Accepted Manuscript
Version of Record at: https://doi.org/10.1152/ajprenal.00067.2023

Effects of a Mitochondrial Targeted Ubiquinol (MitoQ) on Vascular Function and Exercise Capacity in Chronic Kidney Disease: A Randomized Controlled Pilot Study.

Danielle L. Kirkman[1,2]; Joseph M Stock[2]; Ninette Shenouda[2]; Natalie J Bohmke[1]; Youngdeok Kim[1]; Jason Kidd[3]; Raymond R Townsend[4]; David G Edwards[2]

[1]*Department of Kinesiology and Health Sciences, Virginia Commonwealth University*

[2] *Department of Kinesiology and Applied Physiology, University of Delaware*

[3]*Department of Internal Medicine, Virginia Commonwealth University Health Systems*

[4]*Perelman School of Medicine, University of Pennsylvania*

Corresponding Author

Danielle L Kirkman, PhD

500 Academic Center

1020 West Grace Street

Richmond, VA 23220

Email: dlkirkman@vcu.edu

Keywords

mitochondria; oxidative stress; vascular function; chronic kidney disease

Accepted Manuscript
Version of Record at: https://doi.org/10.1152/ajprenal.00067.2023

ABSTRACT

Background. Mitochondrial derived oxidative stress is implicated in vascular and skeletal muscle abnormalities in chronic kidney disease (CKD). The purpose of this study was to investigate the effects of a mitochondrial targeted ubiquinol (MitoQ) on vascular function and exercise capacity in CKD.

Methods. In this randomized controlled trial, 18 patients with CKD (Mean±SEM: Age, 62±3years; eGFR, 45±3ml/min/1.73^2) received 4 weeks of 20mg/day MitoQ (MTQ) or placebo (PLB). Outcomes assessed at baseline and follow up included macrovascular function measured by flow mediated dilation; microvascular function assessed by laser Doppler flowmetry combined with intradermal microdialysis; aortic hemodynamics assessed by oscillometery; and exercise capacity by cardiopulmonary exercise testing.

Results. Compared to PLB, MTQ improved flow mediated dilation (baseline vs follow up: MTQ, 2.4±0.3 *vs.* 4.0±0.9%; PLB, 4.2±1.0 *vs.* 2.5±1.0%; p=0.04). MTQ improved microvascular function (ΔCVC: MTQ, 4.50 $\pm$ 2.57 *vs.* PLB -2.22 $\pm$ 2.67%; p=0.053). Central aortic systolic and pulse pressures were unchanged, however, MTQ prevented increases in augmentation pressures that were observed in the PLB group (p=0.026). MTQ did not affect exercise capacity.

Conclusion. This study demonstrates potential for a mitochondrial targeted ubiquinol to improve vascular function in CKD. The findings hold promise for future investigations of mitochondrial targeted therapies in CKD.

Accepted Manuscript
Version of Record at: https://doi.org/10.1152/ajprenal.00067.2023

NEW AND NOTEWORTHY

In this randomized controlled pilot study, we investigated the effects of a mitochondrial targeted ubiquinol (MitoQ) on vascular function and exercise capacity in CKD. Our novel findings show that a 4-week supplementation of MitoQ was well tolerated and improved macrovascular endothelial function, arterial hemodynamics, and microvascular function in patients with Stage 3-4 CKD. Our mechanistic findings also suggest that MitoQ improved microvascular function in part by reducing the NADPH oxidase contribution to vascular dysfunction.

Accepted Manuscript
Version of Record at: https://doi.org/10.1152/ajprenal.00067.2023

INTRODUCTION

Chronic Kidney Disease (CKD), a progressive condition characterized by impairments in renal structure and function, affects 14% of the US population (1). Cardiovascular disease (CVD) that is not fully explained by traditional risk factors remains a major clinical complication of CKD and is the leading cause of hospitalization and death in this patient population (1). The eitiology of CVD in this patient population is unique to the disease and our understanding of its pathophysiology is still incomplete. Impaired endothelial function and microvascular dyfunction, both of which are primary events in the development of atherosclerosis and well established precursors of CVD, are hallmarks of CKD (2-7). CKD-related vascular endothelial dysfunction is characterized by reductions in nitric oxide production and bioavailability that are largely mediated by increased levels of oxidative stress in the earlier stages of the disease(5). Endotheilal dyfunction is the initial pathophysiological event in the development of atheroslerosis and is a mediator in the pathogenesis of ischemic heart disease in CKD(8). Furthermore, the kidney is a highly vascularized organ that recieves at least 20% of resting cardiac output and requires substatntial oxygenation to meet its high energy demands. Therefore, impairments in vascular endothelial function have been implicated in renal dysfunction and progression of CKD(9). In addition, increased central blood pressure, arterial stiffness and abnormal arterial hemodynamics are all evident in CKD and associated with the development and progression of heart failure, one of the most prevalent CKD related CVDs (10, 11). Superimposed on the vascular endotheilal consequences of CKD is a reduced cardiorespiratory fitness that is associated with exercise intolerance (12-14). Indeed, vascular endotheilal dysfunction may contibute to exercise intolerance by hampering blood flow delivery to the working muscle (13, 15). Furthermore, endothelial dysfunction has also been correlated with an exaggerated pressor response to exercie in patients with CKD which could further limit functional capacity (16). The reduction in exercise capacity further exacerbartes the already

high CVD risk (17) and is also an independent predictor of hospitalization and poor survival in these patients (18). Thus, investigations into the biological mechanisms of CKD related endothelial dysfunction and exercise intolerance are urgently needed.

Patients with CKD have impaired mitochondrial function that is both a cause and a consequence of increased reactive oxygen species (ROS) that exceed the organelle's antioxidant defenses (19-22). Increased mitochondrial derived oxidative stress due to mitochondrial dysfunction could play a role in impairing both vascular function (22) and skeletal muscle health (20, 23, 24), with downstream consequences for CVD and exercise intolerance in CKD. Our group has previously implicated mitochondrial derived ROS (mtROS) in CKD related vascular dysfunction by demonstrating that a local delivery of a mitochondrial targeted antioxidant acutely restores microvascular function (22). However, the chronic effects of reducing mtROS on vascular function and exercise intolerance warrant further exploration. Therefore, the purpose of this pilot study was to investigate the effects of a chronic (4 weeks) oral supplementation of a mitochondrial targeted ubiquinone, MitoQ, on vascular function and exercise capacity in Stage 3-4 CKD before the development of overt CVD. Utilizing a parallel, randomized controlled design, we tested the hypothesis that compared to a placebo, MitoQ would significantly improve conduit artery and microvascular function and cardiorespiratory fitness.

METHODS

Participants

Ethical approval was provided by the University of Delaware Institutional Review Board and the study met the guidelines set forth by the Declaration of Helsinki. All participants provided written informed consent. Patients were recruited during routine outpatient Nephrology clinics between 2017 and 2019. Participant eligibility was confirmed during a screening visit that included a medical history, a physical examination and routine clinical blood work and urinalysis. Participants were included if they had a medical diagnosis of stage 3-4 non-dialysis CKD and were >18 years of age. Kidney function was calculated using the Modification of Diet in Renal Disease [MDRD] equation). Participants were excluded if they had a history of CVD (defined as coronary artery disease; myocardial infarction; heart failure; peripheral artery disease; or a cerebrovascular accident/transient ischemic attack); uncontrolled hypertension or hyperlipidemia; a current pregnancy; were receiving hormone replacement therapy; currently used tobacco products; presented with liver or autoimmune disease; currently used antioxidants >300mg/day; and were unable to or refused consent. Participants that used <300mg of antioxidants per day were required to undergo a 2 week wash out period before starting the trial and were asked to withhold antioxidant use throughout the duration of the trial. The exclusion of participants with a history of CVD allowed us to investigate the role of mtROS on vascular function and exercise capacity prior to the development of overt CVD.

Study Design and Intervention

This double-blind pilot study (NCT02364648) utilized a randomized controlled parallel design. Participants were randomly allocated in 1:1 manner according to a computer-generated permuted block sequence (randomization.com), stratified by sex, to receive 28 days of MitoQ (MTQ) or Placebo (PLB) capsules. Preparation, implementation and allocation of the randomization strategy was performed by an independent investigator blinded to order of

enrollment (BM). Participants were blinded to group allocation. MitoQ and placebo capsules were prepared and provided by MitoQ (MitoQ Limited). Participants allocated to the MTQ group received a 20mg/day dose of MitoQ. The dose was determined based on manufacturer recommendations and supported by studies that have demonstrated physiological efficacy(25). PLB received identical placebo capsules. A duration of 4 weeks was chosen as circulating plasma levels of mitoquinone peak after ~15 days of MitoQ administration and plateau thereafter (26). We therefore estimated that a 4week intervention would allow sufficient time for circulating MitoQ levels to peak and plateau and for subsequent physiological vascular adaptations to occur. Participants were instructed to take daily capsules orally before breakfast each morning. If participants forgot to take the capsule at breakfast, they could take it any time later in the day if they remembered. However, if a full day or more was missed, participants were instructed not to ‘double dose’ on subsequent days. Any missed capsules were returned at the 4 week follow up testing visit to document compliance.

Outcome Measures

Outcome measures were assessed at baseline and at 4 weeks follow up during two separate study visits: a vascular and an exercise testing visit. The outcome measure assessors were blinded to group allocation. Within-subject visits occurred at the same time of day for each participant. Participants were instructed to avoid exercise and alcohol 24 hours before each visit and caffeine at least 12 hours before each visit. Participants were asked to fast >6hours and to refrain from taking morning medications before the vascular testing visit. Participants rested in the supine position in a quiet, temperature-controlled laboratory for at least 30 minutes before any vascular assessments were made.

Conduit Artery Endothelial Function

Brachial artery flow mediated dilation (FMD) was performed according to established guidelines as an *in vivo* measure of nitic oxide dependent conduit artery endothelial function(27). Longitudinal B-Mode images of the brachial artery and simultaneous Doppler measures of blood velocity were obtained with duplex ultrasound (uSmart 3300, Terason, MA). FMD was reported as the percent change from baseline arterial diameter that was induced by reactive hyperemia following 5 minutes of forearm occlusion (E20 Rapid Cuff Inflation System, Hokanson, WA) at 200mmHg (or at least 50mmHg above resting systolic blood pressure [SBP] if SBP was >150mmHg). Shear stimulus is reported as the area under the curve of the shear rate (s^{-1}) profile from release of forearm occlusion to peak diameter. Images were analyzed offline using edge detection software (Cardiovascular Suite, Quippu, Italy).

Central Blood Pressure and Arterial Hemodynamics

Central aortic blood pressures were derived from brachial artery waveforms acquired with oscillometery and the use of the generalized transfer function (SphygmoCor XCEL, Atcor Medical, Australia). Carotid to femoral pulse wave velocity was assessed by the simultaneous acquisition of the carotid pulse via applanation tonometry and the femoral pulse via oscillometery (SphygmoCor XCEL, Atcor Medical, Australia).

Microvascular Function

The skin blood flow response to local heating was coupled with intradermal microdialysis to assess microvascular function. Following 10 minutes of analgesic icing on the ventral forearm, a 23-gauge needle was used as a canula guide to place two subcutis microdialysis fibers

(CMA Microdialysis AB, Sweden) for the local delivery of pharmacological substances to the cutaneous circulation. Multifiber laser probes fixed in small local heaters were situated above each microdialysis membrane (Temperature Monitor SHO_2, Moor Instruments) for the measurement of skin blood flow, represented by red blood cell flux, at each site. Following microdialysis fiber insertion, Ringer's solution was infused through both fibers at 2ul/min for 60 – 90 minutes (Bee Hive controller, Baby Bee microinfusion pumps, Bioanalytical Systems) to allow for the resolution of any insertion related hyperemia. Blood pressure was recorded on the contralateral arm every 15 minutes throughout the experimental procedure.

A standardized local heating protocol was performed to experimentally assess microvascular function(22, 28, 29). Microdialysis sites were randomly allocated receive an 2ul/min infusion of either Ringer's solution (Control) or 100uM Apocynin (NADPH Oxidase inhibitor; Sigma Aldrich) throughout the duration of the heating protocol. Local heaters were set at 33°C to collect baseline red blood cell flux for ~30 minutes. Once a stable baseline was recorded, temperature was increased rapidly (1°C/s) to 42°C and held for the duration of the protocol. The biphasic blood flow response to local heating is comprised of a rapid axon mediated initial peak, followed by a slower, steady increase to a plateau that has been reported to be predominantly mediated by nitic oxide(30). Following a stable plateau, maximum vasodilation at each site was obtained through the simultaneous infusion of 28mM sodium nitroprusside (SNP; U.S. Pharmacopeia) at 4uL/min and an increase in temperature to 43°C. Microvascular function was reported as cutaneous vascular conductance (CVC) calculated as red blood cell flux /mean arterial pressure. Baseline and plateau values were averaged over a 10minute period and the initial peak was average over one minute. To normalize data between sites, CVC was reported as a percentage of the maximum CVC (CVC_{max}) at each site.

Exercise Capacity

A symptom-limited, maximal effort graded cardiopulmonary exercise test (CPET) was performed on an upright electromagnetic braked cycle ergometer (Corival, Lode, The Netherlands). Cycling commenced at 25 Watts (W) and was increased by 25W every three minutes, maintaining a cadence above 60rpm until volitional fatigue. Breath-by-breath gas analysis averaged over 10s intervals was acquired with an automated gas analyzer (TrueOne 2400, Parvo Medics). Twelve lead ECG (Case, GE), rating of perceived exertion and blood pressures (Tango M2, SunTech, NC) were recorded at rest, throughout the exercise protocol and during recovery. CPET derived variables including minute ventilation/oxygen and carbon dioxide production slopes (V_E/VO_2, V_E/VCO_2, respectively) oxygen uptake efficiency slope (OUES) and O_2 pulse were calculated as previously described (12).

Plasma MitoQ and Oxidative Stress Analyses

EDTA-treated plasma quantification of MitoQ at follow up was performed by the Virginia Commonwealth University Metabolomics Core. Measures were made by liquid chromatography/mass spectrometry with the use of deuterated MitoQ as an internal standard. EDTA-treated plasma samples were also analyzed for F2-isoprostanes and isofurans with negative ion gas chromatography mass spectroscopy (Agilent 9690n Network GC; Agilent Laboratories, Torrance, CA) by the Vanderbilt University Eicosanoid Core Laboratory (31).

Adverse Events

Information on adverse events was collected as and when they occurred and documented during the trial period only.

Statistical Analyses

The purpose of this study was to identify the physiological changes associated with the chronic suppression of mtROS with MitoQ rather than the efficacy of MitoQ as a therapeutic treatment in CKD. Therefore, *per protocol* rather than intention to treat analyses were performed. Statistical analyses were performed using SPSS (v27, IBM). Shapiro-Wilk's test of normality was used to confirm normal distribution of the data. Parametric tests were performed if all assumptions were met. Differences in participant characteristics between groups at baseline were assessed with the use of Student's independent samples *t*-tests and Fisher's Exact tests. Intervention compliance rates and plasma MitoQ levels at follow up were compared with Student's independent samples *t*-tests. Changes over time in outcome measures were compared between groups with a two-way repeated measures ANOVA. Following a significant omnibus test, post hoc independent and paired samples *t*-tests were performed. For microdialysis measures, change scores in CVC in the Ringer's control site were compared between groups with independent samples *t*-tests. Within-subject comparisons between drug sites were compared with paired samples *t*-tests. Descriptive data pertaining to the study population presented as mean (range). All other data are presented as mean ± standard error of the mean (SEM). Statistical significance was set at $p \leq 0.05$. Cohens effect sizes (*d*) were calculated for main outcome measures. A post hoc power analysis (G-power) indicated a power of 99% for the primary outcome of conduit artery endothelial function.

RESULTS

Participants

Participant flow through the study is reported in **Figure 1**. There were no differences between groups in participant characteristics at baseline (**Table 1**). Biochemistry and hematology values were within clinically recommended ranges.

Intervention Compliance, Tolerability and Safety

Participants were highly compliant with both MitoQ and Placebo interventions with no significant differences between groups (MTQ *vs.* PLB, 99.60 ± 0.40% *vs.* 97.76 ± 2.24 %; p=0.12). Circulating serum MitoQ levels at follow up were significantly higher in MTQ compared to PLB (30 ± 7 *p*mol/mL *vs.* 0 ± 0 *p*mol/mL; p=0.001). MTQ administration was well tolerated with no expected or serious adverse events reported. With regards to renal safety, there was no significant change in kidney function (estimated glomerular filtration rate, eGFR) after either intervention (baseline *vs.* follow up: MTQ, 51 ± 4 *vs.* 57 ± 5 ml/min/1.73m^2; PLB, 45 ± 4 *vs.* 52 ± 7 ml/min/1.73m^2; interaction p = 0.68).

Conduit Artery Endothelial Function

There was no difference in baseline brachial artery diameter between groups across time (baseline *vs.* follow up: MTQ, 4.99 ± 0.29 *vs.* 4.98 ± 0.29 mm; PLB, 5.16 ± 0.45 *vs.* 5.01 ± 0.49 mm; interaction p = 0.50). There was a significant group x time interaction for FMD (MTQ, 2.36 ± 0.31 *vs.* 3.99 ± 0.88 %; PLB, 4.23 ± 1.01 *vs.* 2.54 ± 0.99 %; interaction p = 0.04; d=0.76) with post hoc analyses showing a significant increase in FMD following MTQ (p = 0.04; **Figure 2.**). There was no difference between groups across time in shear AUC (MTQ, 8991 ± 1871 *vs.* 11553 ± 2134 mm; PLB, 8387 ± 2274 *vs.* 8273 ± 1929 AU; interaction p = 0.37), confirming a similar stimulus for vasodilation between groups across time.

Peripheral and Central Blood Pressure and Arterial Hemodynamics

There were no significant changes between groups in peripheral systolic (baseline *vs.* follow up: MTQ, 141 ± 5 *vs.* 137 ± 6 mmHg; PLB, 136 ± 4 *vs.* 134 ± 6 mmHg; $p = 0.70$; d=0) or diastolic pressures (MTQ, 82 ± 3 *vs.* 81 ± 3 mmHg; PLB, 81 ± 3 *vs.* 78 ± 4 mmHg; $p = 0.58$; d=-0.06). As shown in **Figure 3A & B**, there were no differences between groups across time in central systolic (MTQ, 128 ± 5 *vs.* 123 ± 6 mmHg; PLB, 124 ± 3 *vs*. 123 ± 5 mmHg; interaction $p = 0.44$; d=0.1) or pulse pressures (MTQ, 44 ± 4 *vs.* 41 ± 4 mmHg; PLB, 44 ± 4 *vs.* 45 ± 4 mmHg; interaction $p = 0.11$; d=0.1). There was a significant group x time interaction for augmentation pressure (MTQ, 11 ± 2 *vs.* 8 ± 2 mmHg; PLB 11 ± 2 *vs.* 14 ± 2 mmHg; $p = 0.026$; d=0.4; **Figure 3C**) and augmentation index (MTQ, 23 ± 4 *vs.* 19 ± 3 %; PLB, 25 ± 4 *vs*. 29 ± 4 %; d=04; $p = 0.050$; **Figure 3D**). Follow up tests showed significant increases from baseline to follow up in augmentation pressure ($p = 0.006$; **Figure 3C**) and augmentation index ($p = 0.04$; **Figure 3D**) in PLB. There was no difference between groups across time in heart rate (baseline *vs.* follow up: MTQ 65 ± 4 *vs.* 64 ± 4 bpm; PLB 63 ± 5 *vs.* 67 ± 6 bpm; interaction $p = 0.07$). There was a significant interaction (p=0.037) and main effect for time (p=0.008) for cfPWV. Follow up tests showed significant reductions in cfPWV in the PLB group (baseline *vs*. follow up: MitoQ, 9.30 ± 0.83 *vs.* 9.11 ± 0.67 m/s; p = 0.25; PLB, 10.49 ± 0.93 *vs.* 9.16 ± 0.95 m/s; p = 0.01).

Microvascular Function

Ringers' Control Site. Two subjects from the PLB group were excluded from the analysis (drug contamination to a Ringers site, n=1; refused consent for microdialysis procedure, n=1). There was no difference in the change score between groups in baseline (baseline *vs.* follow

up: MTQ 14 ± 3 *vs.* 10 ± 2 %; PLB 8 ± 2 *vs.* 12 ± 3 %; $p = 0.063$), initial peak (MTQ 69 ± 4 *vs.* 66 ± 2 %; PLB 68 ± 7 *vs.* 50 ± 11 %; $p = 0.14$) or maximal CVC (MTQ 1.12 ± 0.21 *vs.* 1.32 ± 0.29 AU; PLB 0.96 ± 0.22 *vs.* 1.53 ± 0.22 AU; $p = 0.46$). Compared to PLB, there was a nominal increase and with a trend for statistical significance ($p = 0.053$; $d = 0.7$) on the plateau CVC response to local heating in the MTQ group, signifying improved microvascular function following MitoQ supplementation (**Table 2; Figure 4A**).

Ringer's vs Apocynin. Due to a small data sample in the PLB group, we have only investigated the effects of Apocynin infusion before and after the intervention in the MTQ group to provide preliminary insights into mechanistic changes associated with the administration of a mitochondrial targeted antioxidant. One subject was excluded from the MTQ group at baseline due to drug contamination at the Apocynin site. In the MTQ group, Apocynin delivery significantly improved microvascular function at baseline, however, after 4 weeks of MTQ, apocynin was no longer effective at improving microvascular function (**Figure 4B**) suggesting that MitoQ supplementation may reduce NADPH oxidase contributions to CKD-related microvascular dysfunction. There was no difference in the CVC_{max} between sites at baseline (Ringers *vs.* Apocynin: 1.12 ± 0.21 *vs.* 1.39 ± 0.33 AU; $p = 0.49$) or follow up (Ringers *vs.* Apocynin: 1.32 ± 0.29 *vs.* 1.19 ±0.23; $p = 0.73$) confirming that differences between sites were not due to differences in maximal dilatory capacity.

Exercise Capacity

One subject from the MitoQ group (equipment/technical error at follow up test) and one subject from the Placebo group (contraindication to exercise testing at follow up) were excluded from the analyses. MitoQ had no effect on maximal exercise capacity reported as VO_{2peak} (baseline *vs.* follow up: MTQ 17.95 ± 2.91 *vs.* 17.34 ±2.89 ml/kg/min; PLB 19.07 ± 2.18 *vs.* 17.27 ± 2.19

ml/kg/min; interaction p = 0.27; d=0.08) or total exercise time (MTQ, 726 ± 83 *vs.* 691 ± 90 s; PLB, 844 ± 153 *vs.* 780 ± 145 s; interaction p = 0.66; d=0.05). There were no differences between groups in CPET markers of peripheral oxygen extraction and efficiency: OUES (MTQ, 1.53 ± 0.13 *vs.* 1.61 ± 0.04 AU; PLB, 1.69 ± 0.21 *vs.* 1.60 ± 0.21 AU; interaction p = 0.16), and V_E/VO_2 (MTQ, 43 ± 2 AU *vs.* 43 ± 3 AU; PLB, 47± 4 *vs.* 44 ± 5 AU; interaction p = 0.20). There was a significant group x time interaction in the CPET indicator of ventilation-perfusion matching, V_E/VCO_2 (p = 0.007), with post hoc analyses showing a significant increase in the V_E/VCO_2 slope from baseline to follow up in MTQ (34 ± 1 AU *vs.* 35 ± 1 AU, p = 0.04) and a decrease in PLB (37 ± 2 *vs.* 34 ± 3 AU; p = 0.02). There was no significant change in the pressor response to exercise between groups or across time points (peak SBP: MTQ, 192 v 12 *vs.* 186 ± 13 mmHg; PLB, 198 ± 14 *vs.* 192 ± 15 mmHg; interaction p=0.69).

Plasma Biomarkers of Oxidative Stress

There was no significant change in the ratio of F2-isoprostanes to isofurans (baseline *vs.* follow up: MTQ, 1.60 ± 0.21 *vs.* 1.78 ± 0.23 AU; PLB, 1.37 ± 0.22 *vs.* 1.68 ± 0.25 AU; interaction p=0.70).

DISCUSSION

The findings of our pilot trial show that a 4-week supplementation of a mitochondrial targeted ubiquinol (MitoQ) was safe, well tolerated and improved macrovascular endothelial function, arterial hemodynamics, and microvascular function in patients with Stage 3-4 CKD without overt CVD. However, MitoQ supplementation had no effect on cardiorespiratory fitness in this patient cohort.

Our pilot findings in CKD show that MitoQ had a beneficial effect on macrovascular endothelial function. These findings support previous investigations demonstrating significant improvements in FMD in older adults following six weeks of MitoQ supplementation of the same dose (20mg) (25). In addition, acute improvements in FMD have been observed following a single higher dose of MitoQ (80-160mg) in older adults and patients with peripheral artery disease(25, 32). The improvements in endothelial function with MitoQ supplementation are also supported by preclinical models of aging, hypertension and chemotherapy associated endothelial dysfunction that have all reported restored vascular function following chronic MitoQ administration(33-35). As endothelial dysfunction is recognized as the initial pathophysiological event in the development of atherosclerosis, our pilot findings may have significant implications for future clinical trials utilizing mitochondrial targeted interventions for the primordial prevention of CVD in the earlier stages of CKD.

Although MitoQ had no effect on peripheral or central blood pressure in this pilot study, we demonstrated that MitoQ supplementation prevented increases in augmentation pressures and the augmentation index that were observed over time in the placebo group. The augmentation index is a surrogate measure of left ventricular afterload and is influenced by arterial stiffness. Therefore, our findings are clinically noteworthy as aberrant arterial hemodynamics are implicated in kidney disease progression by increasing end organ pulsatility (36), in addition to the pathogenesis of heart failure by increasing left ventricular late systolic pulsatile load(37). We cannot be certain about the exact functional parameters that MitoQ altered to improve arterial hemodynamics in this study. Chronic supplementation of MitoQ has previously been shown to reduce arterial stiffness in translational studies of aging(34, 38). However, arterial

stiffening in CKD is more difficult to reverse and is unlikely over a duration of 4 weeks(28). It is possible that MitoQ mediated improvements in arterial hemodynamics are due to beneficial changes in cardiac function and/or peripheral resistance, however, this warrants investigation in future trials.

Microvascular function, assessed by cutaneous vascular conductance, showed a trend for improvement (with a large effect size) following MitoQ. This evidence builds on previous findings in Stage 3- 4 CKD reporting augmented microvascular function following an acute, local intradermal delivery of the mitochondrial targeted antioxidant, MitoTempo(22). Our preliminary findings showed that Apocynin, a NADPH oxidase inhibitor, was no longer effective at augmenting microvascular function after 4 weeks of MitoQ. This suggests that MitoQ altered microvascular function in part by reducing the NADPH oxidase contribution to vascular dysfunction. This is supported by multiple studies that demonstrate crosstalk between mtROS and NOX in the vasculature whereby mtROS stimulate the production of NADPH oxidase derived ROS and visa-versa(39). Previous studies have also implicated NADPH oxidase derived ROS as mediators of CKD-related microvascular function in Stage 3-4(3). As Apocynin is a non-specific inhibitor of NADPH oxidase, we cannot determine whether the benefits of MitoQ are mediated through one specific NOX isoform (NOX2 or NOX4). It is also possible that MitoQ altered vascular function by improving overall mitochondrial health and integrity. This could result in subsequent reductions in excessive electron leak; improvements in mitochondrial membrane potential and integrity; augmented mitochondrial quality control; and improved regulation of mitochondrial calcium balance, all of which have been shown to play an important homeostatic role in vascular health and function(39). The effects of MitoQ on these mechanisms could be investigated in future CKD studies.

Contrary to our hypothesis, MitoQ had no effect on cardiorespiratory fitness. Studies that have utilized MitoQ to improve exercise capacity have shown varied results. In healthy, athletic males, a recent trial showing improvements in cycling time trial performance following 6 months of MitoQ supplementation(40) is countered by an earlier trial reporting no benefit of MitoQ in skeletal muscle adaptations or cardiorespiratory fitness following 3 weeks of supplementation (41). The difference in findings could potentially be explained by the duration of the intervention. Future trials could investigate the effects of mitochondrial targeted therapies on skeletal muscle factors that increase oxygen utilization. In addition, studies should investigate if the improvements that we observed in resting vascular function translate to improved oxygen delivery to the working muscle during exercise. Future studies should also ascertain if MitoQ supplementation of longer duration is efficacious at improving submaximal exercise capacity that relates to the intensity of activities of daily living and thus portents to translational improvements in quality of life. We have previously demonstrated that vascular adaptations to exercise training are attenuated in patients with CKD(28, 42). Therefore, it would be interesting to investigate if MitoQ supplementation combined with exercise training could augment training adaptations in this patient cohort.

There are several limitations to this study. First, although circulating levels of MitoQ were evident in the treatment group, this study is lacking in a direct assessment of mtROS and we therefore cannot directly conclude that the observed benefits were due to a reduction in mitochondrial derived oxidative stress. Due to the rapid half-life of free radicals, accurate measurements of generic ROS production *in vivo* in human subjects is notoriously difficult and is further complicated when attempting to define the specific source of ROS. Future studies

employing *ex-vivo* mechanistic methodologies could potentially provide a better understanding of the underlying physiological mechanisms of the observed benefits. Our microvascular data is limited by a small sample size in the placebo group. However, the results reported in the MTQ group alone are important and offer some valuable and interesting mechanistic insights into the observed improvements in microvascular function. Although this study included both men and women, we were insufficiently powered to address potential sex differences in responses to MitoQ. We have recently reported that older women with Stage 3-4 CKD have impaired microvascular function and arterial hemodynamics compared with their male counterparts(43). In addition, preclinical studies have shown mitochondrial protective effects of estrogen and excessive mtROS production is postulated to contribute to post-menopausal declines in vascular function(25, 39, 44). Unfortunately, we did not document menopause status, however, all female participants were >60years of age. The findings of this study can be leveraged by future studies to examine if there is a sex dependent response to MitoQ. In this study, we did not include healthy control comparison groups. Although similar findings have been reported in studies of healthy aging, we have no direct comparison to a healthy control response in this study. Finally, we did not have access to etiology of CKD, therefore we are unable to determine if the response to MitoQ differed between kidney diseases.

In summary, our pilot study demonstrates the potential for a mitochondrial targeted ubiquinol to improve vascular function in patients with Stages 3- 4 CKD. These benefits were observed prior to the development of overt CVD and therefore, the findings hold promise for larger, future clinical trials that aim to utilize mitochondrial targeted strategies for the primordial prevention of CKD related CVD. The study did not demonstrate an effect of 4-week MitoQ supplementation on cardiorespiratory fitness. Future trials should investigate if longer

treatment durations have effects on functional capacity or submaximal activities that are more reflective of activities of daily living.

ACKNOWLEDGEMENTS

We would like to acknowledge Bryce Muth for his assistance with the randomization strategy and allocation. We would like to acknowledge the study participants.

FUNDING

Supported by funding from the American Heart Association (17GRNT33670462) awarded to DGE.

CONFLICT OF INTEREST

None to declare

REFERENCES

1. **System USRD**. 2021 USRDS Annual Data Report: Epidemiology of kidney disease in the United States National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD.

2. **Dupont JJ, Farquhar WB, Townsend RR, and Edwards DG**. Ascorbic acid or L-arginine improves cutaneous microvascular function in chronic kidney disease. *J Appl Physiol (1985)* 111: 1561-1567, 2011.

3. **DuPont JJ, Ramick MG, Farquhar WB, Townsend RR, and Edwards DG**. NADPH oxidase-derived reactive oxygen species contribute to impaired cutaneous microvascular function in chronic kidney disease. *Am J Physiol Renal Physiol* 306: F1499-1506, 2014.

4. **Katulka EK, Hirt AE, Kirkman DL, Edwards DG, and Witman MAH**. Altered vascular function in chronic kidney disease: evidence from passive leg movement. *Physiol Rep* 7: e14075, 2019.

5. **Martens CR, Kirkman DL, and Edwards DG**. The Vascular Endothelium in Chronic Kidney Disease: A Novel Target for Aerobic Exercise. *Exerc Sport Sci Rev* 44: 12-19, 2016.

6. **Kenney WL**. Edward F. Adolph Distinguished Lecture: Skin-deep insights into vascular aging. *J Appl Physiol (1985)* 123: 1024-1038, 2017.

7. **Heiss C, Rodriguez-Mateos A, Bapir M, Skene SS, Sies H, and Kelm M**. Flow-mediated dilation reference values for evaluation of endothelial function and cardiovascular health. *Cardiovasc Res* 119: 283-293, 2023.

8. **Kuczmarski JM, Martens CR, Lennon-Edwards SL, and Edwards DG**. Cardiac function and tolerance to ischemia-reperfusion injury in chronic kidney disease. *Nephrol Dial Transplant* 29: 1514-1524, 2014.

9. **Li Y, Cui R, Liu K, Eshak ES, Cui M, Dong J, Imano H, Muraki I, Kiyama M, Kitamura A, Okada T, Yamagishi K, Umesawa M, Ohira T, and Iso H**. Relationship between

468 Endothelial Dysfunction and Prevalence of Chronic Kidney Disease: The Circulatory Risk in
469 Communities Study (CIRCS). *J Atheroscler Thromb* 28: 622-629, 2021.
470 10. **Chirinos JA, Khan A, Bansal N, Dries DL, Feldman HI, Ford V, Anderson AH,**
471 **Kallem R, Lash JP, Ojo A, Schreiber M, Sheridan A, Strelsin J, Teal V, Roy J, Pan Q, Go**
472 **AS, and Townsend RR**. Arterial stiffness, central pressures, and incident hospitalized heart
473 failure in the chronic renal insufficiency cohort study. *Circ Heart Fail* 7: 709-716, 2014.
474 11. **Townsend RR**. Arterial stiffness and chronic kidney disease: lessons from the Chronic
475 Renal Insufficiency Cohort study. *Curr Opin Nephrol Hypertens* 24: 47-53, 2015.
476 12. **Kirkman DL, Muth BJ, Stock JM, Townsend RR, and Edwards DG**.
477 Cardiopulmonary exercise testing reveals subclinical abnormalities in chronic kidney disease.
478 *Eur J Prev Cardiol* 25: 1717-1724, 2018.
479 13. **Kirkman DL, Bohmke N, Carbone S, Garten RS, Rodriguez-Miguelez P, Franco RL,**
480 **Kidd JM, and Abbate A**. Exercise intolerance in kidney diseases: physiological contributors
481 and therapeutic strategies. *Am J Physiol Renal Physiol* 320: F161-f173, 2021.
482 14. **Macdonald JH, Fearn L, Jibani M, and Marcora SM**. Exertional fatigue in patients with
483 CKD. *Am J Kidney Dis* 60: 930-939, 2012.
484 15. **Sprick JD, Downey RM, Morison DL, Fonkoue IT, Li Y, DaCosta D, Rapista D, and**
485 **Park J**. Functional sympatholysis is impaired in end-stage renal disease. *Am J Physiol Regul*
486 *Integr Comp Physiol* 316: R504-r511, 2019.
487 16. **Downey RM, Liao P, Millson EC, Quyyumi AA, Sher S, and Park J**. Endothelial
488 dysfunction correlates with exaggerated exercise pressor response during whole body
489 maximal exercise in chronic kidney disease. *Am J Physiol Renal Physiol* 312: F917-f924,
490 2017.

17. **Howden EJ, Weston K, Leano R, Sharman JE, Marwick TH, Isbel NM, and Coombes JS**. Cardiorespiratory fitness and cardiovascular burden in chronic kidney disease. *J Sci Med Sport* 18: 492-497, 2015.

18. **Sietsema KE, Amato A, Adler SG, and Brass EP**. Exercise capacity as a predictor of survival among ambulatory patients with end-stage renal disease. *Kidney Int* 65: 719-724, 2004.

19. **Tirichen H, Yaigoub H, Xu W, Wu C, Li R, and Li Y**. Mitochondrial Reactive Oxygen Species and Their Contribution in Chronic Kidney Disease Progression Through Oxidative Stress. *Front Physiol* 12: 627837, 2021.

20. **Thome T, Kumar RA, Burke SK, Khattri RB, Salyers ZR, Kelley RC, Coleman MD, Christou DD, Hepple RT, Scali ST, Ferreira LF, and Ryan TE**. Impaired muscle mitochondrial energetics is associated with uremic metabolite accumulation in chronic kidney disease. *JCI Insight* 6: 2020.

21. **Galvan DL, Green NH, and Danesh FR**. The hallmarks of mitochondrial dysfunction in chronic kidney disease. *Kidney Int* 92: 1051-1057, 2017.

22. **Kirkman DL, Muth BJ, Ramick MG, Townsend RR, and Edwards DG**. Role of mitochondria-derived reactive oxygen species in microvascular dysfunction in chronic kidney disease. *Am J Physiol Renal Physiol* 314: F423-f429, 2018.

23. **Gamboa JL, Roshanravan B, Towse T, Keller CA, Falck AM, Yu C, Frontera WR, Brown NJ, and Ikizler TA**. Skeletal Muscle Mitochondrial Dysfunction Is Present in Patients with CKD before Initiation of Maintenance Hemodialysis. *Clin J Am Soc Nephrol* 15: 926-936, 2020.

24. **Avin KG, Chen NX, Organ JM, Zarse C, O'Neill K, Conway RG, Konrad RJ, Bacallao RL, Allen MR, and Moe SM**. Skeletal Muscle Regeneration and Oxidative Stress Are Altered in Chronic Kidney Disease. *PLoS One* 11: e0159411, 2016.

25. **Rossman MJ, Santos-Parker JR, Steward CAC, Bispham NZ, Cuevas LM, Rosenberg HL, Woodward KA, Chonchol M, Gioscia-Ryan RA, Murphy MP, and Seals DR**. Chronic Supplementation With a Mitochondrial Antioxidant (MitoQ) Improves Vascular Function in Healthy Older Adults. *Hypertension* 71: 1056-1063, 2018.

26. **Gane EJ, Weilert F, Orr DW, Keogh GF, Gibson M, Lockhart MM, Frampton CM, Taylor KM, Smith RA, and Murphy MP**. The mitochondria-targeted anti-oxidant mitoquinone decreases liver damage in a phase II study of hepatitis C patients. *Liver Int* 30: 1019-1026, 2010.

27. **Thijssen DHJ, Bruno RM, van Mil A, Holder SM, Faita F, Greyling A, Zock PL, Taddei S, Deanfield JE, Luscher T, Green DJ, and Ghiadoni L**. Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. *Eur Heart J* 40: 2534-2547, 2019.

28. **Kirkman DL, Ramick MG, Muth BJ, Stock JM, Pohlig RT, Townsend RR, and Edwards DG**. Effects of aerobic exercise on vascular function in nondialysis chronic kidney disease: a randomized controlled trial. *Am J Physiol Renal Physiol* 316: F898-f905, 2019.

29. **Alba BK, Greaney JL, Ferguson SB, and Alexander LM**. Endothelial function is impaired in the cutaneous microcirculation of adults with psoriasis through reductions in nitric oxide-dependent vasodilation. *Am J Physiol Heart Circ Physiol* 314: H343-h349, 2018.

30. **Bruning RS, Santhanam L, Stanhewicz AE, Smith CJ, Berkowitz DE, Kenney WL, and Holowatz LA**. Endothelial nitric oxide synthase mediates cutaneous vasodilation during local heating and is attenuated in middle-aged human skin. *J Appl Physiol (1985)* 112: 2019-2026, 2012.

31. **Milne GL, Gao B, Terry ES, Zackert WE, and Sanchez SC**. Measurement of F2-isoprostanes and isofurans using gas chromatography-mass spectrometry. *Free Radic Biol Med* 59: 36-44, 2013.

32. **Park SY, Pekas EJ, Headid RJ, 3rd, Son WM, Wooden TK, Song J, Layec G, Yadav SK, Mishra PK, and Pipinos, II**. Acute mitochondrial antioxidant intake improves endothelial function, antioxidant enzyme activity, and exercise tolerance in patients with peripheral artery disease. *Am J Physiol Heart Circ Physiol* 319: H456-h467, 2020.

33. **Clayton ZS, Brunt VE, Hutton DA, VanDongen NS, D'Alessandro A, Reisz JA, Ziemba BP, and Seals DR**. Doxorubicin-Induced Oxidative Stress and Endothelial Dysfunction in Conduit Arteries Is Prevented by Mitochondrial-Specific Antioxidant Treatment. *JACC CardioOncol* 2: 475-488, 2020.

34. **Gioscia-Ryan RA, LaRocca TJ, Sindler AL, Zigler MC, Murphy MP, and Seals DR**. Mitochondria-targeted antioxidant (MitoQ) ameliorates age-related arterial endothelial dysfunction in mice. *J Physiol* 592: 2549-2561, 2014.

35. **Graham D, Huynh NN, Hamilton CA, Beattie E, Smith RA, Cochemé HM, Murphy MP, and Dominiczak AF**. Mitochondria-targeted antioxidant MitoQ10 improves endothelial function and attenuates cardiac hypertrophy. *Hypertension* 54: 322-328, 2009.

36. **Hsu JJ, Katz R, Chirinos JA, Jacobs DR, Jr., Duprez DA, and Peralta CA**. Arterial wave reflections and kidney function decline among persons with preserved estimated glomerular filtration rate: the Multi-Ethnic Study of Atherosclerosis. *J Am Soc Hypertens* 10: 438-446, 2016.

37. **Chirinos JA, and Sweitzer N**. Ventricular-Arterial Coupling in Chronic Heart Failure. *Card Fail Rev* 3: 12-18, 2017.

38. **Chirinos JA, and Segers P**. Noninvasive evaluation of left ventricular afterload: part 1: pressure and flow measurements and basic principles of wave conduction and reflection. *Hypertension* 56: 555-562, 2010.

564 39. **Kirkman DL, Robinson AT, Rossman MJ, Seals DR, and Edwards DG**.
565 Mitochondrial contributions to vascular endothelial dysfunction, arterial stiffness, and
566 cardiovascular diseases. *Am J Physiol Heart Circ Physiol* 320: H2080-h2100, 2021.
567 40. **Broome SC, Braakhuis AJ, Mitchell CJ, and Merry TL**. Mitochondria-targeted
568 antioxidant supplementation improves 8 km time trial performance in middle-aged trained male
569 cyclists. *J Int Soc Sports Nutr* 18: 58, 2021.
570 41. **Shill DD, Southern WM, Willingham TB, Lansford KA, McCully KK, and Jenkins**
571 **NT**. Mitochondria-specific antioxidant supplementation does not influence endurance exercise
572 training-induced adaptations in circulating angiogenic cells, skeletal muscle oxidative capacity
573 or maximal oxygen uptake. *J Physiol* 594: 7005-7014, 2016.
574 42. **Kirkman DL, Ramick MG, Muth BJ, Stock JM, Townsend RR, and Edwards DG**. A
575 Randomized Trial of Aerobic Exercise in Chronic Kidney Disease: Evidence for Blunted
576 Cardiopulmonary Adaptations. *Annals of Physical and Rehabilitation Medicine* In Press, 2020.
577 43. **Kirkman DL, Ramick MG, Muth BJ, Stock JM, Townsend RR, and Edwards DG**.
578 Sex differences in microvascular function and arterial hemodynamics in nondialysis chronic
579 kidney disease. *Am J Physiol Heart Circ Physiol* 323: H1130-h1136, 2022.
580 44. **Razmara A, Sunday L, Stirone C, Wang XB, Krause DN, Duckles SP, and**
581 **Procaccio V**. Mitochondrial effects of estrogen are mediated by estrogen receptor alpha in
582 brain endothelial cells. *J Pharmacol Exp Ther* 325: 782-790, 2008.
583
584

585 **Figure 1.** Participant flow through study

586 CPET, cardiopulmonary exercise testing

587

588 **Figure 2.** Compared with Placebo**,** 4 weeks of MitoQ supplementation significantly improved
589 flow mediated dilation, a *in vivo* assessment of conduit artery endothelial function, in patients
590 with Stage 3- 4 CKD.

591 MTQ, MitoQ; PLB, Placebo; Data are mean ± SEM; *, $p < 0.05$

592

593 **Figure 3.** There we no significant changes between placebo and treatment groups in central
594 systolic pressure (A) and central pulse pressure (B). MitoQ supplementation mitigated a
595 significant increase in augmentation pressure (C) and augmentation index (D) that was
596 observed in the Placebo group.

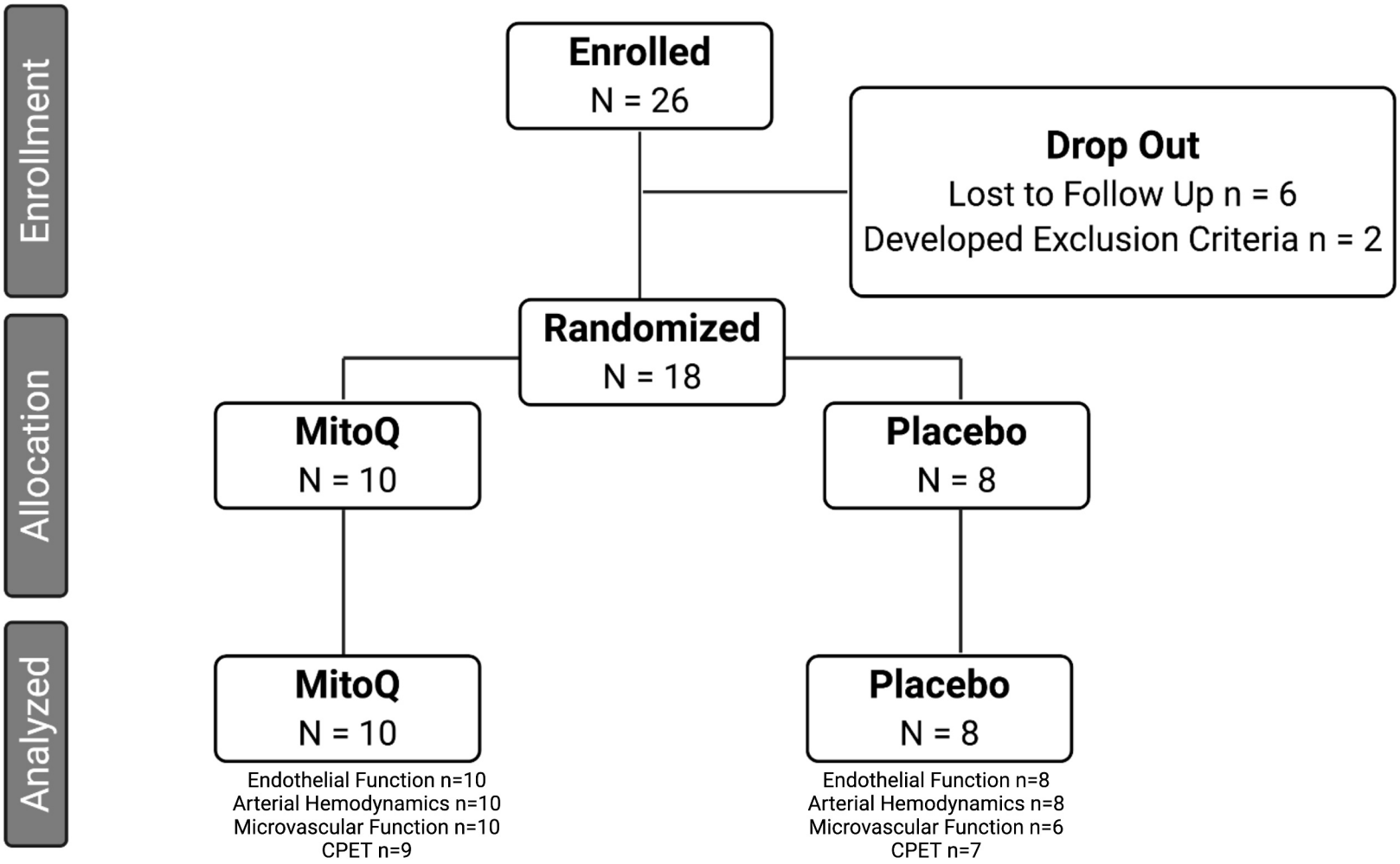
597 MTQ, MitoQ; PLB, Placebo; Data are mean ± SEM; *, $p < 0.05$.

598

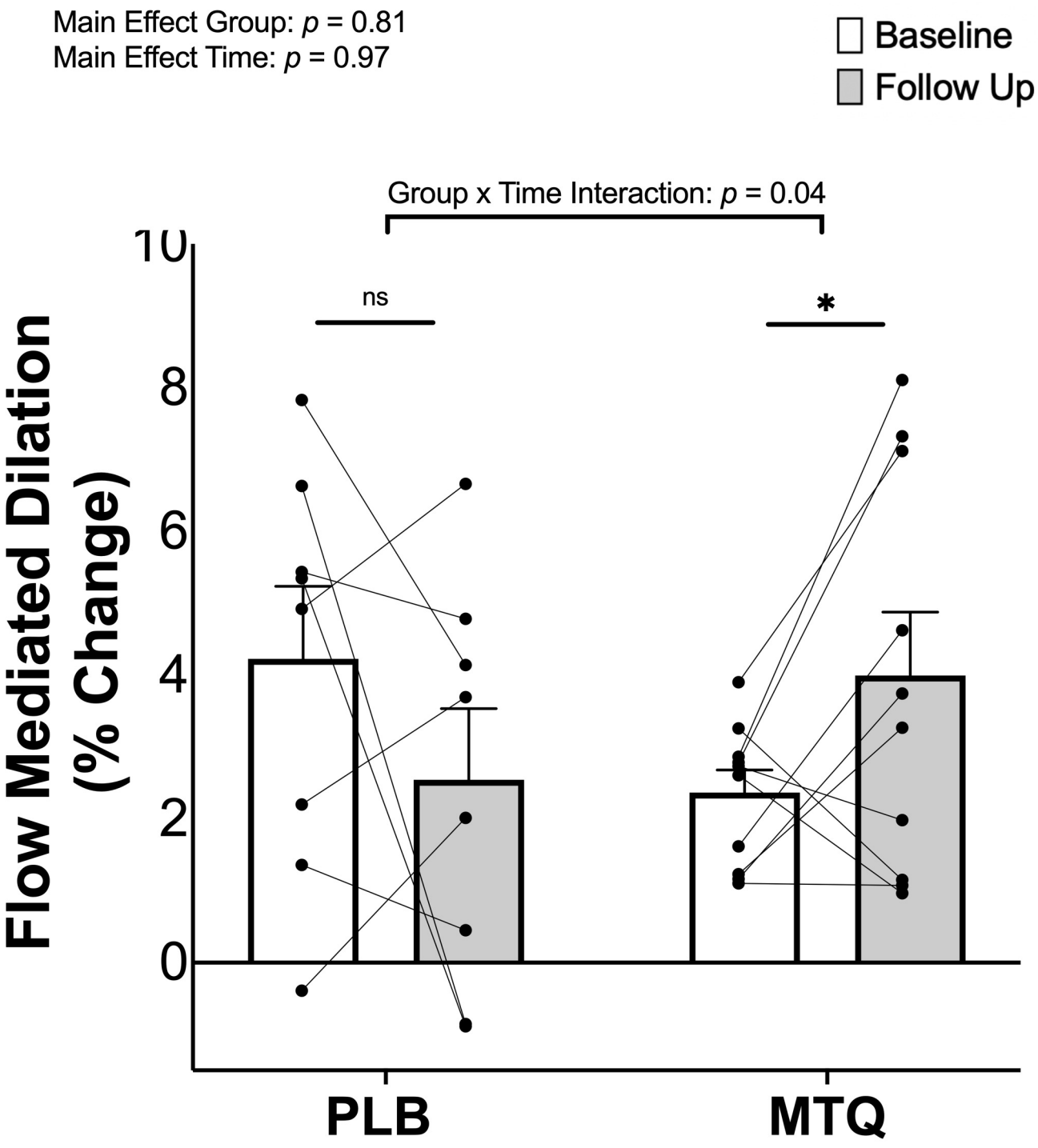
599 **Figure 4.** Four weeks of MitoQ supplementation improved microvascular function, assessed
600 by the cutaneous vasodilatory response to local heating at the Ringer's control site, compared
601 with placebo (A). In patients that received MitoQ, panel (B) shows that at baseline, the local
602 delivery of Apocynin significantly augmented microvascular function, reproducing previous
603 findings that NADPH oxidases contributes to CKD related microvascular dysfunction(3).
604 However, at follow up, Apocynin was no longer effective at increasing microvascular function,
605 suggesting that the beneficial effects of MitoQ on microvascular function may partly be
606 mediated by a reduction in the detrimental effects of NADPH oxidases.

607 MTQ, MitoQ; PLB, Placebo; Data are mean ± SEM; *, $p < 0.05$ compared to Baseline Ringer's

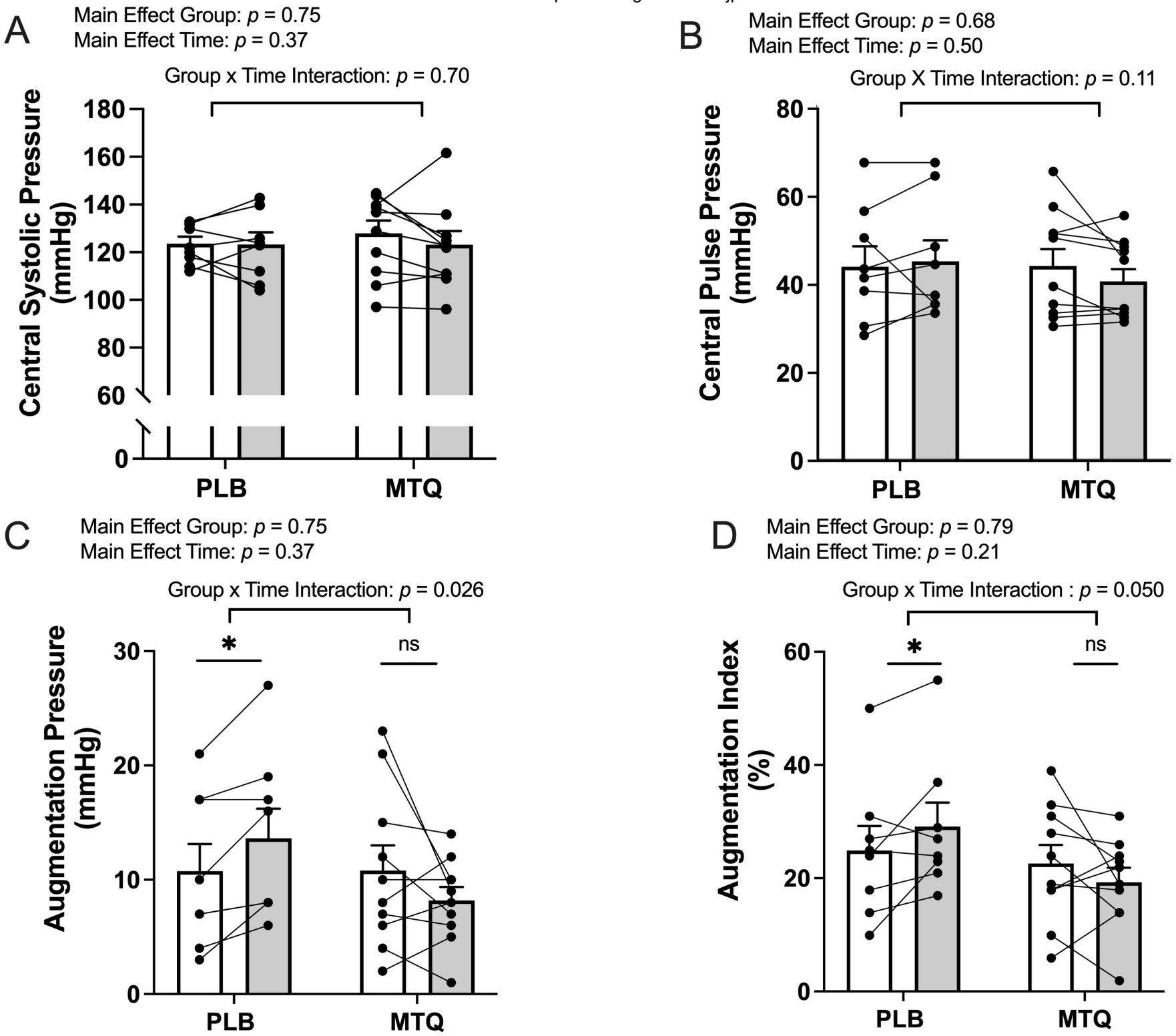
Accepted Manuscript
Version of Record at: https://doi.org/10.1152/ajprenal.00067.2023



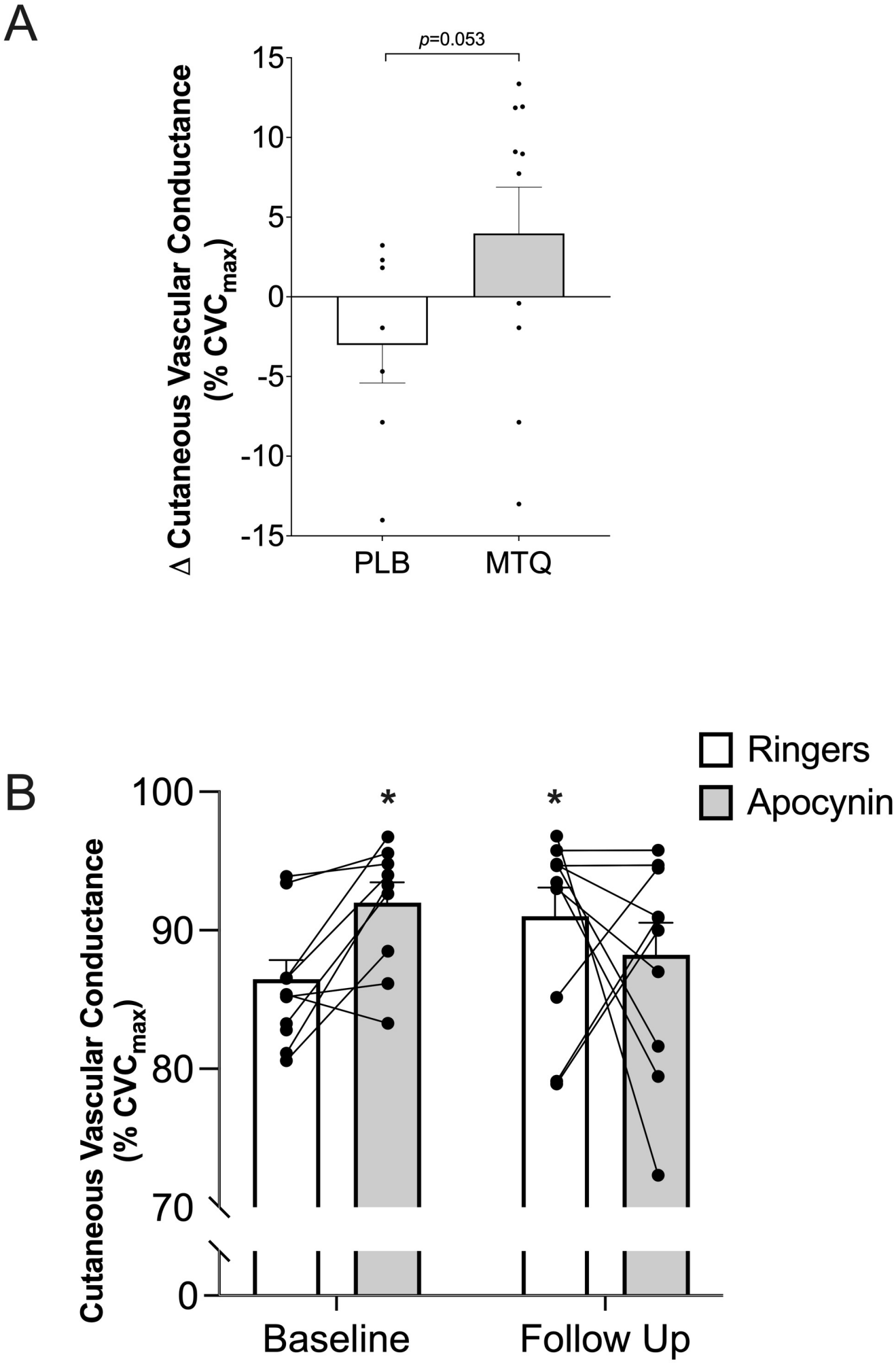
Accepted Manuscript
Version of Record at: https://doi.org/10.1152/ajprenal.00067.2023



Accepted Manuscript
Version of Record at: https://doi.org/10.1152/ajprenal.00067.2023



Accepted Manuscript
Version of Record at: https://doi.org/10.1152/ajprenal.00067.2023



Accepted Manuscript
Version of Record at: https://doi.org/10.1152/ajprenal.00067.2023

Table 1. Participant characteristics.

	Placebo	MitoQ	*p*
Demographics			
n	8	10	
Age (yr)	64 (33-78)	61 (32-75)	0.68
Sex (n)			0.39
Female	2	1	
Male	6	9	
Ethnicity (n)			0.28
African American/Black	3	1	
Caucasian/white	5	9	
Body Mass Index (kg/m^2)	32 (25 -45)	31 (19-43)	0.70
Hemodynamics			
Systolic Blood Pressure (mmHg)	131(119-148)	127(109-148)	0.52
Diastolic Blood Pressure (mmHg)	81(62-90)	77 (61-86)	0.51
Mean Arterial Pressure (mmHg)	97(86-109)	95(78-104)	0.48
Hematology and Biochemistry			
eGFR (ml/min/1.73m^2)	45(30-58)	51(25-67)	0.85
Hemoglobin A1c (%)	6.1(5.1-7.5)	6.0(5.2-7.3)	0.89
Total Cholesterol (mg/dL)	183(109-272)	184(135-271)	0.97
High-Density Lipoprotein (mg/dL)	46(30-60)	48(35-76)	0.74
Low-Density Lipoprotein (mg/dL)	108(54-175)	109(65-178)	0.94
Medication (n)			
Beta-Blockers	1	2	0.58
ACE-I	2	1	0.41
ARB	2	1	0.55
Calcium Channel Blocker	0	4	0.06
Diuretic	2	2	0.61
Glucose Lowering Agent	2	2	0.61
Statin	3	3	0.60

ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; eGFR, estimated glomerular filtration rate. Values are mean (Range).

Accepted Manuscript
Version of Record at: https://doi.org/10.1152/ajprenal.00067.2023

Table 2. Microvascular plateau response to local heating at the Ringer's site. The change in cutaneous vascular conductance from baseline to follow up was different between Placebo and MitoQ groups.

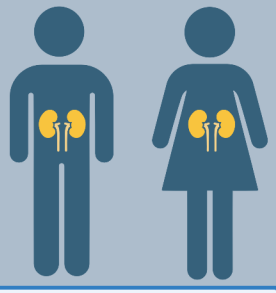
	Baseline	Follow Up	Δ CVC
Placebo	87.08 ± 3.58	84.86 ± 3.85	-2.22 ± 2.67
MitoQ	86.47 ± 1.38	91.98 ± 1.46	4.50 ± 2.57 *

CVC, cutaneous vascular conductance reported as $\%_{max}$; Data are mean ± SEM
*, p = 0.053 between groups

Effects of a Mitochondrial Targeted Ubiquinol (MitoQ) on Vascular Function and Exercise Capacity in Chronic Kidney Disease: A Randomized Controlled Pilot Study

METHODS

18 men and women with non-dialysis CKD



Randomized

Mito Q
20mg/day
4 weeks

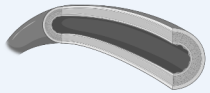
Placebo
1 capsule/day
4 weeks

OUTCOME MEASURES

Assessed at Baseline & 4 Weeks

OUTCOME

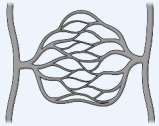
Improved conduit artery endothelial function



Maintained arterial hemodynamics, namely augmentation pressure

Mitochondrial Targeted Ubiquinol

Did not affect exercise maximal exercise capacity



Improved microvascular function, potentially through a NADPH oxidase related mechanism

CONCLUSIONS

- 4 weeks of MitoQ supplementation was safe a well tolerated in patients with non dialysis chronic kidney disease
- This pilot study demonstrates the potential for mitochondrial targeted strategies to improve vascular function.
- MitoQ had no effect on exercise capacity.