Randomized controlled trial of glutamine in chronic kidney disease

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LIST OF ABBREVIATIONS AND ACRONYMS

AE	Adverse Event
CKD	Chronic kidney disease
EAE	Expedited Adverse Event
EC	ethics committee
FDA	(United States) Food and Drug Administration
GCP	Good Clinical Practices
IRB	Institutional Review Board
LDMS	Laboratory Data Management System
LL	Local Laboratory
MRS/OS	^{31}P Magnetic resonance spectroscopy and optical spectroscopy
NIDDK	United States) National Institute of Diabetes and Digestive and Kidney
NIH	(United States) National Institutes of Health
PAT	Pulse amplitude tonometry
ROC	Regulatory Operations Center
SAE	Serious Adverse Event
SDMC	Statistical and Data Management Center
SUSAR	Suspected Unexpected Serious Adverse Reaction
SOP	Standard Operating Procedures
SSP	Study Specific Procedures
TCA	Tricarboxylic acid (Krebs) cycle

Randomized controlled trial of glutamine in chronic kidney disease

SCHEMA

Purpose: Study the impact of oral glutamine supplementation on mitochondrial function and endothelial cell function measured by ^{31}P MRS/OS among persons with moderate-severe CKD.

Design: **Randomized, placebo controlled cross-over trial**

Study Population: Moderate-severe CKD with eGFR by CKD (EPI equation) $\leq$ 60ml/min/1.73m^2

Study Size: **20**

Treatment Regimen: - Oral glutamine 0.4gm/kg/day
- Placebo

Study Duration: 1 year

Primary Objectives:

- To test if glutamine supplementation improves mitochondrial function and vascular function as measured by ^{31}P MRS/OS.

Secondary Objectives:

- To test the effects of oral glutamine supplementation on metabolic response profile.
- To test if glutamine supplementation reduces inflammatory and oxidative stress biomarkers.
- To test if glutamine improves objective isometric muscle fatigue.
- To test if glutamine supplementation reduces proteinuria (albuminuria).
- To test if glutamine supplementation reduces 24 hour urine urea nitrogen.

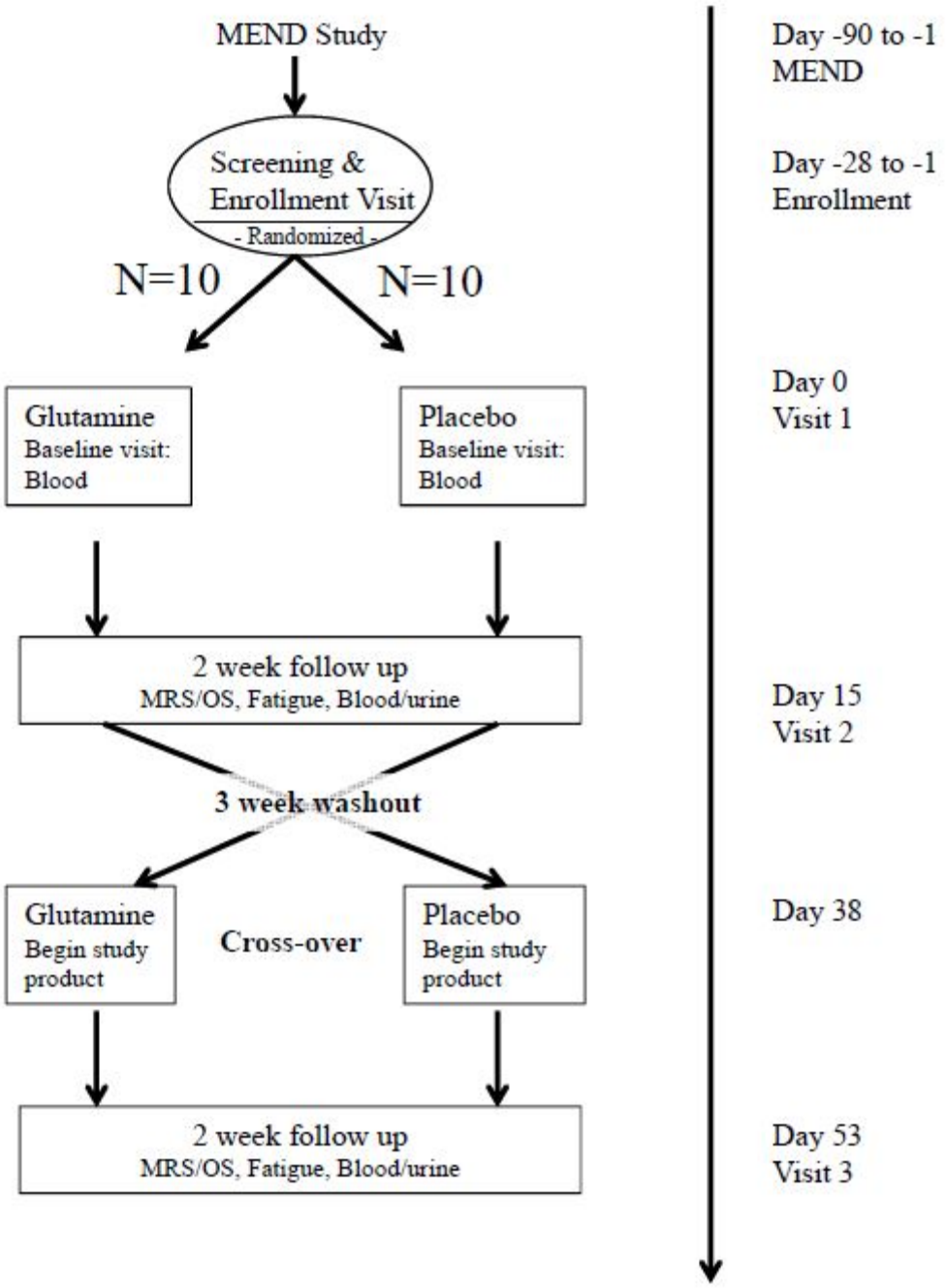
Study Sites:

University of Washington:

- University of Washington Medical Center Translational Center for Metabolic Imaging
- Kidney Research Institute:
  - KRI at Harborview Medical Center
  - KRI at Northwest Kidney Centers-Havilland
  - KRI at UWMC

Randomized controlled trial of glutamine in chronic kidney disease

SCHEMA



1.0 INTRODUCTION

1.1 Background and Prior Research

Chronic kidney disease is associated with vascular dysfunction leading to substantial cardiovascular and cerebrovascular disease burden. A major mechanism of vascular dysfunction is thought to be endothelial cell dysfunction due to the accumulation of homocysteine, asymmetric di- and mono-methylarginines (ADMA), reactive oxygen species, and/or guanidine-compounds. Current standard of care for treating vascular disease in patients with CKD focuses on addressing of hypertension and proteinuria, both of which are detected late in the disease. The observation that endothelial cell dysfunction (ECD) develops at earlier, pre-uremic stages of CKD highlights the critical need for investigations into earlier preclinical mechanisms contributing to ECD.

Defects in endothelial nitric oxide synthase (eNOS) and/or deficiency of bioavailable nitric oxide are the hallmarks of endothelial cell dysfunction leading to vascular disease and defective angiogenesis. Accumulation of toxins found in renal disease may impact endothelial cell nitric oxide bioavailability and eNOS function. Indeed, exposure of endothelial cells to asymmetric dimethylarginines (ADMA) has been shown to result in the redistribution of eNOS from the plasma membrane to the mitochondrion as well as result in uncoupling of eNOS with enhanced generation of reactive oxygen species. Furthermore substantial evidence indicates nitric oxide bioavailability is linked to mitochondrial biogenesis. *Recent observations of a proteomic signature in early ECD modeled by using chronic L-NMMA treatment akin to elevation on ADMA suggest underlying mitochondrial dysfunction*. Under these conditions early ECD is characterized by reductions in aconitase-2 and ECHS-1 (key enzymes in the mitochondrial TCA cycle and fatty acid beta oxidation pathways). Reactive oxygen species have been shown to play a central role in inactivation of aconitase. *These findings may suggest that inhibition of the Krebs cycle and mitochondrial bioenergetics may augur the development of ECD. Deficiency in these enzymes and reduction in mitochondrial mass may result in a metabolic block of normal mitochondrial oxidative phosphorylation.*

Glutamine is a potent anaplerotic (capable of replenishing intermediates of the Kreb's cycle) and a potential therapeutic agent bypassing the metabolic block associated with reduced TCA cycle activity and impaired mitochondrial function (figure 1). It is also the substrate for glutamate important for antioxidant glutathione synthesis. It has been demonstrated to confer metabolic benefits in other populations, but the data on its effect in patients with chronic kidney disease are unknown. Here we assemble a multi-disciplinary team of investigators with expertise in physiology, biophysics, and nephrology to investigate the impact of glutamine on mitochondrial energetics and vascular function.

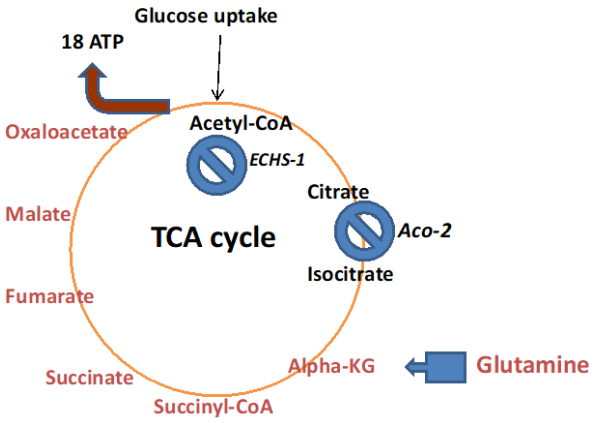


Figure 1. Supplementation with glutamine: its conversion to alpha-ketoglutarate bypasses the enzymatic block in ECD and reinforces the truncated Krebs cycle.

We propose a phase II randomized, controlled cross-over trial of glutamine supplementation on mitochondrial energetic and endothelial function measured by non-invasive ^{31}P magnetic resonance spectroscopy coupled with optical spectroscopy (MRS/OS) and pulse amplitude tonometry among persons with moderate-severe chronic kidney disease.

1.2 Rationale

Chronic kidney disease is associated with endothelial cell dysfunction and muscle wasting contributing to the heightened risk of cardiovascular morbidity and mortality and functional limitation. Accumulation of toxins in renal disease may adversely impact endothelial cell nitric oxide bioavailability and endothelial Nitric Oxide Synthase (eNOS) function consequently heightening oxidative stress and suppressing mitochondrial biogenesis. To date no studies have investigated potential therapies for endothelial and muscle dysfunction in renal disease target mitochondrial metabolic and energetic processes.

Animal studies of uremia underscore mitochondrial dysfunction as a potential precursor for endothelial dysfunction. In particular, uremia has been linked to a proteomic signature indicative of metabolic blockage of TCA cycle activity and fatty acid beta-oxidation. Both of these processes are localized to the mitochondria and may suggest that decreased mitochondrial mass or function may augur endothelial dysfunction in renal disease.

Glutamine, an anaplerotic agent and precursor to the antioxidant glutathione, is a potential therapeutic agent bypassing the metabolic block associated with reduced TCA cycle and improving antioxidant reserve. A recent dose-finding study and randomized controlled trial of glutamine in patients with critical illness demonstrated that an IV dose of 0.35g/kg/day by ideal body weight was associated with an increased muscle mitochondrial mass[1] and well tolerated without any serious clinical or biochemical adverse events compared to placebo.[2]

The primary goal of proposed investigation is to study the impact of oral glutamine supplementation on muscle mitochondrial and endothelial cell function measured mitochondrial energetics and vascular function using ^{31}P MRS/OS among persons with moderate-severe CKD. The secondary objective is to describe the impact of oral glutamine supplementation on mitochondrial metabolic profile as well as inflammatory and oxidative stress biomarkers among persons with chronic kidney disease.

2.0 STUDY OBJECTIVES AND DESIGN

2.1 Primary Objectives

The primary objectives of this study are to:

- To test if glutamine supplementation improves mitochondrial function and vascular function as measured by ^{31}P MRS/OS.

2.2 Secondary Objectives

The secondary objectives of this study are to:

- To test the effects of oral glutamine supplementation on metabolic response profile.
- To test if glutamine supplementation reduces inflammatory, oxidative stress biomarkers and redox proteome
- To test if glutamine improves objective isometric muscle fatigue.
- To test if glutamine supplementation reduces proteinuria (albuminuria).
- To test if glutamine reduces 24 hour urine urea nitrogen.

2.3 Study Design

Overview of study design

This is a placebo-controlled, double-blind crossover trial. There are 2 phases 14 days each separated by a 3-week washout period

A brief screening visit will assess eligibility criteria, and will include informed consent. Qualified participants will be stratified by diabetes status and randomly assigned (1:1) to 14 days of placebo followed by 14 days weeks of oral L-glutamine, or 14 days of oral L-glutamine followed by 14 days of placebo, each separated by a 3-week wash-out period.

Qualified participants will participate in 3 study visits, plus a screening visit. Study outcomes will be assessed at each of these 3 visits.

Subjects will receive 0.4 g/kg/day of L-glutamine in three divided daily doses. This dose was chosen based on a review of studies in the literature evaluating biological activity of glutamine on immune function and modulation of muscle turnover. Placebo will consist of identical appearing maltodextrin powder.

3.0 STUDY POPULATION

Study participants will be selected from patients with moderate-severe CKD not treated with dialysis from the Muscle Mitochondrial Energetics and Dysfunction (MEND) study, a cross-sectional clinic-based study of CKD. MEND has recruited CKD and end stage renal disease (ESRD) patients from Nephrology clinics at Harborview Medical Center, University of Washington Medical Center, and the Northwest Kidney Centers. MEND includes a diverse population, representing the greater population of those with kidney disease. Exclusion criteria for MEND include kidney transplantation, non-English speaking, unable to provide informed consent, or dementia.

Participants will be selected for this study from MEND according to the criteria in Section 3.1 and 3.2. They will be recruited, screened, and enrolled as described in Section 3.3 [and assigned to a study treatment/intervention group as described in Section

7.4]. Issues related to participant retention and withdrawal from the study are described in Sections 3.5 and 3.6, respectively.

3.1 Inclusion Criteria

Criteria included in MEND:
Additional Criteria:

- Adults between 20 and 79 years of age
- Diagnosis of moderate-severe CKD, defined in this study as an estimated glomerular filtration rate (eGFR) of ≤60ml/min/1.73m^2 using the Chronic Kidney Disease Epidemiology Collaboration equation

3.2 Exclusion Criteria

Criteria included in MEND:

- Pregnant
- Kidney transplantation
- Weight >400 lbs
- HIV infection
- End stage liver disease with cirrhosis
- Have physical immobility (defined by wheelchair use, oxygen-dependent COPD, shortness of breath after <100 steps)
- Have implants incompatible with MRI
- Institutionalization, or inability to consent
- Use medications interfering with muscle or mitochondrial function, including steroids, anti-psychotic, antivirals, muscle relaxants, oral calcineurin inhibitors, and anti-epileptic drugs
- Active malignancy
- Pacemaker
- Current participation in another interventional trial
- Non-English speaking

Additional Criteria:

- On chronic dialysis
- Expectation to start dialysis within 6 months or dialysis access in place.
- Insulin dependent diabetes
- Exercise limiting cardiopulmonary disease (e.g. angina, severe heart valve disease, severe COPD, coronary ischemia)
- Use of anticoagulation (i.e. warfarin)
- Baseline systolic blood pressure >170 or diastolic blood pressure >100
- Inflammatory conditions (e.g. autoimmune disease, HIV)
- Cirrhosis, active/chronic hepatitis
- Weight >300 lbs
- Personal history or family history of deep vein thrombosis, pulmonary embolism
- Patients hospitalized within the past 60 days for any reason.

3.3 Recruitment Process

Participants enrolled in the MEND study have documented consent approving contact regarding participation in future studies for which MEND will be the baseline. We will recruit patients who have already undergone a CKD-MEND visit. CKD-MEND is a study of muscle mitochondrial energetics and dysfunction recruiting from the SKS and clinics. A comprehensive battery of testing including MRS/OS is performed. Data from the CKD-MEND visit performed within 180 days of entry into the current study will be used as baseline information.

3.4 Co-Enrollment Guidelines

Study participants will not be allowed to enroll in other clinical trials, as the effect of glutamine on this population is not clear.

3.5 Participant Retention

Every effort to retain enrolled participants will be made throughout the duration of the study in order to reduce bias related to loss to follow-up. Strategies for participant retention include:

- Thorough explanation of the importance of all 2 study treatment groups to the overall success of the study
- Detailed explanation of the study visit schedule and procedural requirements during the informed consent process, with re-emphasis at the end of each study visit
- Use of appropriate and timely visit reminder mechanisms. This includes weekly phone calls to assess participant adherence to regimen and remind participants of subsequent follow up visits
- Compensating participants for their time and effort in the study
- Immediate and multifaceted follow-up on missed visits

3.6 Participant Withdrawal

Participants will be allowed to voluntarily withdraw from the study for any reason and at any time. The investigator may withdraw participants from the study in order to protect their safety or if they are unwilling or unable to comply with required study procedures after consultation with the study sponsors and co-sponsors. Participants also may be withdrawn if the study sponsor, government or regulatory authorities, or site IRBs/ECs terminate the study before the planned end date. Reasons for withdrawal from the study will be recorded and a final evaluation will be made if feasible.

Participants who discontinue treatment but remain in the study will continue to be followed-up whenever possible.

4.0 STUDY TREATMENT/PRODUCT/INTERVENTION

4.1 Treatment/Product/Intervention Regimen(s)

- Active treatment will consist of 0.4 g/kg/day of L-glutamine (Nutrestore, EMMAUS Life Sciences, Inc Torrance, CA) in three divided daily doses. This dose was chosen based on a review of studies in the literature evaluating blood absorption of oral glutamine,[3] biological activity of glutamine on immune function and modulation of muscle turnover.

- Placebo will consist of identical appearing maltodextrin powder.

4.2 Treatment/Product/Intervention Supply and Accountability

The Investigational Drug Pharmacy pharmacist must maintain complete records of all study drugs/products received from the Clinical Research Products Management Center and drug manufacturer and subsequently dispensed to study participants. All unused supplies must be returned to the Clinical Research Products Management Center after the study is completed or terminated.

4.3 Adherence Assessment

Adherence to the study treatment will be assessed by study coordinator phone call at day 7 of the study to assess adherence and inventory method to assess remaining medication/placebo at the end of the study. Adherence will be defined as consumption of at least 75% of the prescribed regimen. Up to 30 participants may be recruited to compensate for non-adherence or drop-out with the goal of 10 individuals completing in each treatment arm.

4.4 Toxicity Management

Currently, the mechanism whereby glutamine may cause harm or toxicity is unknown. There have been studies demonstrating increased levels of blood urea in those treated with glutamine. In the event of moderate or serious adverse effects (as defined in section 6.2) requiring treatment for such conditions as nausea, vomiting, confusion or bleeding thought to be related to toxicity from the study drug, the patient will be withdrawn from the study and adverse events reported.

Mild adverse events that do not requiring treatment and are thought to be potentially related to toxicity from the drug will prompt a 50% dose reduction.

4.5 Clinical Management of Pregnancy

All female participants of childbearing age who are sexually active will be asked to use contraception during the duration of the study and for 1 month afterwards as the effects of glutamine on fetal development are not clear.

Any females who become pregnant during the study period will stop taking the study product, but will continue with study procedures with the exception of the MRS/OS procedures. This is because the risks of ischemia in pregnancy are not known.

4.6 Concomitant Medications

Enrolled study participants may continue use of all concomitant medications — except those listed under criteria for exclusion or treatment discontinuation — during this study.

All concomitant medications taken or received by participants within the 4 weeks prior to study enrollment will be reported on applicable study case report forms. In addition to prescribed and over-the-counter medications (vitamins, herbal remedies, and other traditional preparations) will be recorded. Alcohol and recreational or street drug use will be recorded in clinical progress notes if needed for interpretation/documentation of observed participant health status. Medications used for the treatment of AEs that occur during study participation also will be recorded on applicable study case report forms.

5.0 STUDY PROCEDURES

Schedule of visits and procedures

An overview of the study visit and procedures schedule is presented in table 1. Presented below is additional information on visit-specific study procedures. Detailed instructions to guide and standardize all study procedures will be provided in the study-specific procedures manual.

Screening visit

Some components of the initial screening visit may be performed by telephone or in combination with other study visits in order to best accommodate participants' schedules. Once informed consent is obtained, the subject will undergo a baseline screening evaluation including an assessment of demographics, smoking history, medical history, medication inventory, with physical examination by study personnel. If the subjects meet the eligibility criteria the PI or Co-I will contact the subjects' primary physician regarding the subjects' involvement in the trial. The first study visit will be scheduled within 28 days of the screening visit. We will link to laboratory data from the CKD-MEND visit. *The only lab tests that are not collected in CKD-MEND needed for this trial are metabolomics and redox proteome testing (see below).*

Study drug dispensed at Visits 1 and 2

The participant will receive either placebo (maltodextrin powder) or glutamine from the Investigational Drug Service (IDS) and instructed on how to appropriately prepare it. He or she will be instructed to take the medication daily starting on the day after the study visit. They will be instructed to consume no protein for 30 minutes after glutamine or placebo consumption to prevent competitive binding for absorption with other amino acids.

Visit 1 – Visit 3

The participant will have been required to fast for 8 hrs prior to each visit and abstain from caffeine or smoking which can effect endothelial function for 8 hours prior to the visit. The following data will be obtained:

- vital signs,
- fasting blood collection,
- anthropometrics (height and weight),
- a urine pregnancy test will be given to women of child-bearing potential
- The MRS/OS and Muscle Fatigue tests will occur at the diagnostic imaging center at UWMC at visits 2 and 3. We will use the results of the MEND MRS/OS as the baseline for this study. Only 2 MRS/OS and Muscle Fatigue tests will be obtained per patient during this study because it will be assumed that there will be no carry-over effect of glutamine after the 3-week washout period.

  Note: We may ask the subject to repeat the MRS/OS and Muscle Fatigue tests if more than 180 days has passed, or if they have had a change in health status, since their MEND visit.

Procedures:

An overview of the study visit and procedures schedule is presented in Appendix I. Presented below is additional information on visit-specific study procedures. Detailed instructions to guide and standardize all study procedures across sites will be provided in the study-specific procedures manual.

Blood testing for metabolic response profile (oxidative stress, inflammation, and plasma amino acids, metabolites, and electrolytes). Oxidative Stress and Inflammatory Biomarkers include: C-reactive protein and IL-6 levels will be determined using the automated Abbott FLX C-Reactive Protein Assay and by ELISA, respectively. Plasma F2-isoprostanes and isofurans will be measured by GC-MS as described by Roberts and Morrow at Vanderbilt University; Plasma Amino Acids, Metabolites and Electrolytes include: Plasma amino acid concentrations will be determined by reversed-phase HPLC after derivatization with phenylisothiocyanate.

- Plasma samples will be sent to Emory University for liquid chromatography high-resolution mass spectrometry (LC-MS) for untargeted metabolomics analysis.

- Clinical laboratory assays for determination of comprehensive metabolic panel (electrolytes, blood urea nitrogen, creatinine, glucose, albumin, total protein, calcium, alkaline phosphatase, ALT, AST, total bilirubin), CPK, lactate, triglyceride and ammonia levels will be performed at the *KRI Core Research laboratory.*

-*Testing the redox metabolome.* Glutathione (GSH/GSSG) redox and cysteine (Cys/CySS) redox systems are oxidized in association with oxidative stress and in association with cardiovascular disease. Extracellar Cys/CySS redox state has been associated with events of early atherosclerosis, specifically monocyte adhesion to the endothelium and activation of the inflammatory cascade. Furthermore the redox state of the mitochondria represents a unique pool providing potential insight into the oxidative damage eventually contributing to necrosis and apoptosis. *Plasma samples for all these*

assays will be processed and sent to Emory University in Atlanta for analysis of mitochondrial redox control via systems of glutathione and thioredoxin models.

24 hour urine collection for urea nitrogen: Assessment of protein turnover will be performed using a timed 24hr urine collection for assessment of urine urea nitrogen. In order to confirm adequate collection and estimate muscle mass, we will also collect urine creatinine. Specimens less than 1liter in volume will be repeated.

Magnetic Resonance Spectroscopy and Optical Spectroscopy: As mentioned above, only 2 MRS/OS will be obtained as part of this study (Visit 2 and 3). The baseline MRS/OS will be obtained from the MEND study.

MRS/OS will be used to measure mitochondrial energy coupling (P/O), phosphorlylation capacity (ATP_{max}) and oxidative capacity (O_{2max}) of the first dorsal interosseus (FDI) skeletal muscle.[4] The selection of the FDI skeletal muscle is based on the observation of higher oxidative type II muscle fiber content and degree of coupling similar in extent to the vastus lateralis commonly used to study of mitochondrial dysfunction. [5,6] As part of this procedure, two functional tests will be performed: an exercise protocol to help determine ATP_{max} and a resting (ischemia) protocol to determine mitochondrial energy coupling and the maximal rate of mitochondrial O_2 uptake (O_{2max}). Optical spectroscopy measuring the delay in Hemoglobin saturation relative to Myoglobin saturation after the onset of reperfusion will provide an index of vascular function. These functional tests assist to perturb the cell physiological state in order to measure the contents and dynamics of key metabolic compounds important in mitochondrial energy flux. ^{31}P MRS is reliable with a documented coefficient of variation for oxidative phosphorylation of 5.1% and has a strong correlation with V_{O2max}.[7]

Muscle Fatigue: We will test muscle fatigability at each follow-up visit. We will test the FDI muscle and determine the isometric force generated at 70% of maximal voluntary contraction (MVC) against a force transducer. Using labview software to measure and record the force-time waveform of each contraction we will initiate the protocol at 50 contractions per minute at 70% MVC for one minute. At each 1 minute increment will increase the contraction rate by 10 contractions per minute and reassess the subjects relative perceived exertion on a scale of 1-10 until the patient can no longer maintain the contraction waveform.

Vitals and measurements

Day 7 and 45 phone call:

All subjects will receive a phone call by study coordinators to confirm patients are taking their treatment/placebo medication in order to ensure adherence. They will also receive reminder of their subsequent follow up visits.

Visit 2 (day 15):

At visit 2 the participant will be questioned regarding any concerning symptoms noted during the treatment period. Adherence with the treatment or placebo will be confirmed by evaluating the quantity of remaining placebo or glutamine in the dispensed containers. Repeat fasting blood collection. They will also receive a repeat MRS/OS.

Washout (Day 16-37):

After the second visit, the participant will undergo a 3 week washout period prior to initiating the second treatment or placebo for another 2 week period.

Day 38:

The participant will cross-over to the opposite placebo or glutamine therapy for a two week course. We will phone the subject to remind them to begin taking the treatment/placebo.

Visit 3 (Day 53- Final visit):

During visit 3 the participant will be questioned regarding any concerning symptoms noted during the treatment period during visit 3. Adherence with the treatment or placebo will be confirmed by evaluating the quantity of remaining placebo or glutamine in the dispensed containers. Repeat testing will be performed to assess change during period 2 of the study. Fasting blood will be collected. They will also receive a repeat MRS/OS.

Table 1. Table of study treatments and procedures

Treatments/Procedures	Day -90 to 0 (Screening)	Day -1 (Visit 1)	Day 0 - 14	Day 15 (Visit 2)	Day 16-37 Washout	Day 38	Day 39-52	Day 53 (Visit 3)
Visit may take this much time:	1 hour	3 hour		3 hour		(phone call)		3 hour
Informed Consent	X					Begin Study Product for Period 2.		
Inclusion/Exclusion	X							
Medical History	X							
Medication List	X							
Demographics	X							
Brief Physical Exam	X							
Pregnancy test*		X						
Vitals		X		X				X
Fasting Blood Draw		X		X				X
24hr urine collection				X				X
MRS/OS**				X				X
Muscle fatigue				X				X
Study Product Dispensed		X		X				
Study Product collected				X				X
Study Product Adherence				X				X
Phone Call to Participant			Day 7				Day 45	

*Pregnancy test performed on females of childbearing age who are sexually active.

** MRS/OS and Muscle Fatigue for baseline is already done as part of CKD-MEND visit (within 180 days). If more than 180 days has passed, or if subjects have had a change in health status, these tests will be repeated at Visit 1.

6.0 SAFETY MONITORING AND ADVERSE EVENT REPORTING

6.1 Safety Monitoring

All adverse events will be followed by the PI and study personnel until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator will instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator will follow any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. An internal audit will take place each month of the trial to insure that everything is well documented.

As each of the first five subjects completes the cross-over (visit 3), we will submit to the IRB a report on how the drug was tolerated. We will report all side effects, adverse events and subject concerns. In the event that one of the first 5 subjects withdraws from the study for reasons related to the drug, we will report immediately to the IRB via modification.

6.2 Adverse Event Definitions and Reporting Requirements

6.2.1 Adverse Event

Safety information will be assessed initially by subject interview. The clinical research coordinator is responsible for collecting and recording all clinical data. As these results are collected, all adverse events will be identified and reported to the principle Investigators within seven (7) days. Adverse events will be reported as described below (section 6.2.3). The principal investigator (PI) is responsible for evaluating each AE and for determining whether the AE affects the risk/benefit ratio of the study and whether modifications to the protocol and consent form are required. All IRB-approved protocol or consent form revisions that indicate a change in risk for participants will be reported to the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) in a timely manner.

Study participants will be provided a telephone number and instructed to contact the study clinician to report any AEs they may experience, except for life-threatening events, for which they will be instructed to seek immediate emergency care. Where feasible and medically appropriate, participants will be encouraged to seek evaluation where the study clinician is based, and to request that the clinician be paged or otherwise contacted upon their arrival. With appropriate permission of the participant, whenever possible records from all non-study medical providers related to AEs will be obtained and required data elements will be recorded on study case report forms. All participants reporting an AE will be followed clinically, until the AE resolves (returns to baseline) or stabilizes.

Study site staff will document on study case report forms all AEs reported by or observed in enrolled study participants regardless of severity and presumed relationship to study product.

All adverse events will be graded as follows:

Severity

0 = No adverse event or within normal limits
1 = Mild—did not require treatment
2 = Moderate—resolved with treatment
3 = Severe—required professional medical attention
4 = Life-threatening or disabling
5 = Death

Related to study procedures (there is a reasonable possibility that the experience may have been caused by the study procedures)

0 = Unrelated
1 = Unknown
2 = Related

Unexpected event (an AE with specificity or severity not consistent with the risk information in the protocol/application or an AE that has not been previously observed)

0 = No
1 = Yes

Serious (any AE occurring at any dose that results in death; a life-threatening adverse drug experience; inpatient hospitalization or prolongation of existing hospitalization; a persistent or significant disability/incapacity; a congenital anomaly/birth defect; or any important medical event that, based on medical judgment, jeopardizes the subject and may require medical or surgical intervention to prevent one of the above outcomes)

0 = No
1 = Yes

6.2.2 Serious Adverse Event

Serious adverse event (SAE) will be defined per U.S. Code of Federal Regulations (CFR) 312.32 and International Conference on Harmonization (ICH), "Good Clinical Practice: Consolidated Guidance" (E6) and "Clinical Safety Data Management: Definitions and Standards for Expedited Reporting" (E2A), as AE occurring at any dose that:

- Results in death
- Is life-threatening

- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Requires inpatient hospitalization or prolongation of existing hospitalization

This includes important medical events that may not be immediately life-threatening or result in death, or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed above.

Per ICH SAE definition, hospitalization itself is not an adverse event, but is an outcome of the event. The following types of hospitalization do not require expedited reporting:

- Any admission unrelated to an AE (e.g. for labor/delivery, cosmetic surgery, administrative, or social admission for temporary placement for lack of place to sleep)
- Protocol-specified admission (e.g. for procedure required by protocol)
- Admission for diagnosis or therapy of a condition that existed before receipt of study agent(s) and has not increased in severity or frequency as judged by the clinical investigator

6.2.3 Adverse Event Reporting

Any AE that is unexpected, related to study participation, and serious (defined in 6.2.1) will be reported in writing to the IRB within 7 days of the PI's first knowledge of the event. Any AE that is unexpected, related to study participation, and suggests greater risk of harm than previously known or recognized will be reported in writing to the IRB within 7 days of the PI's first knowledge of the event.

The PI will review all expedited adverse event reports. In addition, any deviations from protocol will be reported to the IRB and copied to the Prevention Center.

A summary of all adverse events and any audit reports will be sent to the IRB. Any action resulting in a temporary or permanent suspension of this study (e.g., IRB actions, or actions by the investigators or co-investigators) will be reported to the appropriate NIDDK program official. In addition, the NIDDK will be given notice of any actions taken by the IRB or regulatory bodies regarding the research and any responses to those actions.

7.0 STATISTICAL CONSIDERATIONS

7.1 Review of Study Design

We propose a single-center double blind randomized cross-over trial of glutamine supplementation versus placebo. As part of the study there will be a 4-week washout between treatment and placebo arms.

7.2 Endpoints

Primary endpoints are change in mitochondrial energetics and endothelial function. Secondary endpoints are change in metabolic response profile and markers of oxidative stress and inflammation.

7.2.1 Primary Endpoints

Consistent with the primary study objective to test for glutamine's impact on endothelial function and mitochondrial function, the following endpoints will be assessed:

- Improvement in mitochondrial function as measured by ^{31}P MRS/OS parameters of ATP_{max} and Oxygen uptake (O_{2max}) and the $ATP_{flux}/O_{2uptake}$ ratio representing the efficiency of mitochondria energy production.
- Improvement in vascular function as measured by the difference in the spectroscopic delay in recovery of oxygenated myoglobin and oxygenated hemoglobin after the onset of reperfusion during optical spectroscopy.

7.2.2 Secondary Endpoints

Consistent with the secondary study objective to assess changes in metabolic response profile and markers of oxidative stress and inflammation the following endpoints will assessed:

- Improvement in metabolic response profile suggested by:
    - *Change in metabolomics profile using liquid chromatography high-resolution mass spectroscopy (LC-MS).* (Reference: Frediani JK. (2014) Plasma metabolomics in human pulmonary tuberculosis disease: a pilot study. PLoS ONE 9(10): e108854).
    - Lower levels of lactate, triglycerides.
    - *Assessment of the integrated redox function* (Reference: Jones DP. Measuring the poise of thiol/disulfide couples in vivo. *Free Radical Biology and Medicine.* 47(2009):1329-38.)
    - Plasma F2-isoprostanes will be measured by GC-MS as described by Roberts and Morrow; Plasma amino acids, metabolites and electrolytes. Plasma amino acid concentrations will be determined by reversed-phase HPLC after derivatization with phenylisothiocyanate
- Improvement in proteinuria (albuminuria).
- Assessment of nitrogen balance and anabolism with 24hr urine for urine creatinine, urine urea nitrogen.

7.3 Accrual, Follow-up, and Sample Size

The overall sample size for this project is 20. We anticipate recruiting 1-2 participants weekly for total study duration of 2 years.

Sample size was calculated based on values obtained from an estimated within individual coefficient of variation in values of ATP/O_2 ratio from MRS/OS of approximately 15% and values of test-retest reliability of pulse amplitude tonometry measures of vascular endothelial function from a recent published study. A total of 20 patients will enter this two-treatment crossover study. With this sample size, the probability is over 90% that we will detect a meaningful treatment difference of 20% for ATP/O2 ratio at a one-sided 0.05 significance level (Table 2). This is based on the assumption that the within-patient standard deviation of the response variable is 0.375 with a mean predicted ATP/O2 ratio of 2.5. Sample size was calculated using the following sample size calculator: *http://hedwig.mgh.harvard.edu/sample_size/js/js_crossover_quant.html*

	ATP/O_2 ratio Power	
Minimal detectable difference	**80%**	**90%**
20%	6	7
25%	4	5
33%	4	4
40%	3	4

Table 2. Samples size required to detect treatment effects for ATP/O2 ratio from MRS/OS and reactive hyperemia index (RHI) from PAT in crossover design.

7.4 Random Assignment / Study Arm Assignment

Investigational drug services will be responsible for randomization of participants into the two treatment groups.

7.5 Blinding

Study participants, investigators, and research coordinators will be blinded to the study. The investigational drug pharmacy will receive a receive treatment/placebo designation in a sealed envelope on the day of study drug/placebo initiation. Subjects will not be told of their assignment. Early unblinding will occur to protect patient safety in the event of an adverse event that the investigator believes may be relate to treatment.

7.6 Data Analysis

7.6.1 Primary Analyses

The major assumption in the cross-over trial is that there is no carryover effect of first period into the second period. The assumption is that a 3-week washout will be sufficient

to eliminate any potential carryover effect due to glutamine. The statistical model we assumed for continuous data from the 2 × 2 crossover trial:

Design	**Period 1**	**Period 2**
Sequence Placebo-Gln	A	B
Sequence Gln-Placebo	B	A

From this design we construct these differences for every patient and compare the two sequences with respect to these differences using a two-sample paired t test. Thus, we are testing:

$$H_0 : \mu_{AB} - \mu_{BA} = 0$$

Where: $\mu_{AB} - \mu_{BA} = 2(\mu_A - \mu_B)$

So testing $H_0 : \mu_{AB} - \mu_{BA} = 0$, is equivalent to testing:
$H_0 : \mu_A - \mu_B = 0$

7.6.2 Secondary Analyses

We will test for difference in each marker of metabolic response profile, oxidative stress, and albuminuria in a similar fashion as above. Similar to above, we will use a two-sample paired t-test testing the difference in means comparing treatment to placebo.

8.0 HUMAN SUBJECTS CONSIDERATIONS

The procedures in the study and model informed consent forms for subjects are to be reviewed by the UW Human Subjects Division (HSD).

Subsequent to initial review and approval, the HSD will review the protocol at least annually. The Investigator will make safety and progress reports to the IRB at least annually, and within three months of study termination or completion. These reports will include the total number of participants enrolled in the study, the number of participants who completed the study, all changes in the research activity, and all unanticipated problems involving risks to human subjects or others.

8.1 Informed Consent

Informed consent will be obtained from each study participant. Consent forms will include the purpose of the study, description of procedures, list of risks and benefits, assurance of confidentiality, assurance of withdrawal without prejudice, description of reimbursement for trial participation ($125 plus parking and bus fare reimbursement), and a telephone number for answering questions about the research. Patients are also given information regarding their right to privacy for personal health information based on HIPAA regulations, and the procedures for data sharing using de-identified data will be explained. Participants will be provided with a copy of their informed consent forms if they are willing to receive them.

8.2 Risks

The following stated risks will be reflected in the informed consent form and communicated orally to prospective participants at the study site at the time of obtaining consent.

Potential loss of confidentiality is a risk for all study participants, which is address by procedures at the coordinating center described below.

All patient will have a blood draw regularly during the study for a total of four blood draws. The risks of phlebotomy include bleeding/bruising, infection, and discomfort.

For the MRS study, participants may be at risk if they have implanted or non-removable magnetic objects within or on the body, or if loose magnetic items are attracted to the magnet while the subject is near or in the magnet bore. We carefully screen subjects to exclude anyone who has implanted or attached magnetic items. In addition, access to the magnet is strictly controlled and all magnetic objects are kept away from the magnetic field. There is no intravenous contrast used in the MR study.

During the MR study, subjects will have blood pressure cuff inflated to 50 mm Hg over systolic blood pressure. This will remain in place for 12 minutes, increasing risk of thrombosis. To decrease the risk of this, subjects will be screened for past history of deep venous thrombosis, pulmonary embolism, or history of hypercoagulability. Family history for deep venous thrombosis, pulmonary embolism, or history of hypercoagulability will also be obtained.

8.3 Benefits

There may be no direct benefits to participants in this study, however, participants and others may benefit in the future from information learned from this study. Specifically, information learned in this study may lead to the development of a safe and effective intervention that reduce risk of cardiovascular events and risk of disability in persons with CKD.

8.4 Compensation

Participants will be compensated for their time and effort in this study, and/or be reimbursed for travel to study visits and time away from work. Reimbursement amounts will be specified in the study informed consent forms.

8.5 Confidentiality

1. All study-related information will be stored securely at the Kidney Research Institute (KRI) at the University of Washington in Seattle. Access to the KRI is protected by individual unit keys to the elevator, the main institute, and individual offices to prevent tampering or unauthorized access. The use of a Local Area Network (LAN) provides two additional levels of security in the unlikely event of an unauthorized user finding a

machine unattended or unlocked. The LAN restricts access to the Database files via passwords, and the Database itself requires a password before files may be opened.

All study participants are assigned a unique identifier that does not contain identifiable information.

2. All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in areas with access limited to study staff. All laboratory specimens, reports, study data collection, process, and administrative forms will be identified by a coded number to maintain participant confidentiality. All local databases will be secured with password-protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link participant ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access. Participant's study information will not be released without the written permission of the participant.

8.6 Study Discontinuation

The study also may be discontinued at any time by the IRB.

9.0 LABORATORY SPECIMENS AND BIOHAZARD CONTAINMENT

9.1 Local Laboratory Specimens

As described in section 5, the following types of specimens will be collected for testing at the Kidney Research Institute local laboratory. If the participant provides informed consent, his/her specimen will be stored in the KRI repository for additional studies (see section 9.2):

- Fasting blood samples – approximately 23ml of blood per visit at baseline visit and post-intervention visits during each period of the study. During the study a total of approximately 69 mL will be collected per subject.

Local laboratories will perform Chemistries and pregnancy testing.

9.2 Reference Laboratory Specimens

- Blood for metabolomics testing. Aliquots of EDTA preserved blood will be stored at -80°C and sent to Emory University (c/o Dr. Ziegler). All samples will be de-identified.
- Blood for integrated redox state testing will be collected at baseline and post-intervention visits. A 18-, 21-, or 23-guage butterfly needle will be used to draw up 3mL of blood into a 5ml syringe. Enough blood will be immediately placed in a microcentrifuge tube containing 150µL of preservative solution to attain a total volume of 1.5mL. These samples will be frozen at -80°C and sent in bulk to Emory University (c/o Dr. Jones. See reference for more

details: Jones DP. Measuring the poise of thiol/disulfide couples in vivo. *Free Radical Biology and Medicine.* 47(2009):1329-38.)

- Blood for F2-isofuran and F2-isoprostane assays will be frozen at -80°C and sent in bulk to Vanderbilt University (c/o Dr. Jorge Gamboa).

Purpose	Sample Type	Maximum Amount per Sample	Number Samples	Total Amount
Baseline/Post-intervention	Blood	-	-	23/46 mL
Venous lactate (3 mL grey top)	Blood	3 mL	3	9 mL
Renal Panel, triglycerides (green top)	Blood	4 mL	3	12 mL
F2 isoprostanes/isofurans, metabolomics (EDTA)	Blood	10 mL	3	30 mL
Redox testing (N & S tubes)	Blood	3 mL	3	9 mL
CBC (3ml lavender top)	Blood	3 mL	3	9 mL
Total blood collected during study →				69 mL
Baseline/Post-intervention	Urine	-	-	n/a
Urine Creatinine (24 hour)	Urine	n/a	2	n/a
Urine albumin (24 hour)	Urine	n/a	2	n/a
Urine Urea Nitrogen (24 hour)	Urine	n/a	2	n/a
Urine pregnancy	Urine	10 mL	1	10 mL

9.3 Specimen Storage and Possible Future Research Testing

Study site staff will store all blood and urine collected in this study at least through the end of the study. In addition, study participants will be asked to provide written informed consent for their blood and urine specimens to be stored in the KRI repository after the end of the study for possible future testing. The specimens of participants who do not consent to long-term storage and additional testing will be destroyed at the end of the study.

10.0 REFERENCES

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