Accepted Manuscript
Version of record at: https://doi.org/10.1152/ajpheart.00500.2022

Sex Differences in Microvascular Function and Arterial Hemodynamics in Non-Dialysis Chronic Kidney Disease

Danielle L Kirkman[2]; Meghan G Ramick[3]; Bryce J Muth[4]; Joseph M Stock[1]; Raymond R Townsend[5]; David G Edwards[1].

[1]Department of Kinesiology and Applied Physiology, University of Delaware

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

[3]Department of Kinesiology, West Chester University

[4]School of Health Sciences, Stockton University

[5]Perelman School of Medicine, University of Pennsylvania

Running Head: Sex Differences in CKD Related Vascular Dysfunction

Corresponding Author:

David G. Edwards, PhD

201Q Health Sciences Complex

540 S. College Ave

Newark, DE 19716

Phone: 302 831 3363

Email: dge@udel.edu

Accepted Manuscript
Version of record at: https://doi.org/10.1152/ajpheart.00500.2022

ABSTRACT

PURPOSE Cardiovascular disease (CVD) is the leading cause of death in chronic kidney disease (CKD). Abnormal arterial hemodynamics contribute to CVD, a relationship that can be mediated by microvascular dysfunction. The purpose of this study was to investigate potential sex differences in arterial hemodynamics and microvascular dysfunction in patients with Stage 3-4 CKD.

METHODS Vascular function was assessed in 22 male (Mean±SD: Age, 56±13 years) and 10 female (Age, 63±9 years) patients. Arterial hemodynamics were acquired with combined tonometry and oscillometry. Skin blood flow was utilized as a model of microvascular function. Participants were instrumented with three microdialysis fibers for the delivery of 1) Ringer's solution 2) superoxide dismutase mimetic, tempol 3) nicotinamide adenine dinucleotide phosphate (NADPH) oxidase inhibitor, apocynin. Blood flow was measured via laser Doppler flowmetry during standardized local heating (42°C).

RESULTS Central pulse pressure (Mean±SEM: 62±9 *vs*. 46±3 mmHg; p=0.01) and augmentation index (36±3 *vs*. 26±3 %; p=0.03) were higher in females. There was a trend for higher central systolic pressures in females (146±9 *vs*. 131±3 mmHg; p=0.06). Females reported higher forward (39±4 *vs.* 29±2 mmHg; p=0.004) and reflected wave amplitudes (27±3 *vs.* 19±1 mmHg; p<0.001). Cutaneous vascular function was impaired in females compared with males (77±3 *vs.* 89±1 %, p=0.001). Microvascular function was improved following the delivery of tempol and apocynin in females but not males.

CONCLUSION Female patients with CKD had poorer central hemodynamics and reduced microvascular function compared with their male counterparts. Oxidative stress may contribute to lower microvascular function observed in females.

Accepted Manuscript
Version of record at: https://doi.org/10.1152/ajpheart.00500.2022

Keywords chronic kidney disease, microvascular dysfunction, arterial stiffness, central pulse pressure, sex differences

NEW AND NOTEWORTHY

There is limited data regarding the physiological mechanisms of potential sex differences in central hemodynamics and vascular function in chronic kidney disease (CKD). We report that older female patients with non-dialysis CKD have higher central pulse pressures compared with male patients with CKD. Additionally, older females with CKD have lower microvascular function compared with their male counterparts and oxidative stress contributes to the lower microvascular function in older female patients with CKD.

Accepted Manuscript
Version of record at: https://doi.org/10.1152/ajpheart.00500.2022

INTRODUCTION

Chronic kidney disease (CKD) is a global epidemic affecting ~15% of the US population(1). With an etiology unique to CKD, the incidence of cardiovascular disease (CVD) is substantially higher in these patients compared with the general population. Within the field of biomedical sciences, the mission to produce generalizable scientific studies had led to the fundamental recognition of sex as a biological variable(2). In cardiovascular medicine and nephrology, there has been a drive to better understand the role of sex differences in the underlying pathophysiology of the development and progression of disease(2). Furthermore, in the general population CVD prevalence is substantially higher in males compared with premenopausal females, a trend that is not observed in patients with CKD wherein CVD incidence is almost the same in males and females(1). This suggests that the cardioprotective effect of female sex before menopause is observed in the general population does not convey protection to females with CKD. The pathophysiology of sex differences in CKD and CVD incidence is not yet fully understood.

Vascular dysfunction characterized by impaired microvascular function and arterial stiffness are hallmarks of CKD that contribute to the CVD burden. Increased arterial stiffening and aberrant arterial hemodynamics are consistently reported in this disease state and contribute to development of CVD and the progression of renal dysfunction (3, 4). Specifically, large artery stiffness and abnormal arterial hemodynamics result in an increased left ventricular pulsatile load with the subsequent development of heart failure, a prominent contributor to mortality and morbidity in CKD(3). Furthermore, increased stiffening of the aorta and abnormal arterial hemodynamics promotes increased pulsatility in the microvasculature(4). The microvasculature of the kidney is highly susceptible to the upstream fluctuation in

pulsatility that, in combination with CKD related impaired renal autoregulation, ultimately culminates in end organ damage and therefore a progression of renal disease(5). Oxidative stress has been implicated as a major contributor to CKD related vascular dysfunction, particularly in Stage 3-4 CKD (6). However, there is limited knowledge regarding the physiological mechanisms of potential sex differences in vascular dysfunction in CKD. Therefore, the aim of this study was to determine if there are sex differences in arterial and microvascular function in mild to moderate CKD and explore the role oxidative stress as a mechanistic contributor to potential differences.

METHODS

Participants

This was a retrospective analysis of baseline data collected for a clinical trial (NCT02050035). All procedures were approved by the University of Delaware Institutional Review Board and were performed according to guidelines set forth by the Declaration of Helsinki. All participants provided written informed consent. Patients with non-dialysis CKD were recruited from local Nephrology outpatient clinics. Eligibility criteria were assessed during a comprehensive screening visit that included a medical history, a physical exam, routine clinical blood work and urinalysis. Patients were considered eligible to participate if they presented with Stage 3-4 CKD (estimated glomerular filtration rate [eGFR] <60 ml/min/1.73m^2) and >18 years. Patients were excluded if they were they presented with a history CVD (defined as a diagnosis of coronary artery disease; myocardial infarction; heart failure; peripheral artery disease; or a cerebrovascular accident/transient ischemic attack); uncontrolled hypertension; current cancer, lung or liver disease; currently receiving immunosuppressant, antiretroviral, hormone replacement therapy; current renal replacement therapy; current pregnancy; hemoglobin <11g/dL; current tobacco use; or if they were unable to provide informed consent.

Notably, we excluded patients with a history of CVD as we aimed to investigate vascular dysfunction prior to the development of overt CVD. As this was a retrospective analysis, menopause status and serum sex hormone levels were unavailable.

Experimental Visit

Participants attended one experimental visit to a temperature-controlled laboratory. Participants were asked to refrain from alcohol and exercise for 24 hours and caffeine intake at least 12 hours prior to the scheduled visit. Participants were asked to withhold any morning medications and arrive to the visit having fasted for at least 6 hours.

Experimental Procedures

Cutaneous Microvascular Function.

We utilized the skin blood flow model as a representation of microvascular function. The cutaneous vasculature is an easily accessible vascular bed that is representative of systemic vascular function (7). This model allows an *in vivo* approach to examine underlying mechanistic contributions to microvascular function. The skin blood flow response to local heating was assessed by laser Doppler flowmetry. Simultaneous delivery of pharmacological substances via intradermal microdialysis allowed for the dissection of physiological mechanisms that contribute to microvascular function.

A 23G needle was used as a guide cannula to place three microdialysis probes (CMA Microdialysis, Sweden) on the forearm for the local delivery of pharmacological substances. Once instrumented, Ringers' solution was infused (Bee Hive controller, Baby Bee microinfusion pumps, Bioanalytical Systems Inc, IN) through the probes at 2uL/min for 60–90 minutes to allow resolution of insertion hyperemia. Skin blood flow, represented by cutaneous

red blood cell (RBC) flux, was assessed by laser Doppler flowmetry from a 1.5mm^2 area of skin at each site. Multifiber laser Doppler probes placed in local heating units were fixed over the microdialysis membrane portion of each probe (Temperature Monitor SHO_2, Moor Instruments, UK). Blood pressure was recorded every 15 minutes throughout the protocol on the contralateral arm.

Cutaneous microvascular function was experimentally assessed with a standardized local heating protocol (8-11). Once the insertion hyperemia had subsided, microdialysis sites received an infusion of either Ringers' solution (control), 10μM tempol (superoxide dismutase mimetic; Sigma Aldrich, MO) or 100 μM apocynin (nicotinamide adenine dinucleotide phosphate [NADPH] oxidase inhibition; Sigma Aldrich, MO) at 2μL/minute. Local heaters were set to 33°C and baseline RBC flux was recorded for ~20 minutes. Temperature was then increased at a rate of 1°C/s to 42°C where it was maintained throughout the heating protocol. The biphasic blood flow response to heating is comprised of an initial peak within the first 5 minutes which is primarily mediated by an axon reflex (12), followed by a steady increase to a plateau that is ~60% mediated by endothelial nitric oxide synthase (eNOS) and ~40% mediated by endothelial derived hyperpolarizing factors (12-14). Once a stable plateau was achieved, the maximum cutaneous vasodilation at each site was obtained with the infusion of 28mM sodium nitroprusside (SNP) at 4uL/min and concurrent local heating to 43°C. Skin blood flow was reported as cutaneous vascular conductance (CVC) calculated as RBC flux divided by mean arterial pressure. CVC was reported as a percentage of the maximum CVC obtained during SNP infusion to normalize data between microdialysis sites. Baseline and plateau values were averaged over a 10-minute period and initial peak values were averaged over one minute.

Arterial Stiffness and Central Hemodynamics.

Measures of arterial stiffness and central hemodynamics were performed in the supine position. Arterial stiffness was represented by carotid to femoral pulse wave velocity (PWV). PWV was assessed by simultaneous acquisition of the carotid pulse by applanation tonometry and the femoral pulse by oscillometry (SphygmoCor XCEL, Atcor Medical, Australia). Pulse transit distances were calculated using the subtraction method. Aortic pressure waves were synthesized from brachial artery waveforms acquired with oscillometry and the use of the generalized transfer function (SphymgmoCor XCEL, Atcor Medical, Australia). Augmentation index was calculated as the ratio of the augmentation pressure to pulse pressure using the central pressure waveform. Wave separation analysis was performed on the central pressure waveform to determine forward and reflected wave amplitudes using a modified triangular flow waveform.

Statistical Analyses

Statistical analyses were performed with the use of SPSS (v.27, IBM). Differences between groups were analyzed with Chi-squared and Student's independent sample *t*-tests, ensuring all assumptions of the tests were met. A repeated measures ANOVA was performed to assess differences in blood flow responses to pharmacological substance infusions that were dependent on sex (Sex * Drug). Pairwise comparisons were performed following a significant omnibus test with Bonferroni adjustments for multiple comparisons. Univariate analysis of variance was performed to assess the effect of potential covariates on reported sex differences. Statistical significance was set at $p \leq 0.05$. Participant characteristic data are mean ± standard deviation (SD), all other data are mean ± standard error of the mean (SEM). Cohen's *d* effect sizes between groups were calculated ($[Mean_{male}\text{-}Mean_{female}]/SD_{pooled}$) and

interpreted as small (0.2), medium (0.5) and large (0.8). Power analyses were performed post hoc (G-Power, v3.1).

RESULTS

Participants

Data from 22 males (56 ± 13 years; range, 31-72 years) and 10 females (63 ± 9 years, range, 42-78 years) patients with Stages 3-4 CKD were included. Four participants from the parent trial were not included in the current analysis. One was excluded because the cause of kidney disease was reported as sarcoidosis which could result in a different manifestation of CVD. The skin blood flow data from three participants included statistical outliers. The characteristics of study participants are presented in **Table 1**. As expected, high density lipoprotein cholesterol was higher in females compared with males (15). Otherwise, there were no significant differences in demographics, resting hemodynamics and medication use between sexes. Biochemistry and hematology values were characteristic of stage 3-4 CKD. Thirty-two percent of males and 30% of females were diabetic (p = 0.9).

Microvascular Function

Microvascular assessments are reported for 19 males and nine females. Four data sets were excluded due to missing data points from technical errors (n=2) or unphysiological responses (i.e. plateau response to heating < 60% CVC_{max}; n=2), all from the apocynin microdialysis sites. There were no significant differences in the initial peak response to local heating between sexes or microdialysis drug infusions (Table 2). There was a significant sex * drug interaction for the plateau response to local heating (p = 0.008), indicating that there was a difference in the blood flow response to pharmacologic agent delivery that was dependent on sex (**Figure 1**). Post hoc analysis revealed that the plateau blood flow response to local heating was significantly lower in females compared with males (Ringer's site, female *vs.* male:

77 ± 3 *vs.* 89 ± 1 %, p = 0.001; d = 1.2; power = 81%; **Figure 1**), signifying impaired microvascular function in female patients compared with their male counterparts. This sex difference remained (p=0.04) after adjustment for systolic blood pressure, age, race and antihypertensive medication use. In male patients, the local delivery of tempol (Ringer's *vs.* tempol: 88 ± 1 *vs.* 90 ± 2 %, p =1.0) or apocynin (Ringer's *vs.* apocynin: 88 ± 1 *vs.* 91 ± 1 %, p =0.8; **Figure 1**) had no significant effect on plateau blood flow response to local heating. In contrast, in female patients, the local delivery of both tempol (Ringer's *vs.* tempol: 77 ± 3 *vs.* 90 ± 2 %, p =0.001) and apocynin (Ringer's *vs.* apocynin: 77 ± 3 *vs.* 89 ± 1 %, p =0.002; **Figure 1**) significantly improved plateau blood flow response to local heating. This suggests that oxidative stress may contribute to microvascular dysfunction in female patients with stage 3-4 CKD. Absolute maximum CVC values were not significantly different between sexes or microdialysis drug infusions (**Table 2**), therefore the differences in microvascular function observed herein cannot be attributed to a superior maximal vasodilatory capacity in males, or after antioxidant delivery.

Arterial Stiffness and Central Hemodynamics

Central hemodynamics and arterial stiffness were assessed in 22 males and 9 females. One female dataset was excluded due to a technical error. In comparison to males, females showed a trend towards higher central systolic pressures and significantly higher central pulse pressures (**Table 3**). The central augmentation index was significantly higher in female patients (**Table 3**). Both forward and reflected waveform magnitudes were significantly higher in females compared with males (**Table 3**). There were no significant differences in pulse wave velocity between sexes (**Table 3**).

DISCUSSION

The findings of this study show that compared with their male counterparts, female patients with CKD and no overt CVD have significantly higher central pulse pressures and impaired microvascular function. A novel finding is that NADPH derived oxidative stress contributes the impaired microvascular function observed in females with CKD. A recent investigation into sex differences in vascular function in CKD patients reported better conduit artery endothelial function in younger (<55 years) female patients with CKD compared with males. However, with advancing age the differences in vascular endothelial function between males and females diminished. Furthermore, once females entered a peri and post-menopausal age range, the decline in vascular function was faster in female patients (16). These findings are compatible with the results presented herein that studied a cohort of female patients with a median age of 65 years.

In the absence of overt CVD, females with CKD trended to have higher systolic aortic pressures, and had higher central pulse pressures and augmented forward and reflected pulse waveforms. This is clinically noteworthy as heightened central systolic pressure and pulse pressure have previously been associated with adverse CVD outcomes in patients with non-dialysis CKD (17). Importantly, patients with increased central and pulse pressures are at a higher risk of developing heart failure (3), which is one of the most prevalent CKD related CVDs (1). Arterial wave reflection amplitudes have previously been associated with a decline in kidney function (18). The findings of this study reveal that female patients have higher forward and reflected waveform amplitudes compared males. Following left ventricular (LV) ejection, a forward pulse wave is propagated. The magnitude of the forward wave amplitude can be influenced by the stroke volume, aortic impedance and arterial stiffness and is typically elevated in the setting of high central pulse pressures(19, 20), as indicated by our findings. It is possible that forward wave amplitudes are magnified due to increased re-reflections from the

larger reflected wave(21). Whether the larger forward wave amplitudes observed in the female patients with CKD is a result of higher stroke volumes, greater aortic impedance, greater pulse pressures or increased reflections from the reflected wave warrants further investigation with more in-depth hemodynamic modeling methods. When the forward travelling pressure wave reaches reflection sites such as bifurcations, changes in arterial radius, or sites of mismatched impedance, the proportion of the waveform is reflected and usually arrives at the heart during diastole to facilitate coronary filling(19, 20). In the setting of arterial stiffness or increased peripheral vascular resistance, a faster traveling waveform of greater magnitude arrives at the heart during late systole, subsequently increasing the LV pulsatile load (19, 20). Increases in late systolic LV pulsate load have been implicated in the development of heart failure(22), particularly heart failure with preserved ejection fraction – a syndrome that is more prevalent in females. As there were no significant differences in arterial stiffness between male and female patients in this study, we suggest that the aberrant arterial hemodynamics observed our cohort of female patients are mediated by cardiac or microvascular abnormalities. Based on our findings pertaining to heightened microvascular dysfunction in female patients with CKD, we suggest that microvascular dysfunction is likely to playing a large role. These speculations should be followed up in future investigations.

Our findings also show that microvascular function was significantly impaired in female patients with CKD compared with males. This is noteworthy as microvascular dysfunction is a key mechanism in the pathology of ischemic heart disease with non-obstructed coronary arteries (INOCA) and HFpEF, both of which are disproportionally higher in females(23, 24). Furthermore, our results show significant microvascular dysfunction in female patients with CKD before the development of overt CVD. Therefore, microvascular dysfunction may be an important therapeutic target in the prevention of CVD in females with CKD. The delivery of the

superoxide dismutase (SOD) mimetic tempol, and the NADPH oxidase inhibitor apocynin both augmented microvascular function in females but had no effect in males. This implicate oxidative stress mediated NADPH derived reactive oxygen species as contributing mechanisms to microvascular dysfunction in female patients with CKD. Our findings are consistent with work from others that show a marked decline in vascular function with age in females from both the general and CKD population (16, 25). Recent work from Moreau *et al*. elegantly demonstrated that the loss of ovarian estradiol is implicated in the attenuation of age related vascular endothelial function (25). This work showed that an altered redox balance and the subsequent increase in oxidative stress in response to the loss of estradiol mediated the observed relationship between the sex hormone and vascular function (25). The role of sex hormones in mediating CKD-related vascular dysfunction is an important area of work for the future. The findings of this study provide a rationale for future work that investigates strategies aimed at reducing oxidative stress to improve microvascular function in female patients. Although not statistically significant, females did tend to report higher blood pressures. It is possible that the differences in microvascular function between groups are driven by chronically higher blood pressures in the females. However, recent evidence from hypertensive patients has shown that microvascular dysfunction can persist despite therapeutically mediated reductions in blood pressure(26). Future longitudinal studies or experimental studies that manipulate blood pressure should investigate if the lower microvascular function in females with CKD is a consequence of higher blood pressures.

In contrast to our findings, a recent meta-analysis and systematic review showed marginally greater CV mortality rates in male compared with female patients with CKD (27). A potential explanation for the difference in findings reported in our study is that the majority of studies included in the meta-analyses were focused on the dialysis population (41 studies),

compared with only 4 studies in the non-dialysis population (27). Furthermore, we studied non-dialysis patients without overt CVD. An additional study from the CRIC database reported CVD prevalence and incidence to be higher in female patients with CKD (28). The female patients in this large cohort were slightly younger than those studied here. As previously mentioned, after menopause vascular function appears to deteriorate at a faster rate in female patients, which could potentially explain our findings (16).

There are several limitations of this study. First, the forward and reflected waveform analyses are limited by the use of the triangulated flow method. Although triangulation waveforms are now widely used in the assessment of arterial hemodynamics, they have been shown to underestimate aortic flow and subsequently overestimate reflected waveform amplitudes, particularly in older adults (29). Future studies should aim to confirm these findings with the use of flow waveforms derived from echocardiography or magnetic resonance imaging (29). Second, as this was a retrospective analysis from a previous clinical trial, the findings of this study are limited by the lack of sex hormone analyses. All but one female patient were above the age range of the post-menopausal transition, however, measurements of sex hormones such as ovarian estradiol would have been beneficial in supporting the post-menopausal status in the female participants.

In conclusion, the findings of this study demonstrate that older females with Stage 3-4 CKD present with aberrant arterial hemodynamics and impaired microvascular dysfunction compared with their male counterparts. Importantly, this vascular dysfunction is evident before overt CVD is detected. For the first time, we show that oxidative stress may contribute to the observed sex difference in microvascular dysfunction in CKD. Therapeutic strategies aimed at

334 improving the redox balance should be investigated to improve vascular health in female
335 patients with non-dialysis dependent CKD.
336

Acknowledgments

The authors would like to acknowledge the research participants.

Funding

Supported by the NHLBI of the NIH under award number R01HL113514.

Disclosures

None

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. **Lindsey ML, LeBlanc AJ, Ripplinger CM, Carter JR, Kirk JA, Hansell Keehan K, Brunt KR, Kleinbongard P, and Kassiri Z**. Reinforcing rigor and reproducibility expectations for use of sex and gender in cardiovascular research. *Am J Physiol Heart Circ Physiol* 321: H819-h824, 2021.

3. **Chirinos JA, Khan A, Bansal N, Dries DL, Feldman HI, Ford V, Anderson AH, Kallem R, Lash JP, Ojo A, Schreiber M, Sheridan A, Strelsin J, Teal V, Roy J, Pan Q, Go AS, and Townsend RR**. Arterial stiffness, central pressures, and incident hospitalized heart failure in the chronic renal insufficiency cohort study. *Circ Heart Fail* 7: 709-716, 2014.

4. **Townsend RR**. Arterial stiffness and chronic kidney disease: lessons from the Chronic Renal Insufficiency Cohort study. *Curr Opin Nephrol Hypertens* 24: 47-53, 2015.

5. **Mitchell GF**. Aortic stiffness, pressure and flow pulsatility, and target organ damage. *J Appl Physiol (1985)* 125: 1871-1880, 2018.

6. **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.

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

8. **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.

9. **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.

10. **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.

11. **Greaney JL, DuPont JJ, Lennon-Edwards SL, Sanders PW, Edwards DG, and Farquhar WB**. Dietary sodium loading impairs microvascular function independent of blood pressure in humans: role of oxidative stress. *J Physiol* 590: 5519-5528, 2012.

12. **Minson CT, Holowatz LA, Wong BJ, Kenney WL, and Wilkins BW**. Decreased nitric oxide- and axon reflex-mediated cutaneous vasodilation with age during local heating. *J Appl Physiol (1985)* 93: 1644-1649, 2002.

13. **Holowatz LA, Thompson CS, Minson CT, and Kenney WL**. Mechanisms of acetylcholine-mediated vasodilatation in young and aged human skin. *J Physiol* 563: 965-973, 2005.

14. **McCord GR, Cracowski JL, and Minson CT**. Prostanoids contribute to cutaneous active vasodilation in humans. *Am J Physiol Regul Integr Comp Physiol* 291: R596-602, 2006.

15. **Lew J, Sanghavi M, Ayers CR, McGuire DK, Omland T, Atzler D, Gore MO, Neeland I, Berry JD, Khera A, Rohatgi A, and de Lemos JA**. Sex-Based Differences in Cardiometabolic Biomarkers. *Circulation* 135: 544-555, 2017.

16. **Kruse NT, You Z, Moreau K, Kendrick J, and Jalal D**. Sex differences in endothelial function in chronic kidney disease. *Am J Physiol Renal Physiol* 319: F33-f40, 2020.

17. **Rahman M, Hsu JY, Desai N, Hsu CY, Anderson AH, Appel LJ, Chen J, Cohen DL, Drawz PE, He J, Qiang P, Ricardo AC, Steigerwalt S, Weir MR, Wright JT, Jr., Zhang X,**

and Townsend RR. Central Blood Pressure and Cardiovascular Outcomes in Chronic Kidney Disease. *Clin J Am Soc Nephrol* 13: 585-595, 2018.

18. **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.

19. **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.

20. **Chirinos JA, and Segers P**. Noninvasive evaluation of left ventricular afterload: part 2: arterial pressure-flow and pressure-volume relations in humans. *Hypertension* 56: 563-570, 2010.

21. **Phan TS, Li JK, Segers P, and Chirinos JA**. Misinterpretation of the Determinants of Elevated Forward Wave Amplitude Inflates the Role of the Proximal Aorta. *J Am Heart Assoc* 5: 2016.

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

23. **Del Buono MG, Montone RA, Camilli M, Carbone S, Narula J, Lavie CJ, Niccoli G, and Crea F**. Coronary Microvascular Dysfunction Across the Spectrum of Cardiovascular Diseases: JACC State-of-the-Art Review. *J Am Coll Cardiol* 78: 1352-1371, 2021.

24. **Bairey Merz CN, Pepine CJ, Walsh MN, and Fleg JL**. Ischemia and No Obstructive Coronary Artery Disease (INOCA): Developing Evidence-Based Therapies and Research Agenda for the Next Decade. *Circulation* 135: 1075-1092, 2017.

25. **Moreau KL, Hildreth KL, Klawitter J, Blatchford P, and Kohrt WM**. Decline in endothelial function across the menopause transition in healthy women is related to decreased estradiol and increased oxidative stress. *Geroscience* 42: 1699-1714, 2020.

26. **Fermoyle CC, Broxterman RM, La Salle DT, Ratchford SM, Hopkins PN, Richardson RS, and Trinity JD**. Persistent vascular dysfunction following an acute nonpharmacological reduction in blood pressure in hypertensive patients. *J Hypertens* 40: 1115-1125, 2022.

27. **Shajahan S, Amin J, Phillips JK, and Hildreth CM**. Relationship between sex and cardiovascular mortality in chronic kidney disease: A systematic review and meta-analysis. *PLoS One* 16: e0254554, 2021.

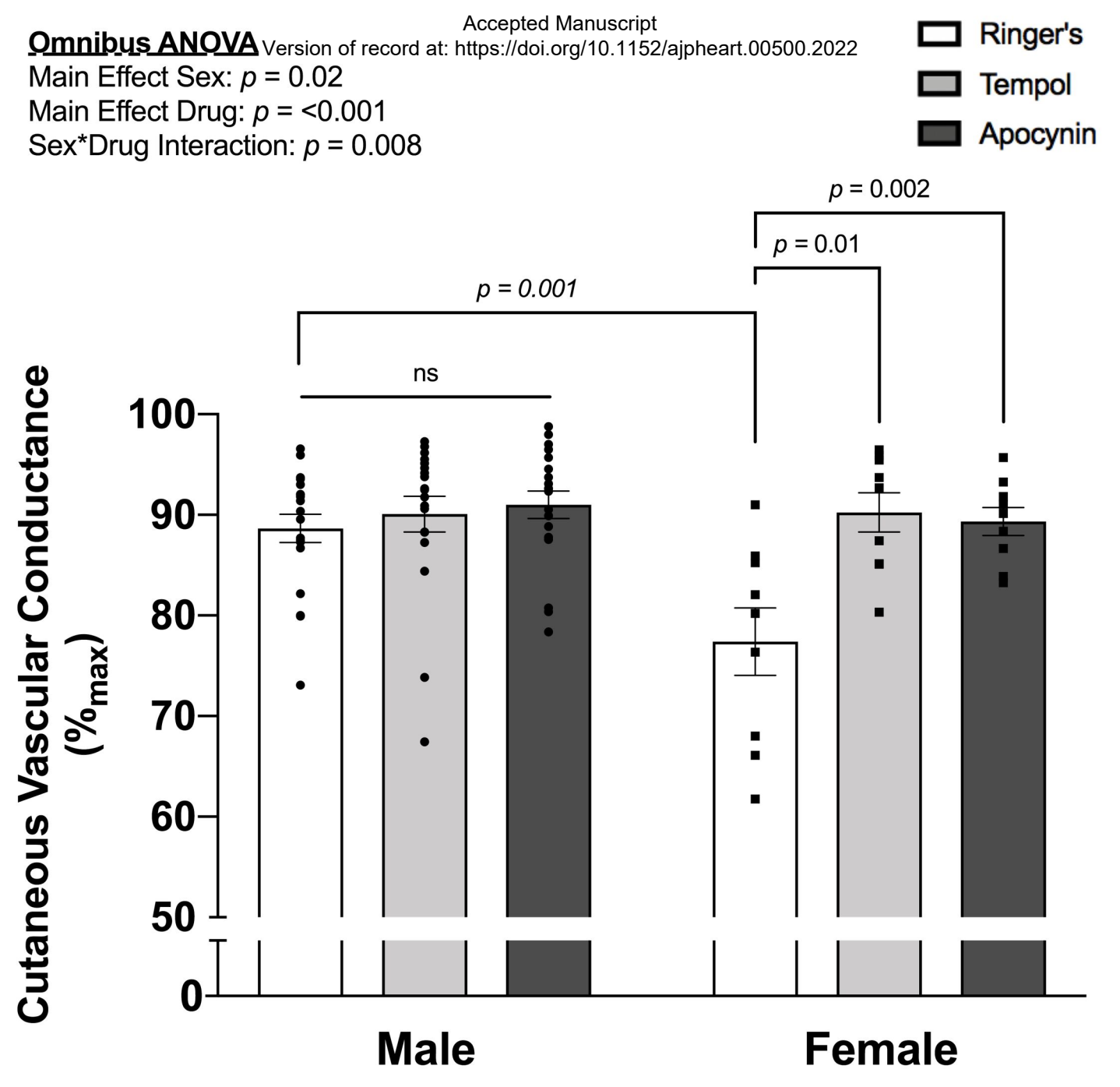
28. **Toth-Manikowski SM, Yang W, Appel L, Chen J, Deo R, Frydrych A, Krousel-Wood M, Rahman M, Rosas SE, Sha D, Wright J, Daviglus ML, Go AS, Lash JP, and Ricardo AC**. Sex Differences in Cardiovascular Outcomes in CKD: Findings From the CRIC Study. *Am J Kidney Dis* 78: 200-209.e201, 2021.

29. **Shenouda N, Stock JM, Patik JC, Chirinos JA, and Edwards DG**. Personalized physiologic flow waveforms improve wave reflection estimates compared to triangular flow waveforms in adults. *Am J Physiol Heart Circ Physiol* 320: H1802-h1812, 2021.

436 **Figure 1.** Cutaneous vascular response to local heating coupled with the local delivery of
437 targeted antioxidants. Microvascular function was impaired in females compared with males as
438 indicated by a lower CVC response to local heating at the Ringer's site. Delivery of the
439 superoxide dismutase mimetic tempol and the NADPH oxidase inhibitor apocynin attenuated
440 microvascular function in females but had no effect in males.
441 CVC, cutaneous vascular conduction
442 Data were analyzed with a repeated measures ANOVA and subsequent post hoc analyses with a Bonferroni
443 correction.

Accepted Manuscript
Version of record at: https://doi.org/10.1152/ajpheart.00500.2022

Omnibus ANOVA
Main Effect Sex: $p = 0.02$
Main Effect Drug: $p = <0.001$
Sex*Drug Interaction: $p = 0.008$



Accepted Manuscript
Version of record at: https://doi.org/10.1152/ajpheart.00500.2022

Table 1. Participant Characteristics

	Male	Female	*p*
Demographics & Anthropometrics			
N	22	10	
Age (years)	58 ± 12	63 ± 9	0.2
Race (n)			
Black	7	4	
White	14	6	
Latin x	1	0	0.7
Body Mass Index (kg/m^2)	32 ± 5	33 ± 7	0.3
Resting Hemodynamics			
Systolic Blood Pressure (mmHg)	133 ± 18	141 ± 23	0.1
Diastolic Blood Pressure (mmHg)	81 ± 12	79 ± 10	0.1
Mean Arterial Pressure (mmHg)	99 ± 14	100 ± 12	0.8
Resting Heart Rate (bpm)	67 ± 11	66 ± 13	0.7
Hematology & Biochemistry			
eGFR (ml/min/1.73m^2)	43 ± 12	43 ±14	0.9
Albumin/Globulin Ratio	1.5 ± 0.2	1.5 ± 0.3	0.3
Blood Urea Nitrogen (mg/dL)	29 ± 13	26 ± 9	0.4
Fasting Blood Glucose (mg/dL)	118 ± 35	111 ± 29	0.6
Hemoglobin A1c (%)	6.6 ± 1.4	6.2 ± 0.7	0.3
Total Cholesterol (mg/dL)	189 ± 36	213 ±75	0.2
HDL (mg/dL)	46 ± 13	68 ± 22	<0.01
LDL (mg/dL)	107 ± 33	119 ± 59	0.5
Medication (n)			
ACE Inhibitor	9	1	0.08
Angiotensin Receptor Blocker	1	5	0.3
Beta Blocker	6	6	1.0
Calcium Channel Blocker	8	3	0.7
Diuretic	6	3	0.8
Anti-diabetic	7	3	0.9
Statin	11	5	0.9

eGFR, estimated glomerular filtration rate; HDL, high density lipoprotein; LDL, low density lipoprotein.
Data are mean ± SD

Accepted Manuscript
Version of record at: https://doi.org/10.1152/ajpheart.00500.2022

Table 2. Axon mediated (initial peak) and maximal vasodilatory blood flow responses to local heating

	Males	**Females**	***p***
Initial Peak (% CVC_{max})			
Ringer's	63 ± 3	58 ± 4	
Tempol	68 ± 3	63 ± 5	
Apocynin	69 ± 2	60 ± 4	0.6
Maximum CVC (AU)			
Ringer's	1.3 ± 0.2	1.5 ± 0.2	
Tempol	1.7 ± 0.2	1.9 ± 0.3	
Apocynin	1.7 ± 0.1	1.3 ± 0.2	0.1

CVC, cutaneous vascular conductance; *p* values are omnibus ANOVA sex*drug interaction.

Accepted Manuscript
Version of record at: https://doi.org/10.1152/ajpheart.00500.2022

Table 3. Comparison of arterial stiffness and central hemodynamic measures between males and females.

	Male	**Female**	***p***	***Effect Size***	**Power**
Peripheral Systolic Pressure (mmHg)	143 ± 3	157 ± 11	0.1	-0.67	0.37
Central Systolic Pressure (mmHg)	131 ± 3	146 ± 9	0.06	-0.85	0.56
Central Pulse Pressure (mmHg)	46 ± 3	62 ± 9	0.01	-0.91	0.66
Augmentation Index (%)	26 ± 3	36 ± 3	0.03	-0.76	0.43
Forward Wave Amplitude (mmHg)	29 ± 2	39 ± 4	0.004	-1.12	0.78
Reflected Wave Amplitude (mmHg)	19 ± 1	27 ± 3	<0.001	-1.13	0.90
Pulse Wave Velocity (m/s)	9.35 ± 0.42	10.10 ± 0.64	0.1	-0.34	0.15