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Relations of Measures of Endothelial Function and Kidney Disease: The Framingham Heart Study

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Abstract

Background—Endothelial dysfunction is prevalent among individuals with end-stage renal disease. Whether endothelial dysfunction is present in moderate chronic kidney disease (CKD) is uncertain.

Study Design—Cross-sectional study.

Settings and Participants—Brachial reactivity measurements were obtained during the seventh examination cycle in 2818 (diameter measurements) and 2256 (flow measurements) Framingham Heart Study Offspring cohort participants (53% women, mean age 61±9 years).

Predictor—Estimated glomerular filtration rate [eGFR] <60 mL/min/1.73m², derived from creatinine- and cystatin-C based estimating equations; microalbuminuria status.

Outcome—Brachial reactivity measurements (baseline brachial diameter, flow-mediated dilation, baseline and hyperemic mean flow).

Measurements—Linear regression models were used to model brachial measures as a function of CKD status and microalbuminuria status.

Results—Overall, 7.3% (n=206) of participants had CKD, and of 2301 with urinary measurements, 10.0% (n=230) had microalbuminuria. Brachial reactivity measures did not differ significantly by CKD status in either creatinine- or cystatin-C based equations, in either age- and sex-, or multivariable-adjusted models. In age- and sex-adjusted models, microalbuminuria was associated with decreased hyperemic mean flow (47.2±1.4 versus 51.4±0.5 mg/g, p=0.005), but the association was not significant after multivariable adjustment (p=0.09).

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Limitations—Predominantly white, ambulatory cohort; results may not be generalizable to other ethnic groups or to individuals with severe CKD.

Conclusions—Endothelial dysfunction was not a major correlate of CKD in our sample.

Keywords

chronic kidney disease; brachial reactivity; cystatin C; Framingham Heart Study

Chronic kidney disease (CKD) affects over 19 million adults in the United States,¹ and is associated with cardiovascular disease (CVD),^{2–5} and its risk factors, including diabetes, hypertension, and dyslipidemia.^{6;7} The mechanisms involved in the relations between CKD and CVD are not fully understood, and may be due to nontraditional risk factors, including endothelial dysfunction.⁸

Endothelial function is associated with multiple CVD risk factors,^{9–13} including hyperlipidemia, hypertension, diabetes, and smoking, and is independently associated with incident CVD events.^{10;14;15} Among individuals with end-stage renal disease (ESRD), reduced production of nitric oxide (NO) has been investigated as a mechanism leading to impaired endothelium-dependent vasodilatation.¹⁶ A recent study of dialysis patients and matched, healthy controls attributed the reduction in endothelium-dependent vasodilatation observed among the dialysis patients to NO impairment.¹⁷ Associations observed between measures of endothelial dysfunction and CVD outcomes and all-cause mortality among individuals with ESRD^{18–23} suggest that impaired endothelial function may be a key mediator of CVD risk in ESRD. Impaired endothelial function, as assessed by circulating biomarkers and brachial measures, has been observed among individuals with ESRD compared to referents without known CKD,^{24–29} and to individuals with less severe forms of CKD.^{26;29}

Limited research exists investigating endothelial function in stage 3 and 4 CKD. Impaired endothelium-dependent vasodilatation has been observed among patients with Stage 4 CKD,³⁰ and circulating biomarkers of endothelial dysfunction were elevated among patients without diabetes but with moderate to severe CKD when compared to normal controls.³¹ In a population-based sample from the Hoorn study, increased levels of circulating biomarkers of endothelial dysfunction were associated with decreasing estimated glomerular filtration rate (eGFR) among participants unselected for CKD.³² However, less is known about the association of endothelial function and Stage 3 CKD. Brachial artery flow-mediated dilation (FMD) is a non-invasive technique that uses ultrasound imaging to measure the degree of endothelial dysfunction.³³ Thus, we sought to test the hypothesis that measures of noninvasively assessed endothelial function are impaired among individuals with Stage 3 CKD in the community. We also assessed the relations between measures of endothelial function and urinary albumin excretion and cystatin C.

Methods

Study sample

The study design and methodology of the Framingham Offspring cohort have been described previously.³⁴ The Offspring cohort was established in 1971, and was comprised of children and the spouses of children of the Original Framingham cohort. Of the 5124 men and women originally in the Offspring cohort, 3539 attended the seventh examination cycle (1998–2001), out of which 2883 had available FMD data and were considered eligible for this study. Participants with missing creatinine data (n=20), eGFR<15mL/min/1.73m² (<0.25mL/sec/1.73m²) (n=3), missing covariate data (n=20), and urinary albumin (mg) to creatinine (g) ratio (UACR) greater than 300mg/g (n=22) were excluded from the analysis. After exclusions, 2818

individuals remained for brachial diameter analyses. Baseline mean flow and hyperemic mean flow were measured in a subset of individuals eligible for this analysis (n=2256); flow measurements were introduced part way through the seventh examination cycle. Participants excluded from the analysis tended to be older, were more likely to be women, and were more likely to have diabetes and be on hypertension treatment. Among those excluded, UACR and cystatin C values were higher (data not shown). Measurements for UACR were available for 2301 participants, and analyses based on UACR were limited to these individuals; there were no statistically significant differences among participants with and without UACR data. The Boston University Medical Center Institutional Review Board approved this study, and all participants supplied written informed consent.

Measurements and definitions

Kidney function was determined using the eGFR based on the 4-variable Modification of Diet in Renal Disease (MDRD) Study equation.^{35;36} The National Kidney Foundation clinical practice guidelines define CKD as the presence of eGFR less than 60 mL/min/1.73m² (1 mL/sec/1.73m²) with or without kidney damage for at least three months.³⁷

The MDRD Study equation estimates the GFR based on an individual's age, sex, race, and serum creatinine level.^{35;36} Serum creatinine was measured using the modified Jaffe method from fasting blood sample collected during the participants' seventh examination cycle. A 2-step calibration process for serum creatinine was implemented due to potential inter-laboratory variability, and this process has been described previously.³⁸ Briefly, a correction factor of 0.23 mg/dL (20μmol/L) was applied to National Health and Nutritional Examination Survey III (NHANES III) serum creatinine values in order to calibrate them to the Cleveland Clinic Laboratory. Our serum creatinine values were then aligned to the age- and sex- specific means of serum creatinine from NHANES III.

Cystatin C concentrations were measured on previously frozen serum samples (stored at -80°C) by nephelometry (Dade Behring Diagnostic, Marburg Germany) and were reported as mg/L. The intra- and inter-assay coefficients of variation were 2.4 and 3.3%, respectively. The range of detection was 0.29 to 7.22 mg/L. Cystatin C was transformed to eGFR using the following equation: $eGFR = 76.7 * cysC^{-1.19}$.³⁹

Microalbuminuria was defined as UACR of at least 30mg/g.³⁷ Spot urine samples were obtained during the examination and kept at -20°C until quantification. Urinary albumin concentration was assessed using immunoturbimetry (Tina-quant Albumin assay; Roche Diagnostics, Indianapolis, IN). Urinary creatinine concentration was measured using a modified Jaffe method; the intra-assay coefficient of variation varied from 1.7–3.8%. The UACR accounts for differences in urine concentrations, has been validated, and is a reliable measure of urinary albumin excretion. The UACR also is correlated with albumin excretion rates determined using a 24-hour urine collection.^{40;41}

FMD assessment

FMD was assessed as previously described.¹³ A Toshiba SSH-140A ultrasound system and 7.5 MHz linear-array transducer were used to image the brachial artery.¹³ A forearm cuff (Hokanson AG101) interrupted arterial flow for 5 minutes at an occlusion pressure of either 200 mm Hg or 50 mm Hg higher than systolic blood pressure, depending on which represented a higher pressure. Digital end-diastolic images were collected with electrocardiographic triggering at baseline and for 2 minutes after deflating the cuff. Flows were analyzed from the digitized Doppler audio data using a semi-automated signal-averaging approach as previously detailed.⁴² Blinded sonographers measured the brachial artery diameter (6- to 10-mm segments) with commercially available software (Brachial Analyzer, Medical Imaging

Applications) as previously described.¹³ Baseline diameter was calculated based on the average of all measured baseline images; the 60-second diameter was calculated based on the average of measurements taken from images between 55 and 65 seconds after the cuff was deflated. FMD was determined as the relative change in brachial artery diameter (FMD %; [60-second diameter – baseline diameter]/[baseline diameter]*100). Higher baseline brachial diameter, higher baseline flow, blunted hyperemic flow and decreased FMD% are associated with CVD risk factors.^{13;43} Decreased FMD% and blunted hyperemic flow are considered indicative of endothelial dysfunction and are associated with worse prognosis.^{44–46}

Covariate assessment

Fasting blood samples were used to measure total cholesterol, high density lipoprotein cholesterol, and fasting blood glucose concentrations. An automatic device was used to measure heart rate. Systolic and diastolic blood pressure measurements were represented by the mean of two readings taken by a physician during the seventh examination. Body mass index was based on anthropometric measurements taken in the clinic and defined as an individual's weight in kilograms divided by height in meters squared. Diabetes was defined as a fasting blood glucose ≥ 126 mg/dL (≥ 7 mmol/L), or as being prescribed medication for treatment of diabetes, including insulin and/or oral hypoglycemic medications. Blinded adjudication panels assessed CVD endpoints based on medical records; diagnostic criteria for CVD events are described elsewhere.⁴⁷ Use of medication for hypertension, lipid control, and hormone replacement therapy was assessed by self-report during the seventh examination. Covariates also included smoking within 6 hours before FMD assessment, and performing a walk test before or after FMD assessment.

Statistical Analysis

Linear regression models were implemented to model each of the four brachial measures (dependent variables) as a function of CKD status (yes/no) defined by both creatinine and cystatin-C based equations, and urinary albumin-creatinine ratio (UACR) greater or equal to 30 mg/g. Models were initially adjusted for age and sex, and then additionally adjusted for examination 7 covariates including systolic blood pressure, diastolic blood pressure, hypertension treatment, heart rate, body mass index, total-to-high density lipoprotein cholesterol ratio, fasting blood glucose, diabetes status, smoking within past 6 hours, prevalent CVD, hormone replacement therapy, lipid lowering medication, and performing the walk test before or after FMD assessment, which were selected a priori. The microalbuminuria models were additionally adjusted for continuous eGFR. We assessed if the assumptions of linear regression were met for models of each of the vascular variables. The normality and homogeneity of the residuals and outliers and influential points were observed through plots of the residuals versus predicted values and by examining the distribution of the residuals. In assessing the assumptions of linear regression for each of the vascular function variables, it appears that there were not any outliers or influential points. The normality of the residuals was not an issue for any of the models, and constant variance was met for most of the variables, although for FMD% and baseline mean flow there were modest departures from variance homogeneity (data not shown).

SAS version 8.1 was used to perform all analyses.⁴⁸ A two-tailed $p < 0.0125$ (0.05/4 brachial measurements) was considered statistically significant.

Results

Overall, 7.3% of the study sample ($n=206$ of 2818) had CKD (Table 1). Of 2301 with urinary measurements 10.0% ($n=230$) had microalbuminuria. The mean eGFR among participants with CKD was 50 ± 8 mL/min/1.73m² (0.83 ± 0.13 mL/sec/1.73m²). Mean cystatin C was 0.96 ± 0.20

mg/L. The age-sex adjusted associations of all four brachial measures among participants with and without $\text{eGFR} < 60 \text{ mL/min/1.73m}^2$ ($< 1 \text{ mL/sec/1.73m}^2$) are shown in Table 1.

CKD and FMD Measurements

Brachial artery measurements did not differ significantly among individuals with or without $\text{eGFR} < 60 \text{ mL/min/1.73m}^2$ ($< 1 \text{ mL/sec/1.73m}^2$) in either age- and sex-, or multivariable-adjusted models (Table 2). In secondary analyses additionally adjusted for log UACR, results were not materially different (data not shown).

Microalbuminuria and FMD Measurements

If stratified by microalbuminuria status, hyperemic mean flow was significantly lower (47.2 ± 1.4 versus 51.4 ± 0.5 , $p = 0.005$) among those with, as compared to those without microalbuminuria after adjustment for age, sex, and eGFR (Table 3). These differences were no longer significant after multivariable adjustment (48.7 ± 1.4 versus 51.2 ± 0.4 , $p = 0.09$). No additional significant relations between microalbuminuria and FMD measures were observed.

Cystatin C and FMD Measurements

There was no association between CKD if eGFR was derived from cystatin-C transforming equations and FMD measures (Table 4).

Secondary Analyses

In secondary analyses, effect modification by age and sex with CKD status was investigated with interaction terms in linear regression models for each of the four brachial measures. No significant interactions between age (≥ 60 years and < 60 years) and CKD status and sex and CKD status were observed in these brachial measure models (all p -values > 0.03).

Discussion

Brachial measures of endothelial function did not differ significantly by CKD status in the Framingham Offspring Cohort. Microalbuminuria was associated with decreased hyperemic mean flow in age- and sex-adjusted linear regression models, but these differences were no longer observed after multivariable adjustment. Therefore, moderate CKD is not a major correlate of endothelial function in the community.

Our findings are in contrast to results observed in studies of individuals with end-stage renal disease (ESRD). Multiple studies have indicated that impaired endothelial function is observed among individuals undergoing hemodialysis or peritoneal dialysis if compared to healthy controls using serum^{24–26} and brachial^{25;27–29} measures of endothelial function. Several studies have also examined the association with endothelial dysfunction and stage 4 CKD.^{25;26;30;31;49;50} In a small sample of pre-dialysis patients with stage 4 and stage 5 CKD ($n = 25$, mean serum creatinine levels $521 \pm 49 \mu\text{mol/L}$ [$5.9 \pm 0.5 \text{ mg/dL}$], range $266–1410 \mu\text{mol/L}$ [$3.0–16.0 \text{ mg/dL}$]), increased concentrations of von Willebrand factor, thrombomodulin, and soluble vascular cell adhesion molecule-1 were observed compared to healthy controls.²⁶ Among a sample of patients with stage 3 and 4 CKD in Uppsala, Sweden ($n = 56$, mean creatinine clearance of $29.4 \pm 24 \text{ mL/min/1.73m}^2$ [$0.49 \pm 0.40 \text{ mL/sec/1.73m}^2$]), impaired endothelium-dependent vasodilatation was observed after methacholine infusion compared to a control sample from the general Uppsala population.³⁰ Taken together these results suggest that impaired endothelial function is present in late Stage 3, Stage 4, and Stage 5 CKD.

The findings in late stage CKD are in contrast to the results observed in the present study, which consisted of participants with predominately early stage 3 CKD. Interpreting our results in the context of the prior literature may signify that impaired endothelial function plays a role

in more severe kidney disease and is not a key component in the initial stages of CKD. A recent report based on a sample from the Hoorn study (n=613, mean eGFR=68±12 mL/min/1.73m² [1.13±0.20 mL/sec/1.73m²]), a community-based study of glucose tolerance and CVD risk factors, may be of particular relevance to our work. An inverse association of biomarkers of endothelial function, including von Willebrand factor, sVCAM-1, urinary albumin excretion, and eGFR was observed.³² Our study is in contrast to these findings, but several potential explanations exist that could explain the differences in these results. In the Hoorn Study, eGFR was used as a continuous exposure in a sample primarily without CKD. The validity of eGFR as a measure of kidney function in individuals without CKD is uncertain.^{35;51} Circulating biomarkers of endothelial function were used in the Hoorn study, whereas the present study was based on brachial measures of endothelial function. The biomarkers assessed in the Hoorn Study are also markers of hemostasis and thrombosis and may reflect other processes in addition to endothelial function. Finally, differences in the study samples may account for the contrasting results. Compared with our participants, individuals included in the Hoorn Study appeared to be more likely to be male, have diabetes, smoke and have higher mean blood pressure.

Statistically significant associations in age- and sex-adjusted models but not in multivariable models were observed for microalbuminuria and hyperemic mean flow, suggesting that this association may be mediated by shared risk factors. Inflammatory pathways have been investigated in the association of microalbuminuria and endothelial function,⁵² and a study of patients with nephropathy indicated that increasing soluble vascular endothelial adhesion molecule-1 serum level, an inflammatory marker as well as a marker for endothelial function, was associated with increasing cystatin C levels.⁵³

There are strengths associated with this study. The Framingham Heart Study is a long-term, community-based sample. The Offspring cohort was established in 1971 and routine ascertainment of CVD events and risk factors has occurred approximately every four to eight years. Prevalent CVD was adjudicated by a panel of investigators, and it was not necessary to rely on self-report of several covariates. Lastly, we had adequate power to detect a modest association.

There are limitations associated with this analysis as well. Classification of CKD was based on eGFR using a single measure of serum creatinine. The MDRD Study equation was used to estimate GFR, and it has been recently reported that this equation underestimates GFR in individuals with normal kidney function.⁵¹ Whereas this underestimation could be an issue if eGFR is analyzed as a continuous variable, it is unclear how it would affect CKD classification if used as a dichotomous exposure. The Framingham Offspring Cohort is also predominately white, which could limit the generalizability of these findings to other ethnic groups. The cross-sectional design of the present analysis is also a drawback, as our findings cannot be extrapolated to longitudinal observations. Lastly, we excluded nearly 20% of our sample who did not undergo brachial reactivity measurements.

Whereas endothelial function is associated with multiple CVD risk factors and has been identified as a potential mediator in the relation of ESRD and CVD outcomes and mortality, the present study indicates that endothelial dysfunction is not a major correlate of moderate CKD among individuals with predominately Stage 3 CKD in the community. These results suggest that it is unlikely that endothelial function in the early stages of CKD explains increased CVD risk in this population, and mechanistic research to link stage 3 CKD with CVD should focus on other mechanisms, such as inflammation and arterial stiffness. Among individuals in the Framingham Offspring Cohort, endothelial dysfunction is not a major correlate of moderate CKD.

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Table 1
 Characteristics and flow-mediated dilation measurements of the study sample by CKD status as defined by estimated GFR. Data represents mean and standard deviations for continuous traits, and percents for dichotomous variables.

	Total Cohort N=2818	CKD N=206	eGFR $\geq$ 60 mL/min/1.73m ² N=2612	Age- and sex-adjusted p-value
Clinical measures				
Age (years)	61 $\pm$ 9	69 $\pm$ 9	60 $\pm$ 9	<0.001
Women (%)	53	59	53	0.05
Systolic blood pressure (mmHg)	127 $\pm$ 19	134 $\pm$ 22	126 $\pm$ 18	0.3
Diastolic blood pressure (mmHg)	74 $\pm$ 10	71 $\pm$ 10	74 $\pm$ 10	0.2
Heart rate (beats/min)	64 $\pm$ 11	64 $\pm$ 12	65 $\pm$ 11	0.8
Body mass index (kg/m ²)	28.1 $\pm$ 5.3	28.8 $\pm$ 5.1	28.1 $\pm$ 5.3	0.01
Total-to-HDL cholesterol ratio	4.1 $\pm$ 1.3	4.3 $\pm$ 1.4	4.0 $\pm$ 1.3	<0.001
Glucose (concentration), mg/dL	104 $\pm$ 27	109 $\pm$ 29	104 $\pm$ 27	0.2
Diabetes (%)	13	23	12	0.02
Smoking within 6 hr (%)	8	4	9	0.4
Cardiovascular disease (%)	12	29	11	<0.001
Hormone replacement therapy (%)	32	32	32	0.2**
Hypertension (%)	44	69	43	<0.001
Hypertension treatment (%)	33	57	31	<0.001
Lipid medication (%)	21	37	19	0.002
Walk test before (%)	38	25	39	0.02
Walk test after (%)	37	31	37	0.6
Kidney disease measure				
eGFR (mL/min/1.73m ²)	85 $\pm$ 19	50 $\pm$ 8	88 $\pm$ 17	<0.001
UACR $\geq$ 30 mg/g (%)	10	25	9	<0.001
Cystatin (mg/L)	0.96 $\pm$ 0.20	1.31 $\pm$ 0.37	0.93 $\pm$ 0.15	<0.001
Flow-mediated dilation measures				
Baseline diameter (mm)*	4.28 $\pm$ 0.87	4.30 $\pm$ 0.88	4.28 $\pm$ 0.86	0.8
FMD (%)*	2.84 $\pm$ 2.76	2.26 $\pm$ 2.46	2.89 $\pm$ 2.78	0.8
Baseline mean flow (cm/s) [†]	8.20 $\pm$ 4.85	7.02 $\pm$ 4.31	8.30 $\pm$ 4.88	0.6
Hyperemic mean flow (cm/s) [†]	50.8 $\pm$ 21.3	41.4 $\pm$ 19.5	51.6 $\pm$ 21.3	0.6

* N=2818 for baseline diameter and FMD%

[†] N=2256 for baseline and hyperemic mean flow

**
HRT only adjusted for age

Abbreviations: HDL cholesterol, high-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; UACR, urinary albumin to creatinine ratio; FMD, flow-mediated dilation.

Note: Glucose in mg/dL may be converted to mmol/L by multiplying by 0.05551; glomerular filtration rate in ml/min/1.73m² may be converted to ml/s/1.73m² by multiplying by 0.01667.

Table 2

Comparison of least square means ($\pm$ standard errors) that are age- and sex-, and multivariable-adjusted flow-mediated dilation markers by CKD status based on eGFR

	CKD N=206	95% CI	eGFR ≥ 60 mL/min/1.73m ² N=2612	95% CI	p-value
Baseline diameter (mm) [*]					
Age and sex	4.29 $\pm$ 0.04	(4.21, 4.38)	4.28 $\pm$ 0.01	(4.26, 4.31)	0.8
Multivariable ^{**}	4.25 $\pm$ 0.04	(4.17, 4.33)	4.29 $\pm$ 0.01	(4.26, 4.31)	0.4
FMD (%) [*]					
Age and sex	2.89 $\pm$ 0.19	(2.52, 3.25)	2.84 $\pm$ 0.05	(2.74, 2.94)	0.8
Multivariable ^{**}	3.03 $\pm$ 0.18	(2.67, 3.39)	2.82 $\pm$ 0.05	(2.73, 2.92)	0.3
Baseline mean flow (cm/s) [†]					
Age and sex	7.99 $\pm$ 0.37	(7.26, 8.72)	8.22 $\pm$ 0.10	(8.01, 8.42)	0.6
Multivariable ^{**}	7.91 $\pm$ 0.36	(7.21, 8.61)	8.22 $\pm$ 0.10	(8.03, 8.42)	0.4
Hyperemic mean flow (cm/s) [†]					
Age and sex	50.1 $\pm$ 1.5	(47.2, 53.0)	50.8 $\pm$ 0.4	(50.0, 51.6)	0.6
Multivariable ^{**}	51.5 $\pm$ 1.4	(48.7, 54.3)	50.7 $\pm$ 0.4	(50.0, 51.5)	0.6

^{*} N=2818 for baseline diameter and FMD%

[†] N=2256 for baseline and hyperemic mean flow; (n=170 with CKD)

^{**} Multivariable models included: age, sex, exam systolic blood pressure, exam diastolic blood pressure, hypertension treatment, heart rate, body mass index, total/high density lipoprotein cholesterol, fasting blood glucose, diabetes, smoking within the past 6 hours, prevalent cardiovascular disease, hormone replacement therapy, lipid lowering medication, and walk test (before or after FMD determination)

Abbreviations: eGFR = estimated glomerular filtration rate; FMD = flow-mediated dilation.

Table 3

Comparison of least square means ($\pm$ standard errors) that are age- and sex- and multivariable-adjusted flow-mediated dilation markers by microalbuminuria status.

	Microalbuminuria (UACR $\geq$ 30 mg/g) N=230	No Microalbuminuria (UACR<30 mg/g) N=2071	P
Baseline diameter (mm) *			
Age-sex	4.37 $\pm$ 0.04	4.29 $\pm$ 0.01	0.05
Multivariable **	4.35 $\pm$ 0.04	4.29 $\pm$ 0.01	0.1
FMD (%) *			
Age-sex	2.67 $\pm$ 0.17	2.88 $\pm$ 0.06	0.3
Multivariable **	2.87 $\pm$ 0.17	2.87 $\pm$ 0.06	0.9
Baseline mean flow †			
Age-sex	7.77 $\pm$ 0.35	8.25 $\pm$ 0.12	0.2
Multivariable **	7.44 $\pm$ 0.34	8.28 $\pm$ 0.11	0.02
Hyperemic mean flow †			
Age-sex	47.2 $\pm$ 1.4	51.4 $\pm$ 0.5	0.005
Multivariable **	48.7 $\pm$ 1.4	51.2 $\pm$ 0.4	0.09

Abbreviations: FMD% = percent-change in flow-mediated dilation; UACR = urinary-albumin-to-creatinine ratio.

* N=2301 for Baseline diameter and FMD%

† N=1838 for Baseline and Hyperemic Mean Flow; (n=184 with microalbuminuria)

** Multivariable models were adjusted for eGFR in addition to the covariates in legend to Table 2

Table 4
Comparison of least square means ($\pm$ standard errors) that are age- and sex-, and multivariable-adjusted flow-mediated dilation markers by CKD status based on eGFR computed from cystatin.

	CKD N=210	95% CI	eGFR $\geq$ 60 mL/min/1.73m ² N=2564 ^{***}	95% CI	P
Baseline diameter (mm) [*]					
Age and sex	4.30 $\pm$ 0.04	(4.22, 4.38)	4.28 $\pm$ 0.01	(4.26, 4.30)	0.7
Multivariable ^{**}	4.21 $\pm$ 0.04	(4.13, 4.29)	4.29 $\pm$ 0.01	(4.27, 4.31)	0.06
FMD (%) [*]					
Age and sex	3.03 $\pm$ 0.19	(2.66, 3.39)	2.81 $\pm$ 0.05	(2.71, 2.91)	0.3
Multivariable ^{**}	3.24 $\pm$ 0.19	(2.88, 3.61)	2.79 $\pm$ 0.05	(2.69, 2.89)	0.02
Baseline mean flow (cm/s) [†]					
Age and sex	8.61 $\pm$ 0.38	(7.87, 9.35)	8.18 $\pm$ 0.10	(7.97, 8.38)	0.3
Multivariable ^{**}	8.35 $\pm$ 0.37	(7.63, 9.07)	8.20 $\pm$ 0.10	(8.00, 8.39)	0.7
Hyperemic mean flow (cm/s) [†]					
Age and sex	50.7 $\pm$ 1.5	(47.7, 53.6)	50.8 $\pm$ 0.4	(50.0, 51.6)	0.9
Multivariable ^{**}	52.8 $\pm$ 1.5	(49.9, 55.6)	50.6 $\pm$ 0.4	(49.9, 51.4)	0.2

* n=2774 for baseline diameter and FMD%

† N= 2228 for baseline and hyperemic mean flow; (n=170 with CKD)

** Multivariable models included: age, sex, exam systolic blood pressure, exam diastolic blood pressure, hypertension treatment, heart rate, body mass index, total/high density lipoprotein cholesterol, fasting blood glucose, diabetes, smoking within the past 6 hours, prevalent cardiovascular disease, hormone replacement therapy, lipid lowering medication, and walk test (before or after FMD determination)

*** 44 people with missing cystatin values

Abbreviations: eGFR=estimated glomerular filtration rate; FMD = flow-mediated dilation.