Cross-over Randomized Controlled Trial of COenzyme Q10 or Nicotinamide Riboside in Chronic Kidney Disease (CoNR trial)

Sponsored by:

National Institute of Diabetes and Digestive and Kidney Diseases

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Randomized controlled trial of Coenzyme Q10 and NR in chronic kidney disease

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LIST OF ABBREVIATIONS AND ACRONYMS

AE	Adverse Event
ATPmax	Muscle mitochondrial phosphorylation capacity by ^{31}P Magnetic Resonance Spectroscopy (MRS)
CKD	Chronic kidney disease
EAE	Expedited Adverse Event
ETC	Electronic Transport Chain
EC	ethics committee
eGFR	Estimated Glomerular Filtration Rate
FDA	(United States) Food and Drug Administration
GCP	Good Clinical Practices
IDS	UW Investigational Drug Services
IRB	Institutional Review Board
KRI	Kidney Research Institute
LDMS	Laboratory Data Management System
LL	Local Laboratory
MRS	^{31}P Magnetic resonance spectroscopy
NAD+	
NIDDK	United States) National Institute of Diabetes and Digestive and Kidney
NIH	(United States) National Institutes of Health
NR	Nicotinamide Riboside
ROC	Regulatory Operations Center
SAE	Serious Adverse Event
SUSAR	Suspected Unexpected Serious Adverse Reaction
SOP	Standard Operating Procedures
SSP	Study Specific Procedures
TA	Tibialis anterior leg muscle
TCA	Tricarboxylic acid (Krebs) cycle

Cross-over Randomized Controlled Trial of COenzyme Q10 or Nicotinamide Riboside in Chronic Kidney Disease (CoNR trial)

SCHEMA

Purpose:	Study the impact of oral nicotinamide riboside vs CoQ10 on aerobic capacity, muscle performance and mitochondrial metabolism among persons with moderate-severe CKD.
Design:	**Randomized, placebo controlled cross-over trial**
Study Population:	Adults age 30-79 with moderate-severe CKD eGFR by CKD (EPI equation) < 50ml/min/1.73m^2
Study Size:	**30 (10 in each of 3 arms).**
Treatment Regimen:	- Nicotinamide riboside 1000mg daily (250mg - 2 tablets twice daily) - Coenzyme Q10 1200mg daily (600mg – 1 tablets twice daily) - Placebo (sugar pill)
Study Duration:	1.5-2 year

Primary Objectives:

- To test if NR or CoQ10 supplementation improves aerobic capacity by cycle ergometry testing compared with placebo.
- To test if NR or CoQ10 improves muscle work efficiency by bicycle ergometry.

Secondary Objectives:

- To test if NR or CoQ10 improves peripheral blood monocyte bioenergetics health.
- To test the effects of oral NR or CoQ10 supplementation on metabolic response profile.
- To test if NR or CoQ10 supplementation reduces inflammatory and oxidative stress biomarkers.
- To test if NR or CoQ10 improves subjective fatigue, self-reported physical function, and heart failure symptoms.

Exploratory objective:

- In-vivo mitochondrial energetics by ^{31}Phosphorus Magnetic Resonance Spectroscopy (MRS) investigating phosphorylation capacity (ATPmax)

Study Sites:

- University of Washington Medical Center
- Fred Hutchinson Cancer Center – Prevention Center
- Kidney Research Institute (KRI): Harborview Medical Center, KRI at Northwest Kidney Centers-Havilland, KRI at UWMC

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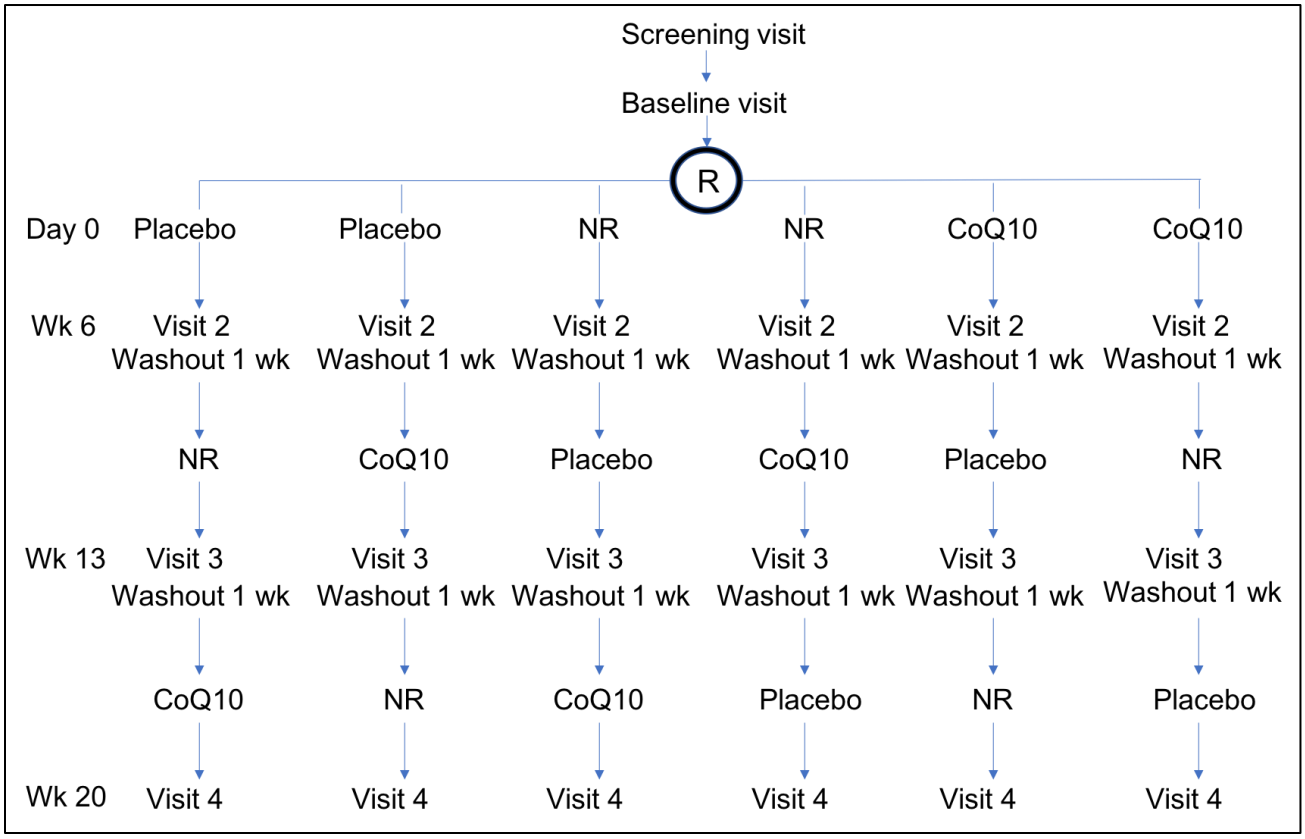
SCHEMA



1.0 INTRODUCTION

1.1 Background and Prior Research

Sarcopenia is a common complication of chronic kidney disease. Sarcopenia is defined by decreased muscle mass or function and is central to the frailty phenotype that is associated with disability, hospitalization, and death. Studies have shown that altered metabolism in CKD leads to well-described catabolic and pro-inflammatory processes with adverse consequences for lipid[1], amino acid[2], and protein metabolism[3,4] that are linked to overall energy metabolism[5]. These events consequently lead to an impaired insulin response, contributing to significant comorbidity among patients living with CKD.

Sarcopenia alters glucose metabolism. Insulin resistance in CKD can contribute to catapleurosis, which in turn increases reliance on the muscle amino acid pool for mitochondrial tricarboxylic acid-cycle respiratory fuel. These events culminate, ultimately, in muscle breakdown, sarcopenia, weakness and fatigue, increasing risk of mobility, disability and frailty.

CKD and sarcopenia alter metabolic pathways. Metabolomic profiling of CKD compared to controls under conditions of fasting and insulin clamp testing reveal marked aberrancy in CoQ10 biosynthesis pathways and niacinamide metabolism respectively. This suggests impaired mitochondrial metabolism as a major contributor in insulin resistance (figure 1). We have observed fasting-state pathway differences in ubiquinone (Coenzyme Q) biosynthesis among patients with CKD compared to controls, suggesting alteration in the integrity or efficiency of the electron transport chain (ETC) of the mitochondria, in turn predisposing to mitochondrial oxidant production resulting in insulin resistance[6,7].

Deficiency in CoQ10 levels has been associated with oxidative stress and impaired oral glucose tolerance in pre-clinical models[8]. This may explain the observed uncoupling of muscle mitochondrial oxidative phosphorylation and oxidative stress in CKD[9-11]. CoQ10 administration in advanced kidney disease patients improved oxidative stress with reduction in isofuran levels (products of lipid peroxidation)[11]. However, little is known about its effects on oxidative stress and mitochondrial function via assessment of mitochondrial bioenergetics using high throughput respirometry assessments in moderate-sever CKD not requiring dialysis.

Nicotinamide Riboside (NR) is implicated in mitochondrial dynamics in those with CKD. NAD+ is important in mitochondrial biogenesis, bioenergetics and redox homeostasis through $NADP^+$. Indeed, administration of nicotinamide riboside (NR), a natural NAD precursor, has been shown to improve insulin sensitivity in obese mice, as well as increase NAD levels, leading to augmented mitochondrial sirtuin (SIRT3) activity and improve muscle endurance along with muscle mitochondrial content[12]. Among patients with CKD not treated with dialysis, insulin clamp testing revealed marked plasma abnormalities in pathways important for mitochondrial TCA cycle function and antioxidant capacity compared with controls. Disruption in insulin response was most notable for nicotinamide metabolism among participants with CKD compare to controls, with a markedly blunted decrease in nicotinamide after insulin challenge. These findings implicate disruption in niacinamide metabolism as a potential mechanism underlying impaired insulin resistance and mitochondrial metabolism in CKD.

Here we propose a phase 2 placebo controlled randomized cross-over trial of nicotinamide riboside and Coenzyme Q10 in CKD investigating the impact on both metabolic and functional consequences using targeted metabolomics, assessment of mitochondrial bioenergetics and bicycle ergometry.

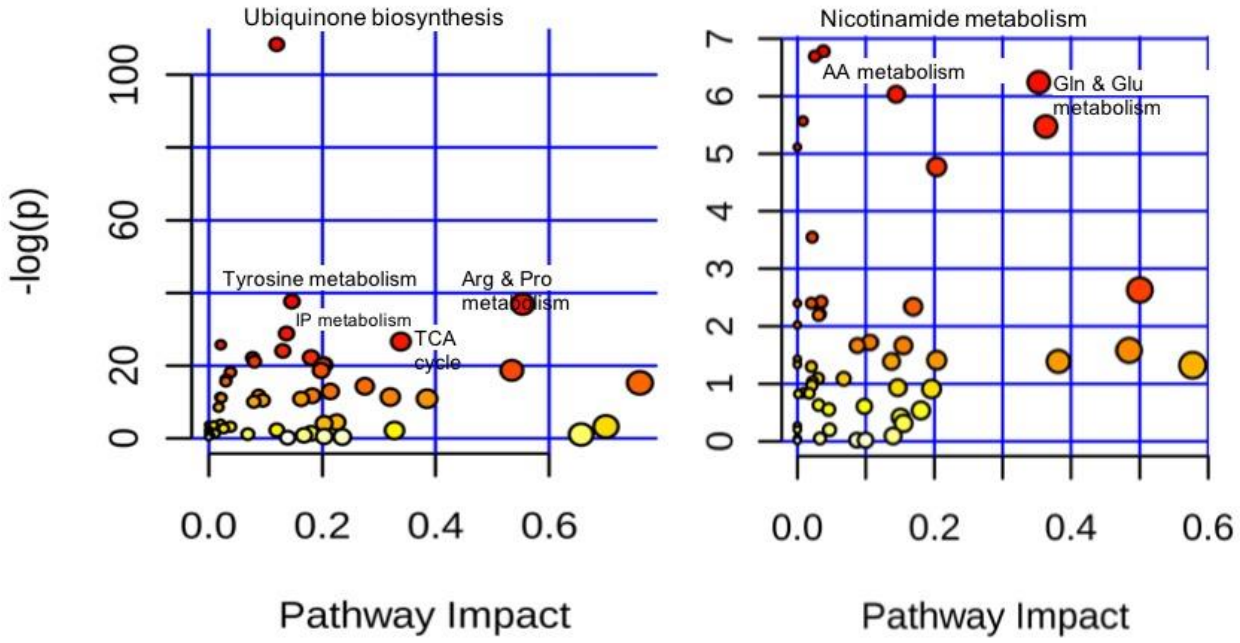


Figure 1. Left panel: Fasting-state metabolic pathway analysis comparing CKD to controls. Right panel: Metabolic pathway differences with insulin challenge comparing CKD to control.

1.2 Rationale

Sarcopenia is common in CKD patients and is associated with adverse metabolic and clinical outcomes. Existing evidence and our preliminary data suggest that mitochondrial dysfunction is a key mechanism underlying sarcopenia in CKD. However, major gaps exist in our understanding of these processes and their physical and social impacts. Altered CoQ10 and NR have both been implicated in mitochondrial dysfunction. Preliminary evidence suggests supplementation may ameliorate these altered metabolic processes. Addressing these knowledge gaps is necessary to shed new light on the pathophysiology of sarcopenia in CKD and suggest future interventions that reduce morbidity and mortality.

1.3 Abstract

Skeletal muscle dysfunction (sarcopenia) is an under-recognized target organ complication of CKD with substantial adverse clinical consequences of disability, hospitalization, and death. Sarcopenia in this proposal is defined by impaired metabolism and physical function associated with decreased skeletal muscle mass or function. Skeletal muscle tissue relies on mitochondria to efficiently utilize oxygen to generate ATP. Impaired mitochondrial energetics is a central mechanism of sarcopenia in CKD. Coenzyme Q10 and Nicotinamide Riboside deficiencies have been implicated in altered mitochondria function, but greater knowledge is needed. We hypothesize that supplementation with oral CoQ10 and

NR will enhance mitochondrial function measured through aerobic capacity, muscle work efficiency, and metabolic profile using cycler ergometry and targeted metabolomics. Secondarily, we seek to describe the impact of these interventions on ex-vivo measures of mitochondrial metabolism, biomarkers of oxidative stress, inflammation and functional outcomes of muscle efficiency by cycle ergometry as well as patient reported fatigue among persons with chronic kidney disease. Lastly, in-vivo muscle mitochondrial phosphorylation capacity by ^{31}P Magnetic resonance spectroscopy (MRS) will test exploratory aspects and descriptive measures of mitochondrial function.

2.0 STUDY OBJECTIVES AND DESIGN

2.1 Primary Objectives

The primary objectives of this study are to:.

- To test if NR or CoQ10 supplementation improves aerobic capacity by cycle ergometry testing compared with placebo.
- To test if NR or CoQ10 improves muscle work efficiency by bicycle ergometry

2.2 Secondary Objectives

The secondary objectives of this study are to:

- To test if NR or CoQ10 improves peripheral blood monocyte bioenergetics health.
- To test the effects of oral NR or CoQ10 supplementation on metabolic response profile.
- To test if NR or CoQ10 supplementation reduces inflammatory and oxidative stress biomarkers.
- To test if NR or CoQ10 improves subjective fatigue, self-reported physical function, and chart failure symptoms.

2.3 Study Design

Overview of study design: This is a placebo-controlled, double-blind crossover trial with 3 arms: placebo, NR, and CoQ10. There are 2 phases 56 days each separated by a 1-week washout period.

A brief screening visit will assess eligibility criteria, and will include informed consent. Qualified participants will be randomly assigned (1:1) to 56 days of placebo followed by 7 days NR or CoQ10, followed another 7 day washout followed by 56 days of either NR or CoQ10.

Qualified participants will participate in at least 4 study visits, plus a screening visit. An optional ^{31}P MRS study visit to assess in vivo muscle mitochondrial phosphorylation capacity will be offered to those who qualify. Including the separate MRS visit will increase the total visits to 8. Study outcomes will be assessed at each of these 4 major visits.

Subjects will receive 1000mg of Nicotinamide riboside (Tru Niagen) or coenzyme Q10 (1200mg/day) for 8 weeks. This dose was chosen based on a review of studies in the literature evaluating biological activity. Placebo will consist of identical appearing tablet.

Objectives	**Population**	**Exposure**	**Design**	**Outcome**
Primary- To test if NR or CoQ10 supplementation improves aerobic capacity and muscle performance	Mod-severe CKD with eGFR < 50 mL/dL (N=30)	Randomized order CoQ10 or NR or Placebo	Placebo-controlled double-blind control crossover	VO2max and Work efficiency
Secondary- To test NR or CoQ10 on descriptive measures of mitochondrial and metabolic functioning	Mod-severe CKD with eGFR < 50 mL/dL (N=30)	Randomized order CoQ10 or NR or Placebo	Placebo-controlled double-blind control crossover	-monocyte bioenergetics (Reserve capacity) -ATPmax (MRS Imaging) -Blood metabolomics & biomarkers -Fatigue & Heart questionnaires

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. Additional patients may be recruited from nephrology clinics in the University of Washington System including Harborview Medical center, and University of Washington Medical Center.

Participants will be selected for this study from the indicated populations 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

- Adults between 30 and 79 years of age
- Diagnosis of moderate-severe CKD, defined in this study as an estimated glomerular filtration rate (eGFR) of <50ml/min/1.73m^2 using the Chronic Kidney Disease Epidemiology Collaboration equation
- 6 minute walking distance<500meters

3.2 Exclusion Criteria

- Pregnant
- On chronic dialysis
- Expectation to start dialysis within 6 months
- Insulin dependent diabetes
- Severe anemia: hemoglobin <8gm/dL
- Hyperkalemia: K>5.7
- Kidney transplantation
- Weight >300 lbs
- HIV
- End stage liver disease with cirrhosis
- Oxygen-dependent COPD
- Unable to walk unassisted from room to room in own house
- Institutionalization, or inability to consent
- Use of immunosuppressive medications (i.e. steroids, calcineurin inhibitors)
- Malignancy requiring active treatment or currently under surveillance at the discretion of the investigator
- Pacemaker
- Current participation in another interventional trial
- Non-English speaking
- Hospitalization for heart attack, stroke, or unstable cardiac chest pain in last 3 months (e.g. myocardial infarction, unstable angina, cerebrovascular accident)
- Any severe medical condition that the investigator feels would disqualify a patient from aggressive exercise.
- Baseline systolic blood pressure >170 or diastolic blood pressure >100
- Persistent or permanent uncontrolled arrhythmia at the discretion of the investigator.

3.3 Recruitment Process

Study coordinators will screen records of previous study participants who have agreed to future contact and UW specialty clinics based on the aforementioned criteria in 3.1 & 3.2.

Previous Studies:

- Participants enrolled in the MEND and the Seattle Kidney Study have documented consent approving contact regarding participation in future studies.

UW Clinics:

- Additional sources of recruitment will include the nephrology clinics in the University of Washington System (University of Washington Medical Center, and Harborview Medical Center), community based nephrology practices, and self-referrals from the Kidney Research Institute Community Connection website.

3.4 Co-Enrollment Guidelines

Study participants will not be allowed to enroll in other clinical trials, as the effect of Coenzyme Q10 and Nicotinamide Riboside 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 3 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 mid-treatment phone call 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):

Participants will be randomized to in treatment order to receive the following medications for 6 weeks plus an additional 1 week in case of logistical difficulties with scheduling study visits.

- **Nicotinamide Riboside** (Tru Niagen by Chromodex) at 1000mg/day in the form of two 250mg tablets twice daily.
- **Coenzyme Q10** will be administered at 600mg twice daily.
- **Placebo** will consist of identical appearing pill. This will be a double dummy with 4 tablets appearing identical to NR and 2 tablets appearing identical to CoQ10.

Randomized Tx Order 1st, 2nd, 3rd	**Total Dosage QD**	**Regimen**	**Duration**
Nicotinamide Riboside	1000 mg	250 mg tabs x2 BID	6 weeks (+/- 7 days)
Coenzyme Q10	1200 mg	600 mg tabs BID	6 weeks (+/- 7 days)
Placebo	N/A	BID	6 weeks (+/- 7 days)

4.2 Treatment/Product/Intervention Supply and Accountability

The University of Washington Investigational Drug Services (IDS) will manage the study drugs. IDS will manage treatment randomization. Study coordinators will alert IDS before a scheduled baseline and subsequent visits and pick up the study drugs for administration.

The IDS 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 midway through each treatment arm 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 36 participants may be recruited to compensate for non-adherence or drop-out with the goal of 10 individuals completing in each treatment arm. Participants will be asked to bring in unused study drugs at each visit to return to IDS.

4.4 Toxicity Management

Currently, the mechanism whereby Coenzyme Q10 and Nicotinamide Riboside may cause harm or toxicity is unknown. There have been studies demonstrating slightly decreasing hemoglobin and potassium in healthy controls treated with NR[13]. In the event of moderate or serious adverse effects (as defined in section 6.2) requiring treatment for such conditions as nausea, vomiting, or abdominal pain 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 Coenzyme Q10 and Nicotinamide Riboside 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 procedures.

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. **All participants will be instructed to avoid altering their level of habitual physical activity during the trial.** Participants who qualify and agree to ^{31}P MRS will receive an additional study visit at UWMC lasting 1-2hrs at baseline and after each treatment arm. Presented below is additional information on visit-specific study procedures. Detailed instructions to guide and standardize all study procedures will be provided in the section titled study procedures/sample collection below.

Screening visit/practice bicycle visit

The screening visit will take place within 28days of the baseline 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. The screening visit will occur at the prevention center. Study coordinators will obtain informed consent prior to any data acquisition or sample collection

Screening visit procedures:

- Verification of inclusion and exclusion criteria as outline in sections 3.1 & 3.2 including
- Vital signs and anthropometrics
- **6-minute walk: Participants able to walk 500 meters or more during 6 minutes will be excluded from the study**
- *Screening labs (if 6 minute walk <500meters or less):* A renal panel & Complete blood count (CBC)
- Bicycle ergometry practice: The exercise trainer will help familiarize the participant with the bicycle to mitigate significant training effects due to lack of exposure to the equipment
- Questionnaires will be handed out to be returned at the baseline visit. Participants may also choose to complete these at the baseline or online via RedCap ITHS
    - Medical History Questionnaire
    - Kansas City Cardiomyopathy Questionnaire

Eligibility for the study will be at the discretion of the principal investigator or study clinician after review of the pertinent labs and inclusion/exclusion criteria.

Baseline visit

The baseline visit will be the same as visits 2-4 with an additional of medical history and anthropomorphic testing. Subject will undergo a baseline evaluation including a detailed assessment of demographics, smoking history, medical history, medication inventory, vital signs and anthropometrics (height, weight, waist to hip ratio). A pregnancy test will be performed on all women of childbearing potential. If the subjects meet the eligibility criteria the study coordinator will contact the subjects' primary physician regarding the subjects' involvement in the trial.

The study drug dispensed at Baseline visit and Visits 2, 3, 4.The participant will receive either placebo or NR or Coenzyme Q10 from the Investigational Drug Service (IDS) and instructed on how to appropriately administer the medications. He or she will be instructed to take the medication daily starting on the day after the study visit.

Baseline visit and Post-treatment visits (2, 3, 4)

The participant will have been required to fast for at least 6 hrs prior to each visit and abstain from caffeine or smoking before bicycle ergometry testing. Patient should avoid exercise 48hours prior to cycle ergometry. Treatment will be dispensed after cycle

ergometry at baseline and post-treatment visits 2,3 and 4. The following data will be obtained:

- Vital signs.
- Fasting blood collection for banked plasma and peripheral blood mitochondrial energetics.
- Anthropometrics (height and weight and waist to hip ratio)
- Urine pregnancy test will be given to women of child-bearing potential

- Resting energy expenditure (REE) and cycle ergometry will be performed at the Prevention center.

- The optional MRS and Muscle Fatigue tests will occur at the diagnostic imaging center at UWMC at baseline and in the same week of visits 2, 3 and 4 while patient remains on study drug. Patient should wait avoid heavy exercise for 48hrs before MRS.

- Questionnaires will be administered (PROMIS fatigue short form 17, PROMIS physical function short form 20a, and Kansas city heart failure questionnaire)

Procedures/Sample collection:

An overview of the study visit and procedures schedule is presented in Appendix I. Presented below is additional information on visit-specific study procedures and Table 4.

Physiologic measurements from cycle ergometry: The main outcome of this study is aerobic capacity (VO2max) using cycle ergometry. Secondary outcome is muscle performance using measures of total work and work efficiency which will be obtained by cycle ergometry using standard protocol measuring oxygen uptake starting at 0 watts (W) at 60 rotations per minute (rpm) increasing by 30W every 3 minutes until volitional exhaustion adapting a prior protocol used in CKD patients[14]. Approximately 30W of activity is required for grocery shopping and 60W is necessary for household chores. Work efficiency will be calculated as the work performed/energy expended (VO2) above that in cycling 0 watts x 100%. The minimum criteria for a successful efficiency calculation will be a rating of perceived exertion (RPE) of 13 (scale 6-20) or 75% of predicted maximal heartrate. The minimum goal for successfully achieving a VO2max is a RPE of 17 or respiratory exchange ratio≥1**.** Recovery will consist of pedaling 5 minutes at 10 watts and 60rpm. **Participants should be fasting for at least 6 hrs prior to this test and not exercise for 48hr prior to this test.**

PBMC mitochondrial bioenergetic stress test: Monocytes are specifically selected due the strong correlation of their bioenergetics properties with skeletal muscle and cardiac bioenergetics in the primate model[15]. Blood samples will be process using an established protocol[16,17]. Sample collection should be coordinated in advance with Dr. David Marcinek (dmarc@uw.edu).

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 (c/o Jorge Gamboa: jorge.gamboa@Vanderbilt.Edu).

Blood Collection:

- NAD samples will be obtained from fasting whole blood collected in one 2.7ml Blue (sodium citrate tube). From the blue tube, after inversion mixing, 0.5ml will be pipetted into 2 cryotubes **on dry ice.** Samples will be immediately frozen on dry ice and placed in -80C ASAP. Samples will be sent to Dr. Laura Shireman in clinical pharmacology (shireman@uw.edu).

- EDTA Plasma samples will be sent to University of California at Davis West Coast Metabolomics for liquid chromatography high-resolution mass spectrometry (LC-MS) for targeted metabolomics analysis. We will perform additional transcriptomics using mRNA samples obtained from Paxgene tubes which will also be sent to West Coast Metabolomics.

- Paxgene mRNA will be obtained at baseline and post-intervention visits.

- 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 will be performed at the *KRI Core Research laboratory.*

Urine collection: **Spot urine** for metabolomics will be collected at baseline and after each treatment period (visits 2-4).

Mid-intervention visits/phone calls:

All subjects will receive a phone call by study coordinators 4 weeks (mid-way) into each intervention arm to confirm patients are taking their treatment/placebo medication in order to ensure adherence. They will also receive a reminder of their subsequent visits.

Week 6, 13, 20 (Visit 2, 3, 4):

The prevention center and Marcinek lab will be contacted to arrange the primary outcome of resting energy expenditure, aerobic capacity and secondary outcomes of monocyte mitochondrial energetics at the end of each treatment period. Patients will also have fasting blood collection and return a 24hr urine collection. If qualified, they will also receive a repeat MRS at UWMC during the same week. **Patient remain on treatment during these visits to ensure drug effect.**

Washout (week 7 and 14):

After the second visit, the participant will undergo a 1 week washout period prior to initiating the subsequent treatment arm for another 6 week period.

Optional Procedures:

A subset of participants (N=20) may choose to undergo the following muscle imaging procedures. Subjects will go to the UW DISC lab for testing +/- 3 days from each scheduled visit (Baseline – visit 4).

Magnetic Resonance Spectroscopy: Leg MRS will serve as a secondary outcome and optional. All participants will be offered the option to receiving leg MRS. As mentioned above, only 4 MRS will be obtained as part of this study (at Baseline, visit 2, 4 and 6). MRS will be used to measure phosphorylation capacity (ATP_{max}) tibialis anterior leg skeletal muscle.[18] 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}.[19] Scheduling of this test should be coordinated with Sophia Liu (sophia21@uw.edu).

Muscle Fatigue: We will test muscle fatigability at each follow-up visit. We will test the TA (tibialis anterior) leg 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.

				Treatment arm 1 (6wk +/-7d)		Treatment arm 2 (6wk +/-7d)		Treatment arm 3 (6wk +/-7d)	
	Screen Day -28	Baseline	Optional MRS visit	Day 1	Visit 2 Week 6 (+/-7d)	Wk 8	Visit 3 Week 13 (+/-7d)	Wk 15	Visit 4 Week 20 (+/-7d)
Time	1-1.5hr	3hr	1-2hr		3hr		3hr		3hr
Location	PC	PC	UWMC		PC		PC		PC
Compensation	$10	$50	$50		$100		$100		$100
Consent	X								
Practice bicycle	X								
Screening labs	X								
Medical history		X							
Medicine inventory		X							
Vital signs/Anthro	X	X			X		X		X
Product dispensed		X			X		X		
Assess AEs					X		X		X
Bicycle Vo2max/work		X			X		X		X
REE		X			X		X		X
PBMC bioenergetics**		X			X		X		X
31P MRS (subgroup)*			X		X (UWMC)		X (UWMC)		X (UWMC)
mRNA (paxgene)		X			X		X		X
Blood		X			X		X		X
Urine		X			X		X		X
Drug level/NAD		X			X		X		X
PROMIS Fatigue/PF PRO	Handout	X			X		X		X

Table 1. Table of study treatments and procedures. Abbreviations: PC – Prevention Center.
*Performed at UWMC. ** Performed in Marcinek lab. Call lab to arrange collection and processing at same time as the bicycle VO2 testing.

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, 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 NR or CoQ10 supplementation versus placebo. As part of the study there will be a 1-week washout between treatment and placebo arms.

7.2 Endpoints

Primary endpoints are change in aerobic capacity using cycle ergometry. Secondary endpoints are change in muscle work efficiency, metabolic response profile and markers of oxidative stress and inflammation.

7.2.1 Primary Endpoints

Consistent with the primary study objective to test NR and CoQ10 improving aerobic capacity, the following endpoints will be assessed:

- *Physiologic measurements from cycle ergometry:* The main outcome of this study is aerobic capacity (VO2max) using cycle ergometry.

7.2.2 Secondary and Exploratory 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:

- Muscle performance using measures of total work and work efficiency which will be obtained by cycle ergometry using standard protocol measuring oxygen uptake starting at 0 watts (W) at 60 rotations per minute (rpm) increasing by 25W every 3 minutes until volitional exhaustion adapting a prior protocol used in CKD patients[14]. 25W of activity is required for grocery shopping and 50W is necessary for household chores. Work efficiency will be calculated as the work performed/energy expended (VO2) above that in cycling 0 watts x 100%. The minimum criteria for a successful efficiency calculation will be a rating of perceived exertion (RPE) of 13 (scale 6-20) or 75% of predicted maximal heartrate. The minimum goal for successfully achieving a VO2max is a RPE of 17 or respiratory exchange ratio≥1.

- PBMC mitochondrial bioenergetic stress test: Monocytes are specifically selected due the strong correlation of their bioenergetics properties with skeletal muscle and cardiac bioenergetics in the primate model[15]. Blood samples will be process using an established protocol[16,17]. Monocyte will be isolated using magnetic-activated cell sorting (MACS) and CD14+ microbead selection. Bioenergetics analysis will be performed using the Seahorse Biosciences XF24 extracellular flux analyzer per protocol[20]. Max-OCR (maximal respiration) is calculated after the addition of FCCP, a mitochondrial uncoupler. RC is calculated as the difference of Max-OCR and Basal-OCR (Figure 2).

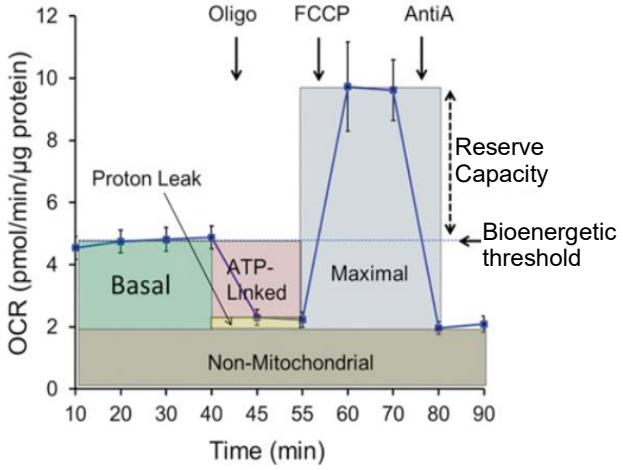


Figure 2. Illustration of monocyte bioenergetic stress test to determine reserve capacity. Abbreviation: oligo=oligomycin, AntiA=antimycin A, FCCP=uncoupler.

- Improvement in metabolic response profile suggested by:
    - *Change in targeted metabolomics profile using liquid chromatography high-resolution mass spectroscopy (LC-MS) using plasma and urine samples*
    - *Oxidative stress biomarkers:* Plasma F2-isoprostanes will be measured by GC-MS as described by Roberts and Morrow.
- Patient reported outcomes: Patient reported fatigue will be assessed using the PROMIS Fatigue Short form 17 and PROMIS Physical function short form 20a. Heart failure symptoms will be assessed using Kanasas City Heart Failure Questionnaire.

Exploratory Endpoints

- *Magnetic Resonance Spectroscopy:* Leg MRS will serve as a secondary outcome. As mentioned above, only 4 MRS will be obtained as part of this study (at Baseline, visit 2, 4 and 6). MRS will be used to measure phosphorylation capacity (ATP_{max}) tibialis anterior leg skeletal muscle.[18] 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}.[19]
- *Muscle Fatigue:* We will test muscle fatigability at each follow-up visit. We will test the TA 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.

7.3 Accrual, Follow-up, and Sample Size

The overall sample size for this project is 30. We anticipate recruiting 1-2 participants weekly for total study duration of 1.5-2 years (Table 2).

Order	N	Period 1	Period 2	Period 3
Sequence Placebo-CoQ10-NR (n=5)	10	A	B	C
Sequence Placebo-NR-CoQ10 (n=5)		A	C	B
Sequence NR-Placebo-CoQ10 (n=5)	10	B	A	C
Sequence NR-CoQ10-Placebo (n=5)		B	C	A
Sequence CoQ10-Placebo-NR (n=5)	10	C	A	B
Sequence CoQ10-NR-Placebo (n=5)		C	B	A

Table 2. Study design and sample size. Given the efficient cross-over design, 30 participants will receive each treatment by the end of the study.

Sample size was calculated based on values obtained from an standard deviation of the difference of VO2max on bicycle ergometry from a prior study of 2.1ml/kg/min. A total of 30 patients will enter this three-treatment crossover study. A total of 30 patients will enter this two-treatment crossover study. The probability is 80 percent that the study will detect a treatment difference at a two-sided 0.05 significance level, if the true difference between treatments is 2.1 (1 SD)ml/kg/min. This is based on the assumption that the standard deviation of the difference in the response variables is 2.1 ml/kg/min (Table 3). Sample size was calculated using the following sample size calculator: *http://hedwig.mgh.harvard.edu/sample_size/js/js_crossover_quant.html*

	VO2max Power	
Minimal detectable difference	**80% (n)**	**90% (n)**
0.5 SD	34	44
0.75 SD	16	21
1 SD	10	13

Table 3. Total samples size required to detect treatment effects for aerobic capacity bicycle ergometry from crossover design. Based on published study demonstrating the effect of a 12mo combined resistance and aerobic exercise program resulting in an increase of +5.7 ml/kg/min VO2peak with a standard deviation of the difference of 2. 1ml/kg/min.

7.4 Random Assignment / Study Arm Assignment

Investigational drug services will be responsible for randomization of participants into the two treatment groups. Every effort will be taken to have an appropriate placebo pill appearing identical to study agent.

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 1-week washout combined with a 2 month treatment will be sufficient to eliminate any potential carryover effect. The statistical model we assumed for continuous data from the 3 × 3 crossover trial. We will use ANOVA to estimate sequence effects, treatment effects, and period effects. Given the short half-life of these drugs, we estimate that the drug washout will occur over 1 week with any residual effects by 3 weeks. According to this model, the coefficient of the interaction term between the treatment and time represents the treatment effect.

Secondary Analyses

We will assess differences in plasma metabolites using a linear mixed model approach with random intercepts, in which the log-transformed metabolite will be regressed on treatment type (Placebo vs Coenzyme Q10 or NR), time point (pre vs post) and the interaction of the two. We will use metaboanalyst to conduct pathway enrichment analysis.

For differential analysis of transcriptomic data, paired analyses of pre- and post-treatment with NR or CoQ10 compared to pre- and post- treatment with placebo will be performed using Significance Analysis of Microarrays (SAM) method implemented in Multi Experiment Viewer (MeV) application (Saeed et al. 2006; Saeed et al. 2003). Genes differentially expressed between two groups with a q-value (False Discovery Rate) below 0.05 were considered significant.

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 ($325 or $525 if volunteering for MRS 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.

NR and CoQ10 have been deemed safe in studies of healthy adults[13]. Among anuric dialysis patients, high dose CoQ10 up to 1800mg was well tolerated[11] in most individuals. We will be using 1200mg daily in our study, well below the threshold for complaints of nausea and abdominal discomfort.

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.

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 60ml of blood per visit at baseline visit and post-intervention visits during each period of the study. During the study a total of approximately 245 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. All samples will be de-identified.
- 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).
- EDTA plasma samples and urine samples used for metabolomics and Paxgene samples for transcriptomics will be sent at the conclusion of the study to Dr. Bob Roshanravan at UC Davis for analysis by West Coast Metabolomics: (bob.roshanravan.wrk@gmail.com)

Tube	Size	Number of tubes	Aliquots for banking	Purpose
Blood Screening only:				
CBC (lavender) Renal panel (green)	3ml 4ml	1 1	none	Send to Qwest labs for hemoglobin and renal panel
Blood banking at baseline and after each treatment				
EDTA whole blood (lavender)	10ml	3	none	Fresh for Dave marcinek lab
EDTA plasma (lavender)	8ml	2	0.5ml x 10 1ml x 3	Metabolomics Lipids F2 isoprostanes/isofurans for Vanderbilt.
Serum (red)	6ml	1	0.5mlx6	biochemistry
ACD (citrated plasma) whole blood (blue top)	2.5ml	1	0.5ml x2 on dry ice	For NAD. Aliquot immediately onto tubes chilled on dry ice
Paxgene tube	2.5ml	1	2.5x1	Banking
Urine banking at baseline and at end of each treatment	10ml		1mlx5	Urine metabolomics Urine urea nitrogen Urine creatinine Urine ammonia

Table 4. List of blood and urine samples to be collected during study.

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.

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