STUDY PROTOCOL

NCT03223883

Aug 19, 2024

STUDY DESIGN

- **Study Subjects:** A total of 88 individuals (age 45-74 years) with stage IIIb and IV CKD will be recruited. Inclusion and exclusion criteria are shown in **Table 1** and discussed in the **Protection of Human Subjects** section. Estimated GFR will be calculated based on the 4- variable Modification of Diet in Renal Disease (MDRD) formula [1]. After obtaining informed consent, individuals with estimated GFR 15-45 mL/min/1.73m^2 who fulfill the inclusion and exclusion criteria will be included in the study. We chose an estimated GFR cut-off of <45 mL/min/1.73m^2 (stage IIIB) for the inclusion in the study based on data by Dr. Tamura that indicate the prevalence of cognitive impairment is significantly increased when estimated GFR is <50 mL/min/1.73m^2 [2]. All subjects will be required to have adequately controlled blood pressure (BP), as our hypothesis is that curcumin reduces vascular dysfunction by targeting non-traditional risk factors for CVD. Adequately controlled BP will be defined as BP <140/90 mmHg in accordance with the current Joint National Committee guidelines [3]. However, if the guidelines change for older adults without DM (based on the recent evidence from Systolic Blood Pressure Intervention Trial (SPRINT) [4]), then we would require a systolic BP <120 mmHg for those patients.

Table 1: Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
Age 45-74 years old	*Consuming a diet rich in curcumin or taking curcumin supplements in the past 12 months*
CKD stage IIIb and IV (estimated GFR: 15-45 mL/min/1.73m^2)	Life expectancy <1 year
BMI <35 kg/m^2 [5]	Pregnant, breastfeeding, or unwilling to use adequate birth control
Able to give informed consent	Uncontrolled hypertension
	Severe liver disease
	Severe congestive heart failure
	Hospitalization within the last 3 months
	Active infection or antibiotic therapy
	Immunosuppressive therapy within the last year

We will also screen for the use of supplements that may improve vascular function including vitamins C and E [6] and resveratrol [7]. Subjects who are taking these supplements but otherwise qualify for the study will be asked to undergo a 6-week wash-out period prior to participating in the study.

- **Experimental Design:** 88 subjects, recruited at the University of Iowa Hospitals and Clinics (UIHC) and The Iowa City VA Medical Center, will be randomized to receive either placebo or curcumin (2000 mg/ day) for 12 months and stratified randomization will be employed based on age ≥ 55 yeas [8-10] and baseline DM status [11]. Block randomization is an alternative for consideration. Study outcomes will be evaluated at baseline, 6 months, and 12 months (except cognitive function will be evaluated at baseline and 12 months only).

- **Curcumin supplementation:** Based on our preliminary data we will use a solid lipid curcumin particle (SLCP, Longvida, Verdure Sciences) at a dose of 2000 mg/d. Based on our preliminary data, we believe this will be an effective dose to improve vascular function in patients with CKD. Longvida will provide the supplement in addition to identical-looking placebo. The randomization table will be generated by Dr. You and provided to Longvida. Once a subject is randomized, Longvida will package and ship the study drug (curcumin or placebo). The package will only state “study drug” in order to guarantee blinding of all the study staff and participants. As we show in the **Preliminary Data** and **Protection of Human Subjects** sections, curcumin was well-tolerated with a small number of adverse events (mainly gastrointestinal discomfort).

- **Rational for the duration of the study:** In specific aim 2, we intend to evaluate whether curcumin improves cognitive function in patients with CKD. Although our preliminary data suggests that curcumin may improve cognition in as little as 12 weeks, we believe a longer duration is necessary to minimize practice effects from repeated cognitive testing [12,13]. Thus, the study duration will be 12 months. In addition, this longer duration will

enhance our ability to detect a benefit on vascular stiffness (specific aim 1). Large elastic artery stiffness reflects structural changes of the extracellular matrix in the vessel wall [14]. Based on this, the longer duration of study will allow the treatment to reverse these structural changes in the vasculature. The vascular measurements will be conducted at baseline, 6 months, and 12 months. In order to reduce practice effects, cognitive function will be measured at baseline and 12 months only.

- **Recruitment Clinics:** The trial will be conducted through CRU of the ICTS at UIHC. Patients will be recruited from 2 clinics with access to >1500 CKD patients from all over the Iowa State area including:

    1) UIHC Renal Clinic: The renal clinics at UIHC see approximately 3600 patient visits annually. At least 2500 patient visits are related to stage III/IV CKD. This is a similarly-sized practice to the prior proposal.

    2) Iowa City VA Medical Center: The VA renal clinics see approximately 1950 patient visits annually the majority of whom have underlying stage 3 and 4 CKD.

Based on the preliminary screening of our database at the UIHC, we expect at least 100 eligible patients per year. Notably, Dr. Jalal has been successful in recruiting patients with CKD to clinical trials, including single center, completing recruitment prior to end of funding period on corresponding grant applications. Additionally, UIHC has been a successful site of recruitment for clinical researchers at the University of Iowa (for example Bradley Dixon).

- **Detailed sessions:** The study design is illustrated in **Figure A**.

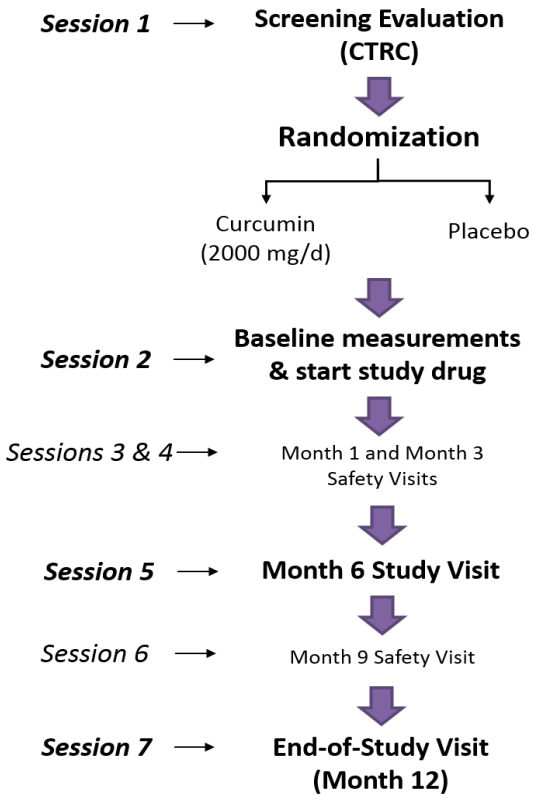
(All study procedures will be conducted at UIHC Clinical Research Unit (CRU))

Session 1: Informed consent and screening questionnaire for inclusion and exclusion criteria including confirmation of stage IIIb/IV CKD on 2 separate occasions in the last 6 months. All women in child-bearing age will undergo pregnancy test at this visit. In addition, all participants will complete a 7-day physical activity recall (International Physical Activity Questionnaire [15]).

Session 2 (baseline measurements after overnight fast):

- Blood pressure at rest
- Endothelial cell sampling for endothelial markers of inflammation and oxidative stress (eNOS and eNOS phosphorylated at Ser 1177, NF-κB, NAD(P)H oxidase, and nitrotyrosine)
- Blood sampling for fasting lipid profile
- Blood sampling for hemoglobin A_{1C} (for participants with DM)
- Blood sampling for levels of curcumin and its metabolites
- Blood sampling for basic metabolic profile and estimated GFR including cystatin C
- Blood sampling for circulating markers of inflammation and oxidative stress (CRP, IL-6, MCP-1, and oxLDL)
- Urine sampling (random) for albumin/creatinine ratio (ACR)
- Evaluation of EDD and EID by measurements of BA-FMD and nitroglycerin-mediated dilation (NMD)
- Aortic PWV
- Assessment of vascular function in response to vitamin C (ascorbic acid) infusion
- Administer NIH Toolbox Cognitive Battery
- Start study drug after this visit

Figure A: Study Design



Sessions 3 and 4 (month 1 and month 3 safety visits):

- Pill count
- Safety monitoring:
    - Completion of an adverse event questionnaire
    - Update medication list

Session 5 (month 6 study visit): will include the same measurements as Session 2 *except* for the NIH Toolbox Cognitive Battery. In addition, it will include the following:

- Physical activity questionnaire
- Pill count
- Safety monitoring:
    - Completion of an adverse event questionnaire
    - Update medication list

Session 6 (month 9 safety visit): identical to Sessions 3 and 4.

Session 7 (month 12 study visit- end of study): identical to Session 2 *including* the administration of the NIH Toolbox Cognitive Battery in addition to the following:

- Physical activity questionnaire
- Pill count
- Safety monitoring:
    - Completion of an adverse event questionnaire
    - Update medication list

OUTCOME MEASUREMENTS

- **BA-FMD (primary outcome):** BA-FMD will be measured at the CRU at UIHC by a trained research assistant using high-resolution ultrasonography (GE Healthcare, Inc.) as described originally by Celermajer *et al*. [16] and by our group [17-20]. Electrocardiogram (ECG) gated end-diastolic ultrasound images and Doppler flow of the brachial artery are acquired during baseline and FMD conditions. For FMD, reactive hyperemia is produced by inflating a pediatric forearm cuff around the forearm to 250 mmHg for 5 minutes followed by rapid deflation. Endothelial independent dilation (EID) is determined by measuring brachial artery dilation for 10 minutes after administration of sublingual nitroglycerin (0.4 mg). A commercially available software package (Vascular Analysis Tools 5.5, Medical Imaging Applications, LLC, Coralville, IA) is used to acquire and analyze ECG-gated brachial artery diameters. *The analysis of the images will take place at Dr. Seals's lab in Boulder.* Brachial artery dilation is determined as mm and % change from baseline [21]. Doppler blood flow velocity is recorded at baseline and for 2 minutes after cuff release. Shear rate will be calculated, and if group- or condition-differences exist, BA-FMD is adjusted accordingly. *We propose to use BA-FMD as opposed to ACh-FBF since BA-FMD is less invasive and considering that BA-FMD has been utilized extensively in patients with CKD [19,22-24].*

- **aPWV:** Large elastic artery stiffness will be determined by aPWV as previously described [19,25-27] , utilizing the Non-Invasive Hemodynamic Workstation (NIHem, Cardiovascular Engineering, Norwood, MA). The subject is supine in a quiet, dim, climate-controlled room. Blood pressure readings are obtained after 20-minute rest. A custom trans-cutaneous tonometer is placed at the carotid and femoral arteries to non-invasively assess aPWV. The distance from the supra-sternal notch to the carotid is subtracted from the distance between the two recording sites. aPWV= distance divided by time between the foot of waveforms recorded at each site. Similar to BA-FMD, the aPWV images will be analyzed by Dr. Seals's group.

- **Vitamin C (ascorbic acid) infusion:** The influence of oxidative stress on BA-FMD and aPWV will be determined by infusing a supraphysiological dose of ascorbic acid (American Regent Laboratories Inc.) or isovolumic saline. A priming bolus of 0.06 g ascorbic acid/kg fat free mass (FFM) dissolved in 100 mL of saline will be infused intravenously at 5 mL/min for 20 minutes, followed immediately by a "drip-infusion" of 0.02 g/kg FFM dissolved in 30 mL of saline administered over 60 minutes at 0.5 mL/min. Vascular measurements will be made at the end of the 20-minute bolus during the "drip infusion" when peak plasma concentrations of ascorbic acid occur. The difference in BA-FMD and aPWV during ascorbic acid *vs.* saline infusion will be taken as a measure of the modulation of EDD/stiffness by oxidative stress. This technique has been developed and well established by the Seals research group [28-31], which included Dr. Gary Pierce. Dr. Pierce directs the Translational Vascular Physiology Lab at UIHC CRU, where several protocols that utilize vitamin C infusion are ongoing. In addition, Dr. Chonchol, who has successfully performed and reported this technique in several clinical trials [20], will remain a co-investigator for the application.

- **Systemic markers of inflammation and oxidative stress:** High sensitivity CRP, IL-6, and MCP-1 will be measured as markers of inflammation. OxLDL will be measured as a marker of systemic oxidative stress. These markers will be measured on the serum or plasma by the UIHC clinical lab.

- **Endothelial cell sampling and cell protein expression:** This technique performed and described by our group of investigators [20,30,32-34] including Dr. Jalal (PI). Briefly, J-wires are advanced into a brachial artery and/or an antecubital vein and withdrawn, and cells are recovered by washing and centrifugation. Collected cells are fixed with 3.7% formaldehyde and plated on slides. After blocking nonspecific binding sites with 5% donkey serum (Jackson Immunoresearch), cells are incubated with the primary antibody. Then, cells are incubated with CY3-conjugated secondary antibodies (Research Diagnostics). Slides are systematically scanned to identify endothelial cells (positive VE-Cadherin), and nuclear integrity is confirmed using 4',6'-diamidino-2-phenylindole hydrochloride staining. Once endothelial cells with intact nuclei are identified, images are captured and then analyzed using Metamorph Software (Universal Imaging, Downingtown, PA) to quantify the intensity of CY3 staining (i.e., average pixel intensity). Values for each sample are reported as ratios of endothelial cell protein expression/human umbilical vein endothelial cell (HUVEC) to account for any variation in the staining procedure. Technicians are blinded to subject identity during the staining and analysis procedures. The following proteins will be evaluated: eNOS (BD Biosciences) and eNOS phosphorylated at Ser 1177 (BD Biosciences) as an indicator of eNOS activity [20,35], NF-κB (Santa Cruz) as a marker of pro-inflammatory signaling [32,36], and NAD(P)H oxidase (Upstate) [30,33,34] and nitrotyrosine (Abcam) [30,33,34] as markers of oxidative stress.

- Cognitive function assessment: The NIH Toolbox for the Assessment of Neurological and Behavioral Function was initiated by the NIH Blueprint for Neuroscience Research to develop state-of-the-art measurement tools to for the collection of cognitive data in large cohort studies [37]. The NIH Cognitive Toolbox is a validated multidimensional assessment of cognitive function domains, with minimal floor and ceiling effects, that enables us to evaluate (consistently) cognitive changes in response to our intervention [13]. The cognitive battery of the NIH Toolbox will be administered to our patients by a trained research assistant (blinded to subject randomization) at the CRU UIHC in a quiet and private exam room over approximately 45 minutes. The following table describes the test, subdomain, and tasks that will be applied:

Table 2: NIH Toolbox Cognition Battery Tests

Subdomain	Test name and Task description
Executive function and attention	Indicate direction of central arrow when flanking arrows are pointing in the same or opposite direction. (6 minutes)
Executive function category switching	Alternate sorting according to shape or color. (7 minutes)
Working memory	Repeat the order of items (2-8) according to size in 1 category then repeat the order of items according to size in 2 categories of items. (7 minutes)
Processing speed	Identify items images (side by side) as “same” or “not the same”. (2 minutes)
Episodic memory	Place pictures of individuals performing different tasks in the order they are shown. (9 minutes)
Written word pronunciation	Read printed words aloud. (6 minutes)
Auditory and word-visual picture matching	Match a single word to one of four pictures. (4 minutes)

- **Curcumin Levels:** These will be measured to evaluate the bioavailability of curcumin and its metabolites in CKD. The measurements will be conducted by Dr. Jelena Klawitter on a fee-for-service basis in the iC42 Laboratory in the UC-Denver Anschutz Medical Campus according to the method described by Cao *et al.* [38]. Curcumin (Acros Organics, Morris Plains, NJ), curcumin *O*-glucuronide and curcumin *O*-sulfate (Cell Mosaic, Worcester, MA), demethoxycurcumin and bisdemethoxy-curcumin (Sigma Aldrich), and tetrahydrocurcumin (ChromaDex, Irvine, CA) will be used in to perform liquid chromatography utilizing high-end mass spectrometry systems. The internal standard (IS) hesperetin will be purchased from 4C Pharma Scientific Inc. (ON, Canada). Heparin-treated human plasma, collected from the study participants and stored at -80 °C, will be used.

- **Clinical labs:** These include basic metabolic profile (and estimated GFR), cystatin C, ACR, Hemoglobin A_{1C}, and lipid profile will be measured by the UIHC clinical lab.

REFERENCES

1. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med 1999;130:461-70.

2. Kurella Tamura M, Wadley V, Yaffe K, et al. Kidney function and cognitive impairment in US adults: the Reasons for Geographic and Racial Differences in Stroke (REGARDS) Study. Am J Kidney Dis 2008;52:227-34.
3. James PA, Oparil S, Carter BL, et al. 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). JAMA 2014;311:507-20.
4. Group SR, Wright JT, Jr., Williamson JD, et al. A Randomized Trial of Intensive versus Standard Blood-Pressure Control. N Engl J Med 2015;373:2103-16.
5. Schroeder S, Enderle MD, Baumbach A, et al. Influence of vessel size, age and body mass index on the flow-mediated dilatation (FMD%) of the brachial artery. Int J Cardiol 2000;76:219-25.
6. Ashor AW, Siervo M, Lara J, Oggioni C, Afshar S, Mathers JC. Effect of vitamin C and vitamin E supplementation on endothelial function: a systematic review and meta-analysis of randomised controlled trials. Br J Nutr 2015;113:1182-94.
7. Carrizzo A, Puca A, Damato A, et al. Resveratrol Improves Vascular Function in Patients With Hypertension and Dyslipidemia by Modulating NO Metabolism. Hypertension 2013;62:359-66.
8. Yaffe K, Ackerson L, Kurella Tamura M, et al. Chronic kidney disease and cognitive function in older adults: findings from the chronic renal insufficiency cohort cognitive study. Journal of the American Geriatrics Society 2010;58:338-45.
9. Vaitkevicius PV, Fleg JL, Engel JH, et al. Effects of age and aerobic capacity on arterial stiffness in healthy adults. Circulation 1993;88:1456-62.
10. Mitchell GF, Parise H, Benjamin EJ, et al. Changes in arterial stiffness and wave reflection with advancing age in healthy men and women: the Framingham Heart Study. Hypertension 2004;43:1239-45.
11. Jalal DI, Decker E, Perrenoud L, et al. Vascular Function and Uric Acid-Lowering in Stage 3 CKD. J Am Soc Nephrol 2016.
12. Heaton RK, Akshoomoff N, Tulsky D, et al. Reliability and validity of composite scores from the NIH Toolbox Cognition Battery in adults. Journal of the International Neuropsychological Society : JINS 2014;20:588-98.
13. Weintraub S, Dikmen SS, Heaton RK, et al. The cognition battery of the NIH toolbox for assessment of neurological and behavioral function: validation in an adult sample. Journal of the International Neuropsychological Society : JINS 2014;20:567-78.
14. Palombo C, Kozakova M. Arterial stiffness, atherosclerosis and cardiovascular risk: Pathophysiologic mechanisms and emerging clinical indications. Vascular pharmacology 2015.
15. Craig CL, Marshall AL, Sjostrom M, et al. International physical activity questionnaire: 12-country reliability and validity. Medicine and science in sports and exercise 2003;35:1381-95.
16. Celermajer DS, Sorensen K, Ryalls M, et al. Impaired endothelial function occurs in the systemic arteries of children with homozygous homocystinuria but not in their heterozygous parents. J Am Coll Cardiol 1993;22:854-8.
17. Jablonski KL, Gates PE, Pierce GL, Seals DR. Low dietary sodium intake is associated with enhanced vascular endothelial function in middle-aged and older adults with elevated systolic blood pressure. Ther Adv Cardiovasc Dis 2009;3:347-56.
18. Jablonski KL, Chonchol M, Pierce GL, Walker AE, Seals DR. 25-Hydroxyvitamin D deficiency is associated with inflammation-linked vascular endothelial dysfunction in middle-aged and older adults. Hypertension 2011;57:63-9.
19. Jablonski KL, Decker E, Perrenoud L, et al. Assessment of vascular function in patients with chronic kidney disease. Journal of visualized experiments : JoVE 2014.
20. Jablonski KL, Racine ML, Geolfos CJ, et al. Dietary sodium restriction reverses vascular endothelial dysfunction in middle-aged/older adults with moderately elevated systolic blood pressure. J Am Coll Cardiol 2013;61:335-43.
21. Donald AE, Halcox JP, Charakida M, et al. Methodological approaches to optimize reproducibility and power in clinical studies of flow-mediated dilation. J Am Coll Cardiol 2008;51:1959-64.
22. Caglar K, Yilmaz MI, Saglam M, et al. Short-term treatment with sevelamer increases serum fetuin-a concentration and improves endothelial dysfunction in chronic kidney disease stage 4 patients. Clin J Am Soc Nephrol 2008;3:61-8.
23. Ghiadoni L, Cupisti A, Huang Y, et al. Endothelial dysfunction and oxidative stress in chronic renal failure. J Nephrol 2004;17:512-9.

24. Yilmaz MI, Saglam M, Caglar K, et al. The determinants of endothelial dysfunction in CKD: oxidative stress and asymmetric dimethylarginine. Am J Kidney Dis 2006;47:42-50.
25. Jablonski KL, Fedorova OV, Racine ML, et al. Dietary Sodium Restriction and Association with Urinary Marinobufagenin, Blood Pressure, and Aortic Stiffness. Clin J Am Soc Nephrol 2013.
26. Seals DR, Tanaka H, Clevenger CM, et al. Blood pressure reductions with exercise and sodium restriction in postmenopausal women with elevated systolic pressure: role of arterial stiffness. J Am Coll Cardiol 2001;38:506-13.
27. Mitchell GF, Hwang SJ, Vasan RS, et al. Arterial stiffness and cardiovascular events: the Framingham Heart Study. Circulation 2010;121:505-11.
28. Eskurza I, Monahan KD, Robinson JA, Seals DR. Effect of acute and chronic ascorbic acid on flow-mediated dilatation with sedentary and physically active human ageing. J Physiol 2004;556:315-24.
29. Moreau KL, Gavin KM, Plum AE, Seals DR. Oxidative stress explains differences in large elastic artery compliance between sedentary and habitually exercising postmenopausal women. Menopause 2006;13:951-8.
30. Pierce GL, Lesniewski LA, Lawson BR, Beske SD, Seals DR. Nuclear factor-{kappa}B activation contributes to vascular endothelial dysfunction via oxidative stress in overweight/obese middle-aged and older humans. Circulation 2009;119:1284-92.
31. Walker AE, Kaplon RE, Pierce GL, Nowlan MJ, Seals DR. Prevention of age-related endothelial dysfunction by habitual aerobic exercise in healthy humans: possible role of nuclear factor kappaB. Clin Sci (Lond) 2014;127:645-54.
32. Jalal DI, Jablonski KL, McFann K, Chonchol MB, Seals DR. Vascular endothelial function is not related to serum uric acid in healthy adults. Am J Hypertens 2012;25:407-13.
33. Donato AJ, Eskurza I, Silver AE, et al. Direct evidence of endothelial oxidative stress with aging in humans: relation to impaired endothelium-dependent dilation and upregulation of nuclear factor-kappaB. Circ Res 2007;100:1659-66.
34. Silver AE, Christou DD, Donato AJ, et al. Protein Expression in Vascular Endothelial Cells Obtained from Human Peripheral Arteries and Veins. J Vasc Res 2009;47:1-8.
35. Donato AJ, Gano LB, Eskurza I, et al. Vascular endothelial dysfunction with aging: endothelin-1 and endothelial nitric oxide synthase. Am J Physiol Heart Circ Physiol 2009;297:H425-32.
36. Donato AJ, Pierce GL, Lesniewski LA, Seals DR. Role of NFkappaB in age-related vascular endothelial dysfunction in humans. Aging (Albany NY) 2009;1:678-80.
37. Gershon RC, Wagster MV, Hendrie HC, Fox NA, Cook KF, Nowinski CJ. NIH toolbox for assessment of neurological and behavioral function. Neurology 2013;80:S2-6.
38. Cao Y, Xu RX, Liu Z. A high-throughput quantification method of curcuminoids and curcumin metabolites in human plasma via high-performance liquid chromatography/tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2014;949-950:70-8.