N/A
Generalisability	21	Discuss the generalisability (external validity) of the study results	N/A
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

BMJ Open

Critical Illness Myopathy and Trajectory of Recovery in Acute Kidney Injury Requiring Continuous Renal Replacement Therapy: A Prospective Observational Trial Protocol

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Critical Illness Myopathy and Trajectory of Recovery in Acute Kidney Injury Requiring Continuous Renal Replacement Therapy: A Prospective Observational Trial Protocol

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Abstract

Introduction: Acute kidney injury requiring renal replacement therapy (AKI-RRT) is common in the intensive care unit (ICU) and is associated with significant morbidity and mortality. Continuous RRT (CRRT) non-selectively removes large amounts of amino acids from plasma, lowering serum amino acid concentrations and potentially depleting total-body amino acid stores. Therefore, the morbidity and mortality associated with AKI-RRT may be partly mediated through accelerated skeletal muscle atrophy and resulting muscle weakness. However, the impact of AKI-RRT on skeletal muscle mass and function during and following critical illness remains unknown. We hypothesize that patients with AKI-RRT have higher degrees of acute muscle loss than patients without AKI-RRT and that AKI-RRT survivors are less likely to recover muscle mass and function when compared to other ICU survivors.

Methods and Analysis: This protocol describes a prospective, multicenter, observational trial assessing skeletal muscle size, quality, and function in ICU patients with AKI-RRT. We will perform musculoskeletal ultrasound to longitudinally evaluate rectus femoris size and quality at baseline (within 48 hours of CRRT initiation), day 3, day 7 or at ICU discharge, at hospital discharge, and 1-3 months post-discharge. Additional skeletal muscle and physical function tests will be performed at hospital discharge and post-discharge follow-up. We will analyze the effect of AKI-RRT by comparing the findings in enrolled subjects to historical controls of critically ill patients without AKI-RRT using multivariable modeling.

Ethics and Dissemination: We anticipate our study will reveal that AKI-RRT is associated with greater degrees of muscle loss and dysfunction along with impaired post-discharge recovery of physical function. These findings could impact the in-hospital and post-discharge treatment plan for these patients to include focused attention on muscle strength and function. We intend to disseminate findings to participants, healthcare professionals, the public, and other relevant groups via conference presentation and publication without any publication restrictions.

Strengths and limitations of this study

- This study has several notable strengths including the study design based on multidisciplinary collaboration, the multicenter patient representation, the collection of longitudinal in-hospital and outpatient measures and outcomes, and the wide range of skeletal muscle and physical function tests being performed.
- Due to the pilot nature of this study, we will recruit critically ill patients with acute kidney injury (AKI) requiring continuous renal replacement therapy (CRRT) to collect a broad array of study measures and compare them with available datasets of recent historical controls of critically ill patients without AKI requiring RRT. While the use of these control groups enhances the feasibility of the project, it also represents a limitation.
- The high morbidity and mortality inherent to the study population may introduce competing risk of death and selection bias when assessing the outpatient outcomes.
- Differentiating the exact contributions of AKI, CRRT, and underlying acute illness may not be possible in this observational study due to several confounding variables that could be better addressed with an interventional trial design in the future. Nonetheless, our pilot study will generate critical data for the design and sample size estimations of such future trials.

Introduction

Background and Rationale: Acute kidney injury (AKI) complicates approximately 20% of all hospital admissions and up to 50% of all intensive care unit (ICU) admissions.[1 2] Moreover, 13.5% of critically ill patients develop AKI requiring renal replacement therapy (AKI-RRT).[1] AKI is associated with poor short- and long-term prognoses. Even stage 1 AKI – defined by as little as a 0.3 mg/dL increase or 50% rise in serum creatinine above baseline[3] – is associated with up to a 10-fold increase in the odds of in-hospital mortality.[4] Similarly, AKI-RRT has an in-hospital mortality rate >50%, making it one of the deadliest conditions encountered in the hospital.[5] After discharge, AKI survivors are at increased risk of developing chronic kidney disease (CKD), end-stage kidney disease, cardiovascular disease, and death.[6] Other studies suggest that AKI predisposes to disparate sources of morbidity including infection[7-10], bone fracture[11], stroke[12], GI hemorrhage[13], and dementia.[14] While muscle wasting is well-described in patients with CKD[15 16], the contribution of AKI-RRT to muscle wasting in critically ill patients has not been previously studied.

Acute skeletal muscle wasting occurs in up to 65% of patients admitted to the ICU.[17] Critical illness myopathy (CIM), defined as a deficit in muscle size and strength that develops as a result of an ICU admission, is associated with high rates of short- and long-term mortality and morbidity, including decreased quality of life (QoL) due to persistent functional mobility impairments and inability to perform simple activities of daily living.[18-21] AKI of any stage is known to alter tissue utilization of amino acids, making it plausible that AKI exacerbates CIM.[15 22] Studies have demonstrated that plasma amino acid levels are reduced and multiple non-essential amino acids become conditionally essential in the setting of AKI.[23-25] In addition, AKI leads to a state of increased amino acid oxidation but reduced amino acid transport into muscle.[15] RRT exacerbates this issue through non-selective removal of amino acids from plasma.[15] Amino acids are small and easily filtered during RRT, and, as a result, daily losses of amino acids in effluent can be immense at up to 18 grams daily with CRRT.[26 27]

The gold standards for assessing CIM are muscle biopsy or electrodiagnostic testing.[28] Furthermore, the measurement of psoas muscle area on a single cross-sectional computed tomography

(CT) image at the level of the L3 vertebra has been suggested as a standard clinical measure for skeletal muscle quantification.[29] However, biopsy, electrodiagnostic testing, and CT imaging present challenges which limit their clinical application. Musculoskeletal ultrasound (MSKUS), a relatively inexpensive, noninvasive alternative, has gained significant traction over the last decade for assessing muscle in ICU patients.[30] Studies have demonstrated that MSKUS has excellent inter-rater reliability and high clinical utility and have suggested that MSKUS has strong construct validity.[31-35] Recent data suggest that MSKUS can be reliably performed at the bedside in the ICU.[36]

The primary objective of this study is to characterize longitudinal measures of muscle size [rectus femoris (RF) muscle cross-sectional area (CSA) and muscle thickness (mT)] and quality [echo intensity (EI)] in critically ill adults with AKI requiring CRRT during their ICU stay and in post-discharge follow-up among survivors. This trial protocol was designed using the elements of the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) checklist for cohort studies[9] whenever applicable.

Objectives:

Aim 1. To characterize changes in RF muscle mass and quality at baseline and days 3 and 7 following study enrollment in critically ill adults with AKI requiring CRRT and to compare these measurements with those of historical ICU controls without AKI-RRT.

Hypothesis 1: RF muscle mass and muscle quality will be lower at 7 days in patients with AKI-RRT compared to the corresponding inpatient measurements of historical ICU controls without AKI-RRT.

Aim 2. To characterize changes in RF muscle mass and quality at hospital discharge and within 3 months post-discharge in survivors of AKI requiring CRRT in the ICU and to compare these measurements with those of historical ICU controls without AKI-RRT.

Hypothesis 2: The muscle mass and functional parameters of survivors of AKI-RRT obtained within 3 months of hospital discharge will be worse than the corresponding measurements from

historical controls of ICU survivors without AKI-RRT obtained within a similar post-discharge timeframe.

Aim 3. To examine if changes in plasma or effluent amino acid levels correlate with skeletal muscle loss during CRRT or with skeletal muscle function at 1-3 months post discharge.

Hypothesis 3: The concentrations of amino acids in blood and effluent during CRRT will correlate with MSKUS parameters of muscle mass and will be associated with muscle function following discharge.

Methods: Participants, Interventions, and Outcomes

Trial Design: This is a prospective multicenter observational study to evaluate longitudinal inpatient and outpatient measures of muscle mass and function in critically ill adults with AKI requiring CRRT and to compare these measurements with those of historical ICU controls without AKI-RRT. The study will have two phases, an ICU phase and a recovery phase for subjects who survive to discharge.

Study Setting: This study will be conducted at the adult ICUs at the academic medical centers of the University of Kentucky, University of Iowa, and University of New Mexico. Following discharge, survivors will return for outpatient evaluation of skeletal muscle and physical function.

Eligibility Criteria: To be included in the study, patients are required to be ≥18 years old and have AKI-RRT with enrollment within 48 hours of CRRT initiation. Exclusion criteria include: (1) ICU admission for >7 days; (2) RRT of any kind at any time before ICU admission; (3) CKD with estimated glomerular filtration rate <20 mL/min/1.73 m^2 as calculated by the 2021 CKD-EPI equation[37]; (4) underlying muscle disorders or muscle atrophy such as quadriplegia or hemiplegia, stroke with residual motor deficits, end-stage liver disease, active alcohol use disorder, active malignancy (other than non-melanoma skin cancer) within 1 year, burns, or other baseline neuromuscular disease; (5) pregnancy; (6) concomitant use of other extracorporeal support devices such as ventricular assist devices or extracorporeal membrane oxygenation; or (7) anticipated inability to engage in weight-bearing testing after discharge (e.g., trauma

or orthopedic surgery). For outpatient testing, patients will be ineligible if they remain on RRT in the week prior to the research appointment.

Control Population*:* Given the pilot nature of this study, we will use recent historical controls defined as critically ill adults without AKI-RRT in whom similar measurements of muscle size, quality, and function were collected. Specifically, we have previously collected data on 41 ICU patients, of which 36 did not have AKI-RRT and will serve as the control group for the ICU phase of this study, and have published the results of MSKUS performed in the ICU and functional assessments performed at both ICU discharge and hospital discharge.[38] The controls for the recovery phase will come from an ongoing prospective observational study being performed at the University of Kentucky, which will include outpatient functional assessments performed on 200 ICU survivors (NCT05537298). See **Table 1** for a summary of the demographic and clinical characteristics of the control cohorts which have been published thus far.[21 38 39]

Primary Outcome (ICU phase): The primary outcome is the change in RF CSA, mT, and EI measured by MSKUS at baseline, assessed within 48 hours of CRRT initiation, to ICU days 3 and 7 of study enrollment (or at ICU discharge, if sooner). Operational and standardization procedures have been previously published.[35 38] In brief, patients are positioned supine with the lower extremity in neutral alignment. RF ultrasound images are acquired two-thirds of the distance from the anterior superior iliac spine to the superior border of the patella of the right lower extremity at all timepoints. Sonographers will use a linear probe (5-15 Hz) with the same machine for all timepoints and a minimal-to-no-compression technique. Sonographers will obtain three images per assessment to reduce variations in EI. Ultrasound images will be assessed for CSA, mT, and EI at baseline within 48 hours of CRRT initiation, at days 3 and 7 from enrollment (or at ICU discharge, if sooner). A representative ultrasound image and the techniques for landmarking and probe pressure are provided in **Figure 1.**

Secondary Outcomes (ICU Phase): Patients will have blood collected at baseline (within 48 hours of CRRT initiation), at study days 3 and 7 (or ICU discharge, if sooner). Creatinine and cystatin C will be measured at each timepoint. Additionally, 5-mL blood samples and CRRT effluent samples at each

Table 1. Demographic and clinical characteristics of the cohorts being used as our historical controls for this study.

Cohort Characteristic	Control cohort for ICU phase*[38]	Control cohort #1 for recovery phase†[21]	Control cohort #2 for recovery phase[39]
ICU or admission type	Medical 73%; cardiothoracic 27%	Medical 100%	Mixed
Years of enrollment	2018 to 2019	2018 to 2019	2018 to 2021
N	41*	12†	59
Primary inclusion	Acute respiratory failure or sepsis and anticipated ICU stay >3 days	Survivors of acute respiratory failure requiring >48 hours of mechanical ventilation	Survivors of sepsis and/or acute respiratory failure
Timing of initial assessment	Within 48 hours of admission; median 1.1 days [IQR 0.7-1.4]	N/A	N/A
Timing of outpatient assessment	N/A	4-8 weeks after hospital discharge	3 months after hospital discharge
Age, median years [IQR]	61 [55-68]	58 [45.5-65]	56 [48-64]
Female, no. (%)	18 (44%)	4 (33%)	31 (53%)
No. (%) mechanically ventilated	30 (73%)	12 (100%)	43 (73%)
Duration of mechanical ventilation, median days [IQR]	3.4 [1-7.7]	6.45 [2.8-11.95]	8 [5-14]
No. (%) requiring RRT	5 (12%)*	NR†	0
Disease severity (SOFA) scores	Mean 8.1 [SD ±4.8]	Median 8 [IQR 4.5-10.25]	Median 10 [IQR 8-12]
ICU LOS, median days [IQR]	8 [4]	7.4 [4.3-18.6]	10 [6-15]
Hospital LOS, median days [IQR]	11.2 [8-19]	16.7 [9.4-28.7]	15 [11-22]
Hospital mortality	12%	N/A	N/A

Presented here are only previously published data. The two control cohorts for the recovery phase represented in the table are only a subset of the 200 patients in our ongoing registry that will serve as the control for the recovery phase. *Data for this entire cohort are presented as published, including data from the 5 patients treated with RRT who will be excluded from the control group in our analysis. †Though not reported in the published manuscript, RRT status is available for this cohort and any patients with AKI-RRT will be excluded from the control group in our analysis. Abbreviations: ICU, intensive care unit; IQR, interquartile range; LOS, length of stay; N/A, not applicable; NR, not reported; RRT, renal replacement therapy; SD, standard deviation; SOFA, Sequential Organ Failure Assessment.

timepoint will be sent for gas chromatography-mass spectrometry evaluating a panel of analytes including amino acids, carbohydrates, and fatty acids. Remaining samples will be stored for future analysis, though no genetic analysis will be conducted now or in the future.

Patients will be scored at the same timepoints as above using the ICU Mobility Scale, an 11-point scale ranging from 0-10 which involves the clinician scoring the patient's maximum level of mobility in the prior 24-hour period.[40 41]

Outcomes (Recovery Phase): Survivors will participate in skeletal muscle and physical function testing at hospital discharge and during the outpatient visit at 1-3 months post-discharge. Measurements will include MSKUS to determine RF CSA, mT, and EI. We will also conduct an array of standardized and validated tests including:

- **Medical Research Council Sum-score (MRC-ss):** MRC-ss is a measure of global peripheral muscle strength that is the current clinical standard for diagnosing ICU-acquired weakness (ICU-AW).[42] Muscle strength is assessed by physical exam and rated on an ordinal scale (0-5) at six bilateral muscle groups: shoulder abductors, elbow flexors, wrist extensors, hip flexors, knee extensors, and ankle dorsiflexors. A score <48 is considered indicative of ICU-AW, with a score <36 indicative of severe weakness with the inability to act against resistance.[43]
- **Muscle strength using hand-held dynamometry for knee extension:** Maximal isometric knee extensor strength will be measured as peak force production and rate of force development following previously published standardized positions (**Figure 2**).[44]
- **Muscle strength using hand-grip dynamometry:** Hand-grip dynamometers will be utilized to measure maximum isometric strength of the hand and forearm muscles at previously published standardized positions.[42 43] The patient will undergo three repetitions with both the right and left hand, alternating between hands.

- **Short Physical Performance Battery (SPPB):** Physical function and physical frailty will be measured using the SPPB, a performance-based composite test with a total of 12 points including components of balance (side-by-side stand, semi-tandem stand, and full-tandem stand), chair-to-stand test, and 4-meter habitual gait speed.[45 46]
- **Timed Up and Go (TUG) Test:** The TUG assesses the time (in seconds) for a subject to stand on command from a seated position, walk 3 meters, turn around, walk back to the chair, and sit down. The purpose of TUG is to assess mobility, physical function, and fall risk. TUG has been validated in and recommended for patients with critical illness.[47-49]
- **Six-minute walk test (6-MWT):** The 6-MWT assesses the distance a subject can walk in six minutes, providing a global representation of physical function and cardiopulmonary endurance.[50 51] Meta-analysis provides benchmark data for survivors of critical illness.[52]
- **Quality of Life testing using EuroQol Group 5-dimension 5-level (EQ-5D-5L) questionnaire:** The EQ-5D is a standardized measure of health status developed by the Euro-Qol Group to provide an assessment of health for clinical and economic appraisal.[53] It consists of two sections: the descriptive system and the visual analogue scale (EQ VAS). The descriptive system assesses five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. The EQ VAS records the respondent's self-rated health on a 20-cm vertical visual analogue scale with endpoints labeled the "best health you can imagine" and the "worse health you can imagine." This information can be used as a quantitative measure of health as judged by individual respondents.[51 54 55]
- **Clinical Frailty Scale (CFS):** The CFS is designed for clinical use and has been widely adopted as a judgement-based tool to screen for frailty and to broadly stratify degrees of fitness and frailty.[56 57] It is not a questionnaire, but a way to summarize information from a clinical encounter to roughly quantify an individual's overall health status. While CFS has traditionally been used

specifically in older patients, recent data have demonstrated its utility in an ICU population demographically similar to our target population.[58]

- **Functional Assessment of Chronic Illness Therapy Fatigue (FACIT-F) Questionnaire:** The FACIT-F scale is a 13-item measure that assesses self-reported fatigue and its impact upon daily activities and function.[59 60]
- **36-Item Short Form Health Survey Physical Function Scale (SF-36 Physical Function):** The SF-36 is a 36-item patient-reported survey of health commonly used to evaluate adult patients which contains 8 domains, including a physical function scale based on 10 of the 36 items which has been shown to have high reliability.[61]
- **Additional Events:** Finally, we will document the occurrence of the following events: return to driving, return to work or hobby, hospital readmission, and need for emergency department care.

Assessor Training: This multisite study is an interdisciplinary collaboration with expertise in critical care, nephrology, muscle biology, and physical function. Physical therapist-scientists and exercise physiologists with established expertise in MSKUS and functional testing serve as coinvestigators at each site. Three two-hour sessions of teleconference training will be performed to promote standardization of ultrasound and outcome assessments. In addition, novice sonographers were instructed to perform and practice a minimum of 10 acquisitions of RF muscle images from healthy individuals before study initiation. To better establish inter-rater reliability of image acquisition, the first 5 patients at each site will have ultrasound studies conducted by two team members. The first sonographer will obtain images and leave the room, and the second will enter and repeat the test. After a 10-minute wash-out period, the team members will sequentially repeat the ultrasound measurements, which will allow us to establish both inter- and intra-observer variability. Finally, images will be blinded, coded (rater 1 or rater 2; site location) and sent securely to the University of Kentucky to be reviewed by an expert sonographer (KPM, who has > 6 years of MSKUS experience) to ensure cross-site standardization of measurements.[35] All images will be analyzed for muscle CSA, mT, and EI by the same blinded expert sonographer. The images from the first 5 patients obtained by the two sonographers at each site will be examined with intra-

class correlation coefficient (ICC). Sites with ICC <0.7 will receive additional training to improve reliability at each site. ICC values will be disseminated with our final results.

Participant Timeline: Patients enrolled in the study will participate in up to two phases, an ICU phase and – for those who survive their critical illness – a recovery phase. The schedule of assessments for both phases is delineated in **Table 2**.

Table 2. Planned evaluations at each study timepoint.

Parameter	Timepoint				
	ICU Phase			Recovery Phase	
	Within 48 hours of CRRT start	3 ± 1 days from enrollment	Study day 7 (or ICU discharge)	Hospital Discharge	Outpatient 1-3 months
Rectus Femoris US	X	X	X	X	X
Amino Acids (Blood)	X	X	X		
Amino Acids (Effluent)	X	X	X		
Cystatin C	X	X	X		
ICU Mobility Scale	X	X	X		
Muscle Strength - MRC-ss				X	X
Muscle Strength - HGD				X	X
Muscle Strength - HHD				X	X
SPPB				X	X
TUG test					X
6-MWT					X
EQ-5D-5L					X
Clinical Frailty Scale					X
FACIT-fatigue					X
SF-36 Physical Function					X
Return to driving					X
Return to work or hobby					X
Readmission					X
ED visit					X

Abbreviations: 6-MWT, six-minute walk test; ED, emergency department; EQ-5D-5L, EuroQol Group 5-dimension 5-level version; FACIT-F, Functional Assessment of Chronic Illness Therapy-Fatigue; HGD, hand-grip dynamometry; HHD, hand-held dynamometry; ICU, intensive care unit; MRC-ss, Medical Research Council sum-score; SF-36, 36-Item Short Form Health Survey; SPPB, Short Physical Performance Battery; TUG, Timed Up and Go; US, ultrasound.

Sample Size: Our previous study reported a decrease in muscle RF CSA at ICU day 7 of 18.5%.[38] To detect an absolute difference in percent decrease in muscle size of 6% at 7 days (24.5% change at 7 days in AKI-RRT group vs. 18.5% change in controls), 61 patients per group are needed assuming an alpha of 0.05 and a power of 80%. This will require inclusion of 20 AKI-RRT patients per site that provide all ICU datapoints. Our previous study also reported that RF CSA was 2.47 ± 0.88 cm^2 at ICU day 7, down from a baseline on ICU day 1 of 2.99 cm^2.[38] To detect a full return to baseline in the outpatient setting with an alpha of 0.05 and a power of 80%, 22 patients are required in each group. To detect a 75% recovery to baseline from day 7 values, 40 patients are required. We anticipate a 40-50% in-hospital mortality rate which would leave 31 to 37 patients alive at discharge. Assuming 22 patients are needed, this provides room for attrition or loss to follow-up of 30-40%.

Recruitment: Patients will be recruited in the multidisciplinary adult ICUs of the three sites involved in the study. Patients will be identified through communication with the nephrology consult services at each site, who will independently make decisions regarding indications for and timing of initiation of RRT. The enrollment will occur for a full year following institutional review board (IRB) approval at each site, with the funding dates ranging from August 1, 2022, to July 31, 2023, at the University of Kentucky and University of Iowa and from October 1, 2022, to September 30, 2023, at the University of New Mexico. To promote subject retention after discharge, we will allow for a two-month window in which to schedule the post-discharge follow-up visit and subjects will be contacted by telephone a minimum of 3 times before being considered lost to follow-up. As stipulated in the informed consent form, though subjects may withdraw from the study at any point, all data collected prior to withdrawal will be retained for analysis.

Patient and Public Involvement: No formal patient advisory committee was established and there was no patient or public involvement in the design or planning of the study. However, to inform future study design, we will conduct a brief open-ended post-study survey following the outpatient visit at 1-3 months to help discern which of the patient-reported outcome measures and functional assessments performed appear most valuable to the study subjects (**Supplementary material 1**).

Methods: Data collection, management, and analysis

Data Collection Methods: Ultrasound images will be transferred through secure link to the data coordinating site (University of Kentucky). One expert sonographer with clinical and research experience (KPM) will analyze the images for CSA, mT, and EI. All muscle analyses will be performed blinded with all images coded by a different research coordinator. The blinded assessor will be unaware of the patient identification, the investigator obtaining the images, or the timepoint of the MSKUS. Blood and effluent fluid will be collected at the defined timepoints. Plasma samples will be collected in EDTA tubes, centrifuged at 1,000 g for 10 minutes at 4 °C. Following extraction of supernatant and transfer into storage tubes, samples will be stored at -80 °C. The specimens will be shipped to the biospecimen site (University of Iowa) where we will perform the metabolomic analysis on all samples in one batch. Patient data including demographics, data related to acute illness and comorbidity, and test results will be recorded using standardized case report forms and then uploaded to REDCap.

Data Management: Data will be stored using REDCap software at each site. REDCap is a secure web application for building and managing online databases.[62] REDCap is HIPAA compliant and is specifically geared to support online and offline data capture for research studies and operations.

Statistical Methods: We will summarize descriptive statistics at each timepoint using frequencies and proportions for categorical variables and means and standard deviations or medians and interquartile ranges, as appropriate, for continuous variables. Binary outcome variables include the diagnosis of ICU-acquired weakness (defined as MRC-ss <48/60) and the additional recovery phase events (i.e., return to driving, return to work or hobby, hospital readmission, and need for emergency department care). Continuous variables include MSKUS parameters, strength testing, and physical function testing. Ordinal variables include scores on the EQ-5D-5L, FACIT-F sub-question, and CFS.

- **ICU Phase:** The primary outcome in the ICU phase of the study, corresponding to Aim 1, will be the change in RF CSA, mT, and EI from baseline to day 7 (or ICU discharge) within AKI-RRT patients and in comparison to historical ICU controls without AKI-RRT. Repeated measures

analysis will be used with muscle parameters as fixed effects. Of note, in the cohort to be used as the historical control for the ICU phase, MSKUS was performed on ICU days 1, 3, 5, and 7 and muscle strength assessment (by MRC-ss, hand-held dynamometry, and hand-grip dynamometry) was performed at ICU and hospital discharge.[38] For AKI-RRT patients, time 0 is study enrollment (which must be within 48 hours of CRRT initiation). To account for the resulting differences in timing of assessments, time from ICU admission to MSKUS and muscle strength assessments will be included as covariables in our analyses. We will develop multivariable models for within-group comparisons (in AKI-RRT cases) and between-group comparisons (AKI-RRT cases vs. controls). Additional variables entered in the analyses will include demographic variables including age and sex, Charlson comorbidity score, illness severity as measured by sequential organ failure assessment (SOFA) score[63], and ICU variables (including mechanical ventilation, trauma, sepsis, corticosteroid use, use of paralytic agents, and ICU type). We will conduct univariable analysis to determine if any of these variables are significantly associated with the primary outcomes. To avoid overfitting, only significant variables will be added in the subsequent adjusted model. We will use paired t-test to examine within-group differences and Analysis of Variance (ANOVA) for between-group differences of MSKUS parameters and other continuous measures. Binary outcomes will be compared by chi-square test. Bonferroni adjustment will be utilized for multiple comparisons.

- **Recovery Phase**: The primary outcomes in the recovery phase of the study, corresponding to Aim 2, will include both (1) the detailed characterization of longitudinal changes in muscle mass and quality and functional status in AKI-RRT survivors, including comparison of post-discharge recovery of RF size and quality assessed by MSKUS and of muscle strength in AKI-RRT survivors as measured within 1-3 months of discharge compared to in-hospital baselines, and (2) comparison of MSKUS parameters and of muscle strength assessed at 1-3 months after discharge in AKI-RRT survivors with the same parameters in historical controls of ICU survivors without AKI-RRT. The assessments of muscle strength at this phase will include MRC-ss, hand-held

dynamometry for knee extension, and hand-grip dynamometry. Using the same methods and covariables as outlined for the ICU phase analysis, we will generate multivariable models for within-group and between-group comparisons of MSKUS parameters and muscle strength, with the exception that lengths of ICU and hospital stay and time from hospital discharge to outpatient assessment will be included as covariables in this phase. For the SPPB, TUG, 6-MWT, EQ-5D-5L, CFS, FACIT-F, SF-36, and additional recovery phase events, differences between the AKI-RRT survivors and the historical ICU survivor controls without AKI-RRT will be compared using t-test, Wilcoxon rank sum test, Mann-Whitney U test, and chi-square test, as appropriate.

- **Metabolomic analysis**: The metabolomic analysis will have no control group. The primary outcome of the metabolomic analysis, corresponding to Aim 3, will be the correlation between changes in plasma and effluent amino acid levels measured during CRRT treatment in the ICU phase and both the MSKUS parameters obtained throughout the study and muscle strength measured in AKI-RRT survivors at hospital discharge and 1-3 months post-discharge. The primary analysis will be performed using a mixed effects model with ANOVA using Sidak's multiple comparison test for paired samples to compare baseline to subsequent samples. Correlations with muscle changes based on MSKUS measurements at days 3 and 7 and correlations with muscle mass, strength, and function at hospital discharge and at 1-3 months will be assessed with Pearson correlation test for continuous variables and Spearman Rho test for non-parametric data.

Ethics and Dissemination

Research Ethics Approval: This protocol was approved by the University of Kentucky Office of Research Integrity Medical IRB, which serves as the single IRB for this multisite study according to National Institutes of Health single IRB Policy[64] (IRB #71153; initial approval June 7, 2022; protocol version 2 dated November 27, 2022, approved January 7, 2023). Any further significant protocol revisions will be communicated to the IRB and *BMJ Open* and updated on the clinicaltrials.gov registry.

Consent: Given the expectation that most AKI-RRT patients will be mechanically ventilated, consent will be obtained prior to enrollment from a legally authorized representative if necessary. Consent will be obtained by the local research team, which may include the site principal investigator, coinvestigators, or research assistants. Patients will be identified based on discussion with nephrology consult teams regarding patients about to be initiated on CRRT. The patient or the patient's legally authorized representative will undergo detailed consent and will be given a copy of the signed consent form (**Supplementary material 2**).

Confidentiality: Confidentiality of the data obtained from enrolled participants will be achieved by storing the data using REDCap data management to reduce the risk of accidental loss of confidentiality. Each patient will be assigned a unique research ID, which will be used to identify the REDCap record and the biospecimens in storage for each patient. Once all data are collected, the records will be de-identified by removing any identifying information including medical record numbers, names, and dates of birth and hospital admission.

Data Access: The final de-identified dataset will be made fully accessible upon reasonable request once the results are published.

Dissemination Policy: We intend to disseminate results to participants, healthcare professionals, the public, and other relevant groups via conference presentation and publication and without any publication restrictions. The final manuscript will be drafted by the primary investigators. We plan to grant public access to the full protocol, data collection forms, participant-level dataset, and statistical code upon reasonable request.

Author Contributions:

The study was conceptualized by JPT, BRG, JAN, and KPM. The methodology was developed and revised by JPT, BRG, CAP, FGS, NJ, BMJ, YY, NG, HPI, LG, JAN, and KPM. Resources for developing and carrying out the protocol were provided by JPT, BRG, CAP, FGS, NJ, BMJ, YY, NG, HPI, JAN, and KPM. JPT, BRG, JAN, and KPM wrote the original draft manuscript. Funding for the study was obtained by JPT, BRG, NJ, BMJ, YY, JAN, and KPM. All authors reviewed and edited this final draft. This manuscript and all subsequent publications stemming from this trial protocol will adhere to the authorship eligibility guidelines of the International Committee of Medical Journal Editors (ICMJE) and will not involve professional writers.

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Figure Legends

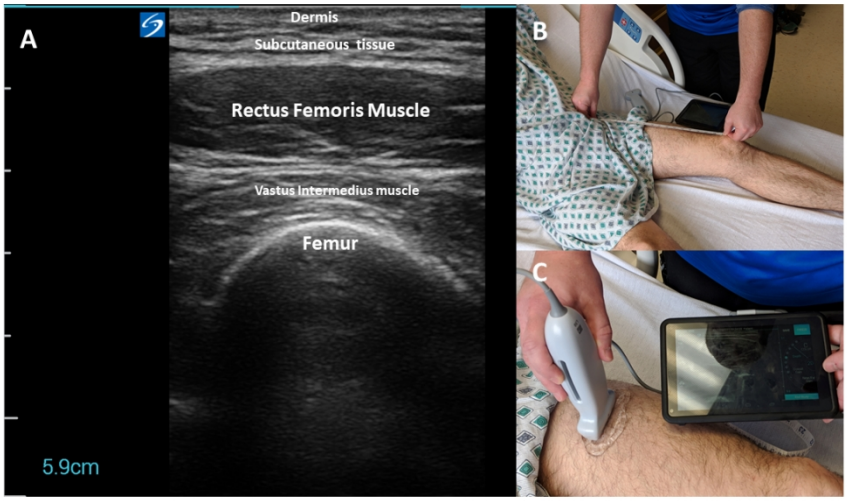
Figure 1: Representative images of ultrasound acquisition techniques and the obtained image of the rectus femoris muscle.

Caption: Panel A is a representative ultrasound image with anatomical structures labeled for the quadriceps muscle. Panel B demonstrates the technique to locate the anatomical landmarks for rectus femoris ultrasound (two-thirds of the distance from anterior superior iliac spine to the superior border of the patella). Panel C depicts the-minimal-to-no-compression technique using the ultrasound probe with adequate ultrasound transmission gel to obtain images.

** These images were staged by the authors to demonstrate appropriate technique and were not taken from a patient encounter.*

Figure 2: Representative image of the performance of hand-held dynamometry to measure isometric knee extensor strength with a subject in the supine position with a towel roll keeping the knee in 20-30 degrees of flexion.

** This image was staged by the authors to demonstrate appropriate technique and was not taken from a patient encounter.*



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End-of-study feedback survey for research study, “The Impact of Renal Replacement Therapy on the Development of Critical Illness Muscle Wasting”

Patient subject no.:_____

Thank you for participating in our study about the effects of acute kidney injury in the intensive care unit on muscle loss and muscle weakness. We ask that you to please fill out this brief survey to help us determine what kind of research would be best in the future.

1. Of all the tests we completed, including the different surveys you took and the different types of functional tests we performed, which one do you think was the most important or valuable to you as a survivor of acute kidney injury? Multiple answers are okay.

2. Is there anything else about muscle strength and physical function that you think we should test or measure in future research in patients like yourself?

Official Title: Critical Illness Myopathy and Trajectory of Recovery in Acute Kidney Injury (AKI) Requiring Continuous Renal Replacement Therapy (CRRT): A Prospective Observational Trial

NCT05287204

Document Date: 1/7/2023

The University of New Mexico Health Sciences Center

Consent and Authorization to Participate in a Research Study

Key Information for "The Impact of Renal Replacement Therapy (RRT) on the Development of Critical Illness Muscle Wasting" (HRRC ID 21-438)

You are being invited to take part in a research study about the impact of Continuous Renal Replacement Therapy (CRRT) on muscle wasting in ICU patients. We are asking you because you have been administered CRRT during your stay in the ICU. This page is to give you key information to help you decide whether to participate. We have included detailed information after this page. Ask the research team questions. If you have questions later, the contact information for the research investigator in charge of the study is below.

WHAT IS THE PURPOSE, PROCEDURES, AND DURATION OF THE STUDY?

By doing this study, we hope to learn if CRRT has an impact on muscle loss in patients admitted to the ICU, a common consequence of ICU admission. The study will consist of 3 parts: (1) ultrasound of a muscle in the leg (rectus femoris) to detect decrease in muscle size, (2) physical function assessments performed by physical therapists or other study members, and (3) collection of blood and CRRT effluent samples for analysis. CRRT effluent is the yellow fluid that is removed the body by CRRT that is similar to urine normally removed from the body by healthy kidneys. Your participation in this research will last about 3 months.

WHAT ARE THE KEY REASONS YOU MIGHT CHOOSE TO VOLUNTEER FOR THIS STUDY?

By participating in this study, you will help provide important information that can add to the quality of care for ICU patients in the future. For a complete description of benefits, refer to the Detailed Consent.

WHAT ARE THE KEY REASONS YOU MIGHT NOT CHOOSE TO VOLUNTEER FOR THIS STUDY?

The study will involve collection of biologic samples, including small amounts of blood, and ultrasound imaging. While the risk of these procedures is minimal, you may experience some discomfort and risk of infection from the blood draw. For a complete description of risks, refer to the Detailed Consent and/or Appendix.

DO YOU HAVE TO TAKE PART IN THE STUDY?

If you decide to take part in the study, it should be because you really want to volunteer. You will not lose any services, benefits or rights you would normally have if you choose not to volunteer.

WHAT IF YOU HAVE QUESTIONS, SUGGESTIONS OR CONCERNS?

The person in charge of this study is Dr. J. Pedro Teixeira of the University of New Mexico Health Sciences Center (UNM HSC), Department of Internal Medicine. If you have questions, suggestions, or concerns about this study or want to withdraw from the study, his contact information is 505-272-4751.

If you have any concerns or questions about your rights as a volunteer in this research, contact staff in the University of Kentucky (UK) Office of Research Integrity (ORI) between the business hours of 8am and 5pm Eastern Time, Monday-Friday at 859-257-9428 or toll free at 1-866-400-9428.

DETAILED CONSENT
Version 2 27Nov2022

Project Title: **The Impact of Renal Replacement Therapy (RRT) on the Development of Critical Illness Muscle Wasting (HRRC ID 21-438)**

Principal Investigator: J. Pedro Teixeira, MD
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MSC10-5550
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505-272-4751

If you are the legally authorized representative of a person who is being invited to be in this study, the word "you" in this document refers to the person you represent. You will be asked to read and sign this document to give permission for the person you represent to participate in this research study.

This consent form describes the research study to help you decide if you want to participate. This form provides important information about what you will be asked to do during the study, about the risks and benefits of the study, and about your rights as a research subject.

- If you have any questions about or do not understand something in this form, you should ask the research team for more information.
- You should discuss your participation with anyone you choose such as family or friends.
- Do not agree to participate in this study unless the research team has answered your questions and you decide that you want to be part of this study.

WHAT IS THE PURPOSE OF THIS STUDY?

This is a research study. We are inviting you to participate in this research study because you have severe acute kidney injury (AKI), and will soon be initiated on dialysis, also called renal replacement therapy (RRT) (Cases).

OR- we are inviting you to participate in this research study because you have illness requiring intubation and are at risk for muscle loss while immobile in the intensive care unit (ICU) (Controls).

The purpose of this research study is to determine the impact of RRT on the rate of muscle loss in the intensive care unit (ICU), and the impact of RRT on the trajectory of muscle recovery following hospital discharge. The loss of muscle mass in the ICU can increase the risk of death while in the hospital, can prolong the time of recovery, and can lead to the development of more health problems in the future following hospital discharge.

HOW MANY PEOPLE WILL PARTICIPATE?

Approximately 20 cases and controls will take part in this study conducted by investigators at the University of New Mexico. Approximately 40 others will be enrolled at the University of Iowa and University of Kentucky.

HOW LONG WILL I BE IN THIS STUDY?

If you agree to take part in this study, your involvement will last for the duration of your hospitalization and for visits at 1 month and 3 months following hospital discharge. The time involved in the study in the hospital will be the time spent completing 2 muscle ultrasounds (each approximately 10-20 minutes in length) and the time spent collecting blood (and effluent for CRRT) samples, which will take 5-10 minutes per day for up to 7 days. We will collect data from the electronic medical record regarding outcomes like the number of days spend in the hospital. At the time of hospital discharge, you would undergo a series of muscle strength tests and muscle ultrasound, that are anticipated to take 30- 60 minutes. These procedures will be repeated at your 1- and 3-month visits to determine the trend in muscle recovery.

WHAT WILL HAPPEN DURING THIS STUDY?

For the case population: Before you initiate RRT, or within 24 hours after initiation, you will undergo an ultrasound (US) to determine your muscle mass. The US will be repeated at 48 hours and again at 7 days or at ICU discharge, whichever comes first. The ultrasound will evaluate the size of your rectus femoris muscle, one of the muscles in your thigh. The ultrasound will take approximately 10-15 minutes to complete, and there should be no discomfort outside mild coolness from the ultrasound gel.

The first blood draw will take place before RRT initiation**.** After RRT is started, we will draw blood from the RRT machine daily while you are on CRRT. These blood draws can be taken directly from the RRT machine or from a central line, and so will not require additional needle sticks. The amount of blood taken at each time point is 5 mL (about 1 teaspoon). We will also collect effluent daily after RRT is initiated. Effluent is the waste fluid generated by the CRRT machine (akin to urine).

At the time of hospital discharge, you will undergo an ultrasound (US) to determine your muscle mass, and a series of muscle strength tests to determine the strength and functionality of your muscles. The ultrasound will take approximately 10-15 minutes to complete, and there should be no discomfort outside mild coolness from the ultrasound gel. The muscle strength testing will take 30-60 minutes, and there should be no discomfort other than discomfort from the use of your muscles. These tests will be repeated at visits scheduled to take place at 1 month and 3 months following hospital discharge.

For the control population: At the time of hospital discharge, you will undergo an ultrasound (US) to determine your muscle mass, and a series of muscle strength tests to determine the strength and functionality of your muscles. The ultrasound will take approximately 10-15 minutes to complete, and there should be no discomfort outside mild coolness from the ultrasound gel. The muscle strength testing will take 30-60 minutes, and there should be no discomfort other than discomfort from the use of your muscles. These tests will be repeated at visits scheduled to take place at 1 month and 3 months following hospital discharge.

For both groups, we would access your medical record to collect basic information about your case, such as why you developed acute kidney injury (cases), your vital signs, use of certain medications, and

laboratory data. We will not collect information like your social security number or any other information not directly related to this project.

Tissue/Blood/Data Storage for Future Use

As part of this study, we are obtaining blood (and effluent in RRT patients) samples and data from you. We may like to study your blood (and effluent in RRT patients) and data in the future, after this study is over without further consent. Your sample, information, and/or data may be stored for later use in a central repository or other national repositories sponsored by the National Institutes of Health or other Federal agencies. If this happens, it may be stripped of identifiers (such as name, date of birth, address, etc.). Other qualified researchers who obtain proper permission may gain access to your sample and/or data for use in approved research studies that may or may not be related to in the purpose of this study.

The samples will not be used for whole genome sequencing.

The tests we might want to use to study your blood or effluent and data may not even exist at this time. Therefore, we are asking for your permission to store your blood and effluent and data so that we can study them in the future. These future studies may provide additional information that will be helpful in understanding critical illness muscle loss, but it is unlikely that what we learn from these studies will have a direct benefit to you. It is possible that your blood and effluent and data might be used to develop products tests, or discoveries that could be patented and licensed. In some instances, these may have potential commercial value and may be developed by the Investigators, University of Nex Mexico, commercial companies, organizations funding this research, or others that may not be working directly with this research team. However, donors of blood and effluent and data do not retain any property rights to the materials. Therefore, there are no plans to provide financial compensation to you should this occur.

Your blood samples and data will be stored with a code which may be linked to your medical record number. If you agree now to future use of your blood samples and data but decide in the future that you would like to have it removed from future research, you should contact Dr. J. Pedro Teixeira. However, if some research with your blood samples and data has already been completed, the information from that research may still be used.

Do you give permission for your identifiable samples (blood, effluent) to be stored, used, and shared for future research?

☐ Yes ☐ No Initials _____

Remember, you can still be in the main study even if you even if you do not wish to allow your information and/or specimens stored or shared for future research.

WILL I BE NOTIFIED IF THERE IS AN UNEXPECTED FINDING IN MY BLOOD (OR EFFLUENT, IN CRRT PATIENTS)?

The results from the blood and effluent and data we collect in this research study are not the same quality as what you would receive as part of your routine health care. The blood and effluent and data results will not be reviewed by a physician who normally reads such results. Due to this, you will not be informed of any unexpected findings. The results of your blood and effluent and data will not be placed in your medical record with your primary care physician or otherwise. If you believe you are having symptoms that may require care, you should contact your primary care physician.

WHAT ARE THE RISKS OF THIS STUDY?

You may experience one or more of the risks indicated below from being in this study. In addition to these, there may be other unknown risks, or risks that we did not anticipate, associated with being in this study.

Blood collection:
For RRT patients:
When RRT is initiated, there will be labs drawn as part of the usual care provided to patients undergoing this procedure. We will draw our first set of labs at the same time, so there shouldn't be any additional venipuncture as part of this study. Subsequent labs after CRRT initiation will be taken directly from the machine circuit or arterial line. The total amount of blood that we take is 5 mL (or about 1 teaspoon) per day. This amount of blood is small and safe. It will not increase your need for a blood transfusion. Accessing the dialysis circuit or arterial line for obtaining the research blood samples may increase the risk of infection. However, we will try as much as possible to combine these draws with labs that will be drawn anyway as part of routine CRRT care, so additional risk should be minimal. Risks related to the muscle strength testing could include muscle injury, although these risks are low, and these tests are generally considered minimal risk within the medical community.

For Controls:
Risks related to the muscle strength testing could include muscle injury, although these risks are low, and these tests are generally considered minimal risk within the medical community.

Effluent collection (for RRT patients):
There should be no discomforts or risks associated with collection of effluent. The effluent will be collected directly from the dialysis circuit.

Ultrasound
Ultrasound is a non-invasive imaging procedure. There may be some mild discomfort related to the application of gel or pressure from the probe, but all discomforts are expected to be minimal. There are no known long-term health risks associated with ultrasound.

Confidentiality:
There is potential risk of loss of confidentiality. Efforts will be made to keep your personal information private and confidential. You will be identified by a code, and personal information from you records will not be released without your written permission. Data will be stored on a secure database (REDCap) supported by the University of Kentucky.

WHAT ARE THE BENEFITS OF THIS STUDY?

You will not benefit from being in this study. The study is designed for the researcher to learn more about the effect of kidney injury on the muscles in patients after CRRT initiation. However, we hope that, in the future, other people might benefit from this study from the knowledge gained.

WILL IT COST ME ANYTHING TO BE IN THIS STUDY?

You will not have any additional costs related to being in this research study. You and/or your medical/hospital insurance carrier will remain responsible for your regular medical care expenses.

WILL I BE PAID FOR PARTICIPATING?

You will not be paid for being in this research study.

WHO IS FUNDING THIS STUDY?

This study will be funded by a grant from the Western States Consortium, composed of six institutions with Clinical and Translational Sciences Award (CTSA) Program institutions. This means that the University of New Mexico is receiving payments to support the activities that are required to conduct the study. No one on the research team will receive a direct payment or increase in salary for conducting this study.

WHAT IF I AM INJURED AS A RESULT OF THIS STUDY?

If you believe you are hurt or if you get sick because of something that is due to the study, you should call Dr. J. Pedro Teixeira at 505-272-4751 immediately. Dr. Teixeira will determine what type of treatment, if any, is best for you at that time. It is important for you to understand that the University of New Mexico does not have funds set aside to pay for the cost of any care or treatment that might be necessary because you get hurt or sick while taking part in this study. Also, the University of New Mexico will not pay for any wages you may lose if you are harmed by this study.

Medical costs related to your care and treatment because of study-related harm will be your responsibility; or

- may be paid by your insurer if you are insured by a health insurance company (you should ask your insurer if you have any questions regarding your insurer's willingness to pay under these circumstances); or
- may be paid by Medicare or Medicaid if you are covered by Medicare or Medicaid (If you have any questions regarding Medicare/Medicaid coverage you should contact Medicare by calling 1-800-Medicare (1-800-633-4227) or Medicaid 1-800-635-2570.).

A co-payment/deductible may be needed by your insurer or Medicare/Medicaid even if your insurer or Medicare/Medicaid has agreed to pay the costs. The amount of this co-payment/deductible may be costly. You do not give up your legal rights by signing this form.

WHAT ABOUT CONFIDENTIALITY?

We will keep your participation in this research study confidential to the extent permitted by law, and all signed consent forms will be retained and securely stored at the University of New Mexico. However, it is possible that other people such as those indicated below may become aware of your participation in this study and may inspect and copy records pertaining to this research. Some of these records could contain information that personally identifies you.

- Federal government regulatory agencies,
- Auditing departments of the University of New Mexico or the University of Kentucky
- The Institutional Review Board (IRB, a committee that reviews and approves research studies) at the University of Kentucky or the Institutional Review Board at the University of New Mexico

To help protect your confidentiality, you will be identified by a code known only to the research team. Data will be stored on a secure database supported by the University of Kentucky. Paper copies of this consent will be stored in a locked drawer in a secured office on the UNM HSC campus. Badge access is needed to enter the building after hours and only the study principal investigator (Dr. Teixeira) has access to the key to the locked drawer. Biospecimens will be stored in a secure lab requiring a UNM

HSC badge for entry. The biospecimens will be stored with a code, so samples cannot be linked to you without the master list secured on the University of New Mexico database site (secured access folder on HSC central IT managed network storage). Electronic data will be stored within REDCap, which is a secure data storage platform operated by the University of Kentucky. If data is transferred for statistical analysis, names and other identifying information will be removed beforehand.

If we write a report or article about this study or share the study data set with others, we will do so in such a way that you cannot be directly identified.

The University of New Mexico Hospital generally requires that we document your participation in research occurring in a UNM HSC facility. This documentation will be in either your medical record or a database maintained on behalf of the institution reflecting that you are participating in this study. The information included will provide contact information for the research team as well as information about the risks associated with this study. We will keep this Informed Consent Document in our research files; it will not be placed in your medical record chart.

IS BEING IN THIS STUDY VOLUNTARY?

Taking part in this research study is completely voluntary. You may choose not to take part at all. If you decide to be in this study, you may stop participating at any time. If you decide not to be in this study, or if you stop participating at any time, you won't be penalized or lose any benefits for which you otherwise qualify.

CAN SOMEONE ELSE END MY PARTICIPATION IN THIS STUDY?

Under certain circumstances, the researchers might decide to end your participation in this research study earlier than planned. This might happen because in our judgment it would not be safe for you to continue, because your condition has become worse.

WHAT IF I HAVE QUESTIONS?

We encourage you to ask questions. If you have any questions about the research study itself, please contact: J. Pedro Teixeira, MD at 505-272-4751.

If you have questions, concerns, or complaints about your rights as a research subject or about research related injury, please contact the Office of Research Integrity, 405 Kinkead Hall, University of Kentucky, Lexington, KY 40506 (859) 257-9428, email: rs_ORI@uky.edu General information about being a research subject can be found by clicking "Participants" on the Office of Research Integrity web site, https://www.research.uky.edu/office-research-integrity. To offer input about your experiences as a research subject or to speak to someone other than the research staff, call the Office of Research Integrity at the number above.

AUTHORIZATION TO USE OR DISCLOSE YOUR IDENTIFIABLE HEALTH INFORMATION

The privacy law, HIPAA (Health Insurance Portability and Accountability Act), requires researchers to protect your health information. The following sections of the form describe how researchers may use your health information.

Your health information that may be accessed, used and/or released includes:

- **Physical exams, blood tests, and other diagnostic and medical procedures, as well as medical history.**
- **Demographic information, including: name, age, height/weight, address/telephone number, and medical record number (MRN)**

<u>The Researchers may use and share your health information with:</u>

- **The Institutional Review Boards at the University of Kentucky or the University of New Mexico;**
- **Law enforcement agencies when required by law;**
- **University of New Mexico representatives;**
- **UNM HSC and their representatives**
- **Health systems outside of UNM for which you have a patient relationship;**
- **Federal regulatory agencies [i.e., the Food and Drug Administration (FDA), National Institutes of Health (NIH)]**
- **Clinical & Translational Science Center (CTSC)**

The researchers agree to only share your health information with the people listed in this document. Should your health information be released to anyone that is not regulated by the privacy law, your health information may be shared with others without your permission; however, the use of your health information may still be regulated by applicable federal and state laws.

<u>You may not be allowed to participate in the research study if you do not sign this form. If you decide not to sign this form, it will not affect your:</u>

- **Current or future healthcare at the University of New Mexico;**
- **Current or future payments to the University of New Mexico;**
- **Ability to enroll in any health plans (if applicable); or**
- **Eligibility for benefits (if applicable).**

After signing the form, you can change your mind and NOT let the researcher(s) collect or release your health information (revoke the Authorization). If you revoke the authorization:

- **Send a written letter to: Dr. J. Pedro Teixeira (MSC10-5550, 1 University of New Mexico, Albuquerque, NM 87131-0001) to inform him of your decision.**
- **Researchers may use and release your health information already collected for this research study.**
- **Your protected health information may still be used and released should you have a bad reaction (adverse event).**

<u>The use and sharing of your information have no time limit.</u>

If you have not already received a copy of the Privacy Notice, you may request one. If you have any questions about your privacy rights, you should contact the UNM HSC Privacy Officer between the business hours of 8am and 5pm MT, Monday-Friday at (505) 272-1493.

INFORMED CONSENT SIGNATURE PAGE (HRRC ID 21-438)

You are participating or are authorized to act on behalf of the participant. This consent includes the following:

- Key Information Page
- Detailed Consent

You will receive a copy of this consent form after it has been signed.

_____ _____

Signature of research subject, *or if applicable, *research subject's legal representative*

Date

_____

Printed name of research subject

_____

**If applicable, printed name of research subject's legal representative*

*If applicable, please explain Representative's relationship to subject and include a description of representative's authority to act on behalf of subject:

_____ _____

Printed name of [authorized] person obtaining informed consent/HIPAA Authorization

Date

_____

Signature of [authorized] person obtaining informed consent/HIPAA Authorization

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym: ***page 1, lines 1-2***
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry: ***page 1, line 34***
	2b	All items from the World Health Organization Trial Registration Data Set: ***See attachment below.***
Protocol version	3	Date and version identifier: ***page 16, line 25***
Funding	4	Sources and types of financial, material, and other support: ***page 18, line 9-16***
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors: ***Title page; page 18, lines 1-7***
	5b	Name and contact information for the trial sponsor: ***see WHO trial registration data set below or clinicaltrials.gov posting***
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities: ***page 18, lines 11-13***
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee): ***N/A*** *(all these tasks will be carried out by the three primary investigators in this pilot trial)*
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention: ***page 4, line 1, through page 5, line 13***

	6b	Explanation for choice of comparators: ***page 7, lines 3-11***
Objectives	7	Specific objectives or hypotheses: ***page 5, line 19, to page 6, line 7***
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)*:* ***page 6, lines 10-13***

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained: ***page 6, lines 14-16***
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists): ***page 6, line 17, to page 7, line 2***
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered: ***N/A*** *(observational study)*
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease): ***N/A*** *(observational study)*
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests): ***N/A*** *(observational study)*
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial: ***N/A*** *(observational study)*
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended: ***page 7, line 13, to page 11, line 11 [skip page 8]***
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure): ***page 12, lines 3-12***
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations: ***page 13, lines 1-10***

Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size: ***page 13, lines 11-21***
Methods: Assignment of interventions (for controlled trials)		
Allocation:		
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions: ***N/A*** *(single-arm non-interventional study)*
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned: ***N/A*** *(single-arm non-interventional study)*
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions: ***N/A*** *(single-arm non-interventional study)*
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how: ***N/A*** *(single-arm non-interventional study)*
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial: ***N/A*** *(single-arm non-interventional study)*
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol: ***page 7, line 8, to page 12, line 5 [skip page 8]; page 14, lines 2-13; page 17, line 16***.
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols: ***page 13, lines 17-21***.
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol: ***page 11, line 12, to page 12, line 2; page 14, lines 5-7 and 14-16***

Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol: ***page 14, line 17, to page 16, line 19***
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses): ***N/A***
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation): ***page 13, lines 19-21***
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed: ***N/A*** *(non-interventional study)*
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial: ***N/A*** *(non-interventional study)*
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct: ***N/A*** *(non-interventional study)*
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor: ***N/A*** *(non-interventional study)*
Ethics and dissemination		
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval: ***page 16, lines 22-24***
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators): ***page 16, lines 25-26***
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32): ***page 17, lines 1-6***
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable: ***see page 5 of supplemental material 2 (ICF)***.

Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial: ***page 17, lines 8-13***
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site: ***page 18, line 18***
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators: ***page 17, lines 14-15***
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation: ***see page 5 of supplemental material 2 (ICF)***.
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions: ***page 17, lines 16-20***
	31b	Authorship eligibility guidelines and any intended use of professional writers: ***page 18, lines 8-10***
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code: ***page 17, lines 14-20***
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates: ***supplemental material 2 (ICF)***
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable: ***page 7, lines 24-26; page 9, lines 1-3; page 14, lines 7-11***

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

WHO Trial Registration Data Set

1. Primary Registry and Trial Identifying Number: NCT05287204

2. Date of Registration in Primary Registry: March 18, 2022

3. Secondary Identifying Numbers: University of Kentucky IRB# 71153 (single IRB), CTSA Project ID #: CTSC006-13

4. Source(s) of Monetary or Material Support: Clinical and Translational Science Awards Inter-Institutional Pilot Project Award

5. Primary Sponsor: University of Iowa

6. Secondary Sponsor(s): University of Kentucky, University of New Mexico

7. Contact for Public Queries:
   Institute for Clinical and Translational Science
   University of Iowa
   200 Hawkins Drive
   Iowa City, IA 52242
   deshauna-jones@uiowa.edu
   319-356-1060

8. Contact for Scientific Queries:
   Benjamin R. Griffin, MD
   Assistant Professor
   Division of Nephrology, Department of Internal Medicine
   University of Iowa Hospitals and Clinics
   200 Hawkins Dr
   Iowa City, IA 52242
   319-384-8197
   benjamingriffin@uiowa.edu

9. Public Title: Impact of CRRT on the Development of Critical Illness Muscle Wasting

10. Scientific Title: Impact of CRRT on the Development of Critical Illness Muscle Wasting

11. Countries of Recruitment: United States

12. Health Condition(s) or Problem(s) Studied: acute kidney injury

13. Intervention(s): N/A (observational study)

14. Key Inclusion and Exclusion Criteria:
    - Inclusion: acute kidney injury requiring continuous renal replacement therapy (CRRT) within 48 hours of admission to the intensive care unit (ICU)
    - Exclusion criteria:
      A. ICU admission for greater than 7 days

B. Renal replacement therapy of any kind at any time before ICU admission
C. Chronic kidney disease with estimated glomerular filtration rate <20 mL/min/1.73 m^2
D. Underlying muscle disorders or muscle atrophy such as quadriplegia or hemiplegia
E. Stroke with residual motor deficits
F. End-stage liver disease
G. Active alcohol use disorder
H. Active malignancy (other than non-melanoma skin cancer) within 1 year
I. Burns
J. Other baseline neuromuscular disease
K. Pregnancy
L. Concomitant use of other extracorporeal support devices such as ventricular assisted devices (VADs) or extracorporeal membrane oxygenation (ECMO)
M. Anticipated inability to engage in weight-bearing testing after discharge (e.g., trauma or orthopedic surgery)

15. Study Type: This is a prospective observational single-arm study (using historical controls) with a purpose of analyzing the effect of acute kidney injury requiring continuous renal replacement therapy (CRRT) on the development of ICU-acquired weakness using 3 methods: (1) muscle ultrasound, (2) assessments of physical function and muscle strength, and (3) analysis of biological samples (metabolomics of blood and CRRT effluent).

16. Date of First Enrollment: August 1, 2022

17. Sample Size: target sample size 60 (20 patients each at 3 sites), to be compared to 60 historical controls; current enrolment: 15 (as of April 15, 2023)

18. Recruitment status of this trial: Recruiting

19. Primary Outcome(s): change in muscle ultrasound parameters (rectus femoris cross sectional area, muscle thickening, and echo intensity) in patients between study enrollment and study day 7 or ICU discharge

20. Key Secondary Outcomes:
    - Physical function assessments (including strength assessment using hand-held and hand-grip dynamometry) at hospital discharge and at 1-3 months after discharge
    - Metabolomic analysis of blood and CRRT effluent (at day 0, day 3, and day 7 or ICU discharge if sooner)

21. Ethics Review:
    - Approved (IRB #71153)
    - Initial Approval June 7, 2022; most recent protocol revision approved January 7, 2023
    - Office Research Integrity at the University of Kentucky (859-257-9428, rs_ORI@uky.edu)

22. Completion date: September 30, 2023

23. Summary Results: pending

24. IPD sharing statement: The final de-identified dataset will be made fully accessible upon reasonable request once the results are published.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5-6
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6, 11-12, 23
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6-7, 11, 23
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-10, 12-14
Bias	9	Describe any efforts to address potential sources of bias	10-11; 12-14
Study size	10	Explain how the study size was arrived at	11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	12-14
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	12-14
		(*b*) Describe any methods used to examine subgroups and interactions	12-14
		(*c*) Explain how missing data were addressed	N/A
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	N/A
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	N/A
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	Report numbers of outcome events or summary measures over time	N/A

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	N/A
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	N/A
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	3
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	N/A
Generalisability	21	Discuss the generalisability (external validity) of the study results	N/A
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.