Association of Contrast-Enhanced Ultrasound–Derived Kidney Cortical Microvascular Perfusion with Kidney Function

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Key Points

- Contrast-enhanced ultrasound is a noninvasive imaging modality that may noninvasively assess kidney cortical microvascular perfusion.
- Contrast-enhanced ultrasound–derived kidney cortical microvascular perfusion is associated with eGFR.

Abstract

Background Individuals with chronic kidney disease (CKD) have decreased kidney cortical microvascular perfusion, which may lead to worsening kidney function over time, but methods to quantify kidney cortical microvascular perfusion are not feasible to incorporate into clinical practice. Contrast-enhanced ultrasound (CEUS) may quantify kidney cortical microvascular perfusion, which requires further investigation in individuals across the spectrum of kidney function.

Methods We performed CEUS on a native kidney of 83 individuals across the spectrum of kidney function and calculated quantitative CEUS-derived kidney cortical microvascular perfusion biomarkers. Participants had a continuous infusion of the microbubble contrast agent (Definity) with a flash-replenishment sequence during their CEUS scan. Lower values of the microbubble velocity (β) and perfusion index (β×A) may represent lower kidney cortical microvascular perfusion. Multivariable linear regression models tested the associations of the microbubble velocity (β) and perfusion index (β×A) with estimated glomerular filtration rate (eGFR).

Results Thirty-eight individuals with CKD (mean age±SD 65.2±12.6 years, median [IQR] eGFR 31.5 [18.9–41.5] ml/min per 1.73 m²), 37 individuals with end stage kidney disease (ESKD; age 54.8±12.3 years), and eight healthy volunteers (age 44.1±15.0 years, eGFR 117 [106–120] ml/min per 1.73 m²) underwent CEUS without side effects. Individuals with ESKD had the lowest microbubble velocity (β) and perfusion index (β×A) compared with individuals with CKD and healthy volunteers. The microbubble velocity (β) and perfusion index (β×A) had moderate positive correlations with eGFR (β: rs=0.44, P<0.001; β×A: rs=0.50, P<0.001). After multivariable adjustment, microbubble velocity (β) and perfusion index (β×A) remained significantly associated with eGFR (change in natural log transformed eGFR per 1 unit increase in natural log transformed biomarker: β, 0.38 [95% CI 0.17 to 0.59]; β×A, 0.79 [95% CI 0.45 to 1.13]).

Conclusions CEUS-derived kidney cortical microvascular perfusion biomarkers are associated with eGFR. Future studies are needed to determine if CEUS-derived kidney cortical microvascular perfusion biomarkers have prognostic value.

Introduction

Decreased kidney cortical microvascular perfusion from worsening arterial and arteriolar sclerosis may lead to chronic hypoxia and subsequent fibrosis (1), which are associated with CKD progression (2). Gold-standard assessments of kidney blood flow, such as para-aminohippurate (PAH) clearance, are invasive and not feasible to incorporate into routine clinical practice (3). Noninvasive methods that quantify kidney cortical microvascular perfusion require further

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development to complement current noninvasive biomarkers, such as eGFR and albuminuria (4), to identify individuals at high risk of CKD progression.

Contrast-enhanced ultrasound (CEUS) is a noninvasive imaging modality that may be able to quantify tissue perfusion without the need for nephrotoxic contrast media or ionizing radiation (5-8). Through the use of ultrasound and gas-filled microbubble contrast agents that remain in the intravascular space, CEUS is able to depict the microvasculature by identifying differences in echogenicity between the gas-filled microbubbles and soft tissues of the body (5,9-11). Because microbubbles remain intravascular and share a similar size and flow rate to red blood cells, quantification of their movement through the kidneys may provide a readout for kidney cortical microvascular perfusion (12-14). Prior animal studies support the use of CEUS to quantify kidney cortical microvascular perfusion (15-17). Confirmation human data are limited to a few studies in healthy volunteers (10,18) and individuals with CKD (19,20). These prior animal and human studies suggest that lower microbubble velocity and perfusion indices may indicate lower kidney cortical microvascular perfusion (12-14). Prior animal studies support the use of CEUS to quantify kidney cortical microvascular perfusion (15-17). Confirmatory human data are limited to a few studies in healthy volunteers (10,18) and individuals with CKD (19,20). These prior animal and human studies suggest that lower microbubble velocity and perfusion indices may indicate lower kidney cortical microvascular perfusion. Because kidney blood flow is intimately linked to eGFR (3,21), we performed CEUS on a native kidney of individuals across the spectrum of kidney function to test the hypothesis that CEUS-derived kidney cortical microvascular perfusion biomarkers would have a significant association with eGFR.

Methods

Study Population

In patients who had CKD or ESKD and a kidney lesion undergoing CEUS, we performed an additional CEUS scan on the kidney parenchyma away from the lesion. We also performed CEUS on a kidney of healthy volunteers who had no kidney lesions. All study participants were ≥18 years old. CKD was defined by an eGFR of <90 ml/min per 1.73 m² with albuminuria or two measurements of eGFR <60 ml/min per 1.73 m² at least 90 days apart. ESKD was defined as the need for kidney replacement therapy (dialysis or transplantation). Healthy volunteers took no prescribed medications and had no history of CKD, diabetes mellitus, hypertension, coronary artery disease, congestive heart failure, liver disease, or autoimmune disease. Exclusion criteria were critically ill patients admitted to an intensive care unit, right-to-left cardiac shunt, severe pulmonary hypertension, acute respiratory distress syndrome, active cardiac disease, unstable neurologic disease, pregnancy, obesity that limited acquisition of acceptable images on B-mode ultrasound, recent kidney procedural intervention, or known hypersensitivity to the microbubble contrast agent. Participants were recruited from an academic tertiary care hospital. All participants provided written informed consent. The study protocol was approved by the Institutional Review Board at the University of North Carolina and is in accordance with the principles of the Declaration of Helsinki.

Imaging Procedure

All participants underwent CEUS of at least one native kidney using an Acuson Sequoia 512 (Siemens Healthcare, Malvern, PA) scanner with Cadence pulse sequencing (CPS) contrast imaging technology (Siemens Healthcare). All images were acquired using a 4C1 curved array, 1–4 MHz transducer (Siemens Healthcare). Ultrasound depth, focal position, and gain were adjusted on a case-by-case basis in order to obtain best possible image quality for visualization of contrast-enhanced images. Imaging frequency was held constant (4.5 MHz for B-mode images and 1.5 MHz for contrast mode). The microbubble contrast agent, Definity (Perflutren lipid microsphere, Lantheus Medical Imaging, Inc., N. Billerica, MA), was used for contrast imaging under Food and Drug Administration IND 127535. Definity was prepared per package insert instructions by mechanical agitation using the Vialmix (Lantheus Medical Imaging, Inc.) no earlier than 5 minutes before use. The entire volume from a single vial of Definity (approximately 1.5 ml) was diluted in 50 ml sterile saline and administered as a continuous infusion (4–8 ml/min) on the basis of the participant’s body mass index (BMI; BMI ≤21 kg/m²: 4 ml/min; 21 ≤BMI ≤30 kg/m²: 6 ml/min; BMI >30 kg/m²: 8 ml/min) using a programmable syringe pump (Smith’s Medical, Dublin, OH).

For each participant, baseline precontrast B-mode and CEUS images were obtained of the native kidney parenchyma at the “mid-plane” of the kidney, defined as the longest cross-sectional measurement of the kidney in the sagittal plane, to allow for the largest cortical area. For the CEUS portion of the exam, the sonographer held the transducer over the mid-plane during the infusion of the microbubble contrast agent. Clip acquisition began simultaneously with infusion of the microbubble contrast agent. The microbubble contrast agent was infused until steady state enhancement was reached (typically around 90 seconds after the start of infusion). The ultrasound mechanical index was maintained between 0.1 and 0.2 during CEUS imaging. After reaching steady state, a “flash,” which consists of an ultrasound pulse at a high mechanical index of 0.8, was applied for 1 second to burst and clear the microbubble contrast signal within the imaging plane of the transducer. Immediately after the flash, microbubbles repurfused into the imaging plane until reaching steady state. Participants were instructed to perform a breath hold during the flash-replenishment sequence. Acquired cine clips consisted of the steady state, flash, and replenishment periods. This flash-replenishment sequence was performed twice for each kidney imaged.

Imaging Analysis

Images and clips were exported in DICOM format and analyzed in Matlab (MathWorks, Inc., Natick, MA). Quantitative image analysis was performed on the kidney cortex of all imaged kidneys. A graphical user interface was developed with the ability to segment regions of interest (ROIs) from a preloaded image. An experienced abdominal radiologist with more than 9 years of experience (L.B.), blinded to the full CEUS clips, outlined ROIs containing the kidney cortex using a single CEUS frame and the corresponding grayscale ultrasound reference image. Images were deemed acceptable if the radiologist was able to define a ROI.

Time-intensity curves (TICs) of the microbubble flash-replenishment sequences were generated on the basis of averaged contrast intensity values as a function of time.
within the ROIs identified by the radiologist. The TIC data for each kidney were fit to a rising function exponential model that quantifies perfusion using CEUS as previously described (Figure 1) (22). The fit provided an initial slope of the curve (\(b\)), which is related to the velocity of the microbubble contrast agent. “A” is the maximum enhancement and relates to the fractional vascular volume. Their product (\(b \times A\)) forms an estimate of tissue perfusion. Supplemental Figure 1 shows representative TIC for healthy volunteers and participants with CKD and ESKD. Finally, a goodness of fit (\(R^2\)), which is based on how well the TIC curve of each kidney fit to the exponential model, was generated (range 0–1, with 0 being poor and 1 being best fit). \(R^2\) values >0.8 are considered strong.

**Exposure and Outcomes**

The exposure was kidney cortical microvascular perfusion, as assessed by two CEUS-derived biomarkers: the microbubble velocity (\(b\)) and perfusion index (\(b \times A\)). Each CEUS-derived kidney cortical microvascular perfusion biomarker was the mean of the first and second flash-replenishment sequence, resulting in a single representative value for the microbubble velocity (\(b\)) and perfusion index (\(b \times A\)), respectively. The primary outcome was eGFR at the time of CEUS. We calculated eGFR by the creatinine-based CKD Epidemiology Collaboration equation (23). We used CKD stage as a secondary outcome. If no eGFR was available in healthy volunteers, we imputed a value of 120 ml/min per 1.73 m². We imputed a value of 9 ml/min per 1.73 m² for all participants with ESKD.

**Ascertainment of Covariates**

We collected participants’ information at the time of CEUS visit, including demographics (age, sex, and race), medical history (hypertension, diabetes mellitus, systemic vasculitis, systemic lupus erythematosus, coronary artery disease, congestive heart failure, cerebrovascular disease, hepatitis C, and malignancy), medication lists (angiotensin converting enzyme inhibitors [ACEi], angiotensin II receptor blocker [ARB], mineralocorticoid receptor antagonist, calcium channel blocker, \(\beta\)-blocker, statins, corticosteroids, or other immunosuppressive medications), serum creatinine, and proteinuria. For proteinuria, we classified participants as having none, microalbuminuria, or macroalbuminuria on the basis of their semi-quantitative or quantified assessments of proteinuria. If a participant had a urinalysis, urine protein-to-creatinine ratio, and urine albumin-to-creatinine ratio, we used urine albumin-to-creatinine ratio. If a participant had only a semi-quantitative assessment of proteinuria from a urinalysis, we used the following scheme: <30 mg/g (negative or trace), 30–300 mg/g (1+ or 2+), >300 mg/g (3+ or 4+). We obtained this information from the electronic medical record on the basis of physician documentation, which was verified with the participant at the CEUS visit.

**Figure 1.** Methods to generate contrast-enhanced ultrasound (CEUS)-derived kidney cortical microvascular perfusion biomarkers.

(A) A representative time-intensity curve (TIC) showing the steady state enhancement (green), flash (purple), and replenishment (pink) stages of a cine clip that was acquired. (B) Zoomed in image of the replenishment section to show the actual replenishment (pink dotted line) and the curve fit (black line). (C) Individual frames of the cine clip demonstrating the steady state enhancement (1 second into the clip), flash frames (that are transiently overlayed with a grayscale color map), and the replenishment frames showing the rise back to steady state enhancement.
**Statistical Analyses**

Descriptive statistics were summarized as count with percentages for categorical variables and mean±SD or median with interquartile range for continuous variables. For skewed data distributions, we performed natural logarithmic transformation as appropriate. We used Spearman correlation coefficients to determine associations between continuous variables and each CEUS-derived kidney cortical microvascular perfusion biomarker. To evaluate associations of CEUS-derived kidney cortical microvascular perfusion biomarkers with baseline characteristics, we used Wilcoxon rank sum or Kruskal-Wallis tests for two-group and multiple-group comparisons, respectively. We used multivariable linear regression to test the association between each CEUS-derived kidney cortical microvascular perfusion biomarker with eGFR after adjustment for age, sex, race, BMI, diabetes mellitus, and ACEi/ARB. As a sensitivity analysis, we repeated the associations with eGFR in participants who had a strong goodness of fit for their CEUS-derived kidney cortical microvascular perfusion biomarkers (R²>0.8) from the TIC. All statistical tests were two sided, and P values <0.05 were considered significant. Statistical analyses were performed using SAS v9.4 (SAS Institute, Inc., Cary, NC).

**Results**

**Study Participants**

We performed 87 CEUS studies without any side effects or adverse events. Of these 87 participants, we excluded four participants due to unsatisfactory images due to difficulties with breath holding that limited the ability to perform image analysis. Baseline characteristics of the study cohort are shown in Table 1. A total of 38 participants with CKD, 37 participants with ESKD, and eight healthy volunteers were included in this study. Participants with CKD and ESKD had a lower frequency of women compared with healthy volunteers. There was a higher frequency of White participants in the healthy volunteer and CKD groups compared with the participants with ESKD. There were no significant differences by BMI across the groups (P=0.72). The most common comorbid conditions were

| Characteristics | Healthy (N=8) | CKD (N=38) | ESKD (N=37) | P Value |
|-----------------|--------------|------------|-------------|---------|
| **Age, yr, mean±SD** | 44.1±15.0 | 65.2±12.6 | 54.8±12.3 | <0.001 |
| **Women, n (%)** | 4 (50) | 14 (36.8) | 15 (39.5) | 0.78 |
| **Race, n (%)** |  |  |  | 0.008 |
| White | 4 (50) | 25 (65.8) | 10 (27.0) |  |
| Black | 3 (37.5) | 12 (31.6) | 26 (70.3) |  |
| Other | 1 (12.5) | 1 (2.6) | 1 (2.7) |  |
| eGFR, ml/min per 1.73 m², a median (IQR) | 117 (106–120) | 31.5 (18.9–41.5) | 9 | <0.001 |
| **Proteinuria** |  |  |  | 0.02 |
| No albuminuria (<30 mg/g) | 8 | 0 | 0 |  |
| Microalbuminuria (30–300 mg/g) | 14 | 9 | 15 |  |
| Macroalbuminuria (>300 mg/g) | 12 | 9 | 7 |  |
| **BMI, kg/m², mean±SD** | 28.9±5.9 | 28.9±5.3 | 30.1±8.1 | 0.72 |
| **CKD stage, n (%)** |  |  |  | <0.001 |
| 1 | 0 | 1 (2.6) | 0 |  |
| 2 | 0 | 5 (13.2) | 0 |  |
| 3 | 0 | 15 (39.5) | 0 |  |
| 4 | 0 | 10 (26.3) | 0 |  |
| 5 | 0 | 7 (18.4) | 0 |  |
| ESKD | 0 | 0 (0) | 37 (100) |  |
| **Comorbidities, n (%)** |  |  |  |  |
| Diabetes mellitus | 0 | 16 (42.1) | 19 (51.3) | 0.02 |
| Hypertension | 0 | 35 (92.1) | 37 (100) | <0.001 |
| Coronary artery disease | 0 | 8 (21.1) | 8 (21.6) | 0.33 |
| Congestive heart failure | 0 | 2 (5.3) | 5 (13.5) | 0.26 |
| Malignancy | 0 | 6 (15.8) | 6 (16.2) | 0.42 |
| **Medications, n (%)** |  |  |  |  |
| ACEi/ARB | 0 | 23 (60.5) | 15 (40.5) | 0.006 |
| Diuretic | 0 | 22 (57.9) | 7 (18.9) | <0.001 |
| β-blocker | 0 | 20 (52.6) | 23 (62.1) | 0.004 |
| Calcium channel blocker | 0 | 17 (44.7) | 21 (56.7) | 0.82 |
| Immunosuppression | 0 | 3 (7.9) | 15 (40.5) | <0.001 |
| Corticosteroids | 0 | 2 (5.3) | 6 (16.2) | 0.15 |
| Calcineurin inhibitor | 0 | 0 (0) | 14 (37.8) | <0.001 |
| Statin | 0 | 17 (44.7) | 15 (40.5) | 0.05 |

CEUS, contrast-enhanced ultrasound; IQR, interquartile range; BMI, body mass index; SLE, systemic lupus erythematosus; ACEi/ARB, angiotensin converting enzyme inhibitor or angiotensin II receptor blocker; MRA, mineralocorticoid receptor antagonist.

*aHealthy volunteers who did not have an eGFR value (n=4) had an eGFR value of 120 ml/min per 1.73 m² imputed. Patients who had ESKD had an imputed eGFR value of 9 ml/min per 1.73 m². Nine participants were missing values for proteinuria.*
diabetes, hypertension, and coronary artery disease in the CKD and ESKD groups.

CEUS-Derived Imaging Characteristics

The imaging characteristics from the CEUS studies are show in Table 2. Kidney sizes were smallest in participants with ESKD (9.0 [7.6–10.4] cm) compared with participants with CKD (10.2 [9.8–11.6] cm) and healthy volunteers (10.0 [9.5–11] cm; P<0.001). Kidney depths were similar across all participants (ESKD: 10 [9–11] cm, CKD: 10 [9–11] cm, healthy: 10 [9–10.5] cm; P=0.99). The microbubble velocity (β; ESKD: 0.38 [0.21–0.62] arbitrary units [au], CKD: 0.52 [0.41–0.69] au, healthy: 1.08 [0.77–1.31] au; P<0.001) and the perfusion index (β×A; ESKD: 0.34 [0.25–0.43] au, CKD: 0.49 [0.38–0.62] au, healthy: 1.00 [0.71–1.10] au; P<0.001) were each lowest in participants with ESKD and highest in healthy volunteers (Figure 2). Participants who had a strong TIC model fit (R²>0.8) had similar differences in the microbubble velocity (β) and the perfusion index (β×A). In all participants (ESKD: 10 [9–11] cm, CKD: 10 [9–11] cm, healthy: 10 [9–10.5] cm; P=0.99). The microbubble velocity (β; ESKD: 0.38 [0.21–0.62] arbitrary units [au], CKD: 0.52 [0.41–0.69] au, healthy: 1.08 [0.77–1.31] au; P<0.001) and the perfusion index (β×A; ESKD: 0.34 [0.25–0.43] au, CKD: 0.49 [0.38–0.62] au, healthy: 1.00 [0.71–1.10] au; P<0.001) were each lowest in participants with ESKD and highest in healthy volunteers (Figure 2). Participants who had a strong TIC model fit (R²>0.8) had similar differences in the microbubble velocity (β) and the perfusion index (β×A). In

![Figure 2](image-url)

**Figure 2.** | CEUS-derived kidney cortical microvascular perfusion biomarkers by disease groups. Boxplots show differences of CEUS-derived kidney cortical microvascular perfusion biomarkers in healthy volunteers, participants with CKD, and participants with ESKD.

Table 2. Baseline imaging characteristics of participants who underwent CEUS

| Characteristics | Healthy (N=8) | CKD (N=38) | ESKD (N=37) | P Value |
|-----------------|--------------|------------|-------------|---------|
| Kidney size, cm | 10.0 (9.5–11) | 10.2 (9.8–11.6) | 9.0 (7.6–10.4) | 0.001   |
| Kidney depth, cm| 10.0 (9–10.5) | 10.0 (9–11) | 10.0 (9–11) | 0.99    |
| β, au           | 1.08 (0.77–1.31) | 0.52 (0.41–0.69) | 0.38 (0.21–0.62) | <0.001 |
| β×A, au         | 1.00 (0.71–1.10) | 0.49 (0.38–0.62) | 0.34 (0.25–0.43) | <0.001 |
| β, au* (if R²>0.8) | 1.07 (0.75–1.20) | 0.50 (0.41–0.68) | 0.37 (0.21–0.44) | <0.001 |

Data presented as median (IQR). P values represent across all group comparisons. CEUS, contrast-enhanced ultrasound; au, arbitrary units.

*Participants included: healthy (n=7), CKD (n=35), ESKD (n=33).
a subset of patients \( n=9 \) who had both left and right kidneys imaged, the microbubble velocity \( \beta \) and the perfusion index \( \beta \times A \) had positive correlations between the left and right kidneys, which did not reach statistical significance \( \beta: r_s=0.45, P=0.22; \beta \times A: r_s=0.67, P=0.05 \). In the 64 participants who had two flash-replenishment sequences performed, the coefficient of variation between the repeated microbubble velocity \( \beta \), relative blood volume \( A \), perfusion index \( \beta \times A \), and eGFR did not correlate significantly with CKD stage.

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### Table 3. Correlation between CEUS-derived kidney cortical microvascular perfusion biomarkers and kidney function

| Kidney Cortical Microvascular Perfusion Biomarker | \( n \) | \( r_s \) | \( P \) Value |
|-----------------------------------------------|------|------|-------------|
| eGFR, ml/min/1.73m²                            |      |      |             |
| \( \beta \) mean                              | 83   | 0.44 | <0.001      |
| \( A \) mean                                  | 83   | −0.12| 0.25        |
| \( \beta \times A \) mean                     | 83   | 0.50 | <0.001      |
| \( \beta \) mean (where \( R^2 > 0.8 \))      | 75   | 0.52 | <0.001      |
| \( A \) mean (where \( R^2 > 0.8 \))          | 75   | −0.17| 0.14        |
| \( \beta \times A \) mean (where \( R^2 > 0.8 \)) | 75  | 0.57 | <0.001      |
| CKD stage                                     |      |      |             |
| \( \beta \) mean                              | 83   | −0.43| <0.001      |
| \( A \) mean                                  | 83   | 0.13 | 0.25        |
| \( \beta \times A \) mean                     | 83   | −0.51| <0.001      |
| \( \beta \) mean (where \( R^2 > 0.8 \))      | 75   | −0.52| <0.001      |
| \( A \) mean (where \( R^2 > 0.8 \))          | 75   | 0.18 | 0.12        |
| \( \beta \times A \) mean (where \( R^2 > 0.8 \)) | 75  | −0.58| <0.001      |

CEUS, contrast-enhanced ultrasound.

**Figure 3.** CEUS-derived kidney cortical microvascular perfusion biomarkers by CKD stage. Boxplots show differences of CEUS-derived kidney cortical microvascular perfusion biomarkers by CKD stage.
volume (A), and perfusion index (β×A) measurements were 33%, 13%, and 25%, respectively.

** Associations of CEUS-Derived Kidney Cortical Microvascular Perfusion Biomarkers with Clinical Characteristics **

There were no significant differences in the microbubble velocity (β) and the perfusion index (β×A) in women compared with men (β: 0.50 [0.39–0.69] versus 0.42 [0.31–0.69] au, P=0.13; β×A: 0.48 [0.35–0.72] versus 0.39 [0.30–0.59] au, P=0.10), participants with a history of diabetes compared with participants without diabetes (β: 0.47 [0.30–0.73] versus 0.45 [0.33–0.75] au, P=0.38; β×A: 0.43 [0.27–0.60] versus 0.45 [0.33–0.65] au, P=0.39), participants with a history of coronary artery disease compared with participants without coronary artery disease (β: 0.47 [0.40–0.66] versus 0.45 [0.31–0.75] au, P=0.35; β×A: 0.44 [0.36–0.58] versus 0.44 [0.28–0.65] au, P=0.40).

There were no significant differences in the microbubble velocity (β) and the perfusion index (β×A) in participants using ACEi or ARB (β: 0.49 [0.37–0.63] versus 0.42 [0.29–0.96] au, P=0.38; β×A: 0.46 [0.36–0.58] versus 0.43 [0.30–0.72] au, P=0.49) or participants using diuretics (β: 0.50 [0.41–0.63] versus 0.42 [0.32–0.77] au, P=0.36; β×A: 0.48 [0.38–0.58] versus 0.41 [0.31–0.66] au, P=0.39).

The microbubble velocity (β) and the perfusion index (β×A) were slightly higher in participants with minimal proteinuria compared with participants who had proteinuria (Supplemental Figure 2). There were no significant correlations of the microbubble velocity (β) and the perfusion index (β×A) with age (β: r_s=0.02, P=0.88; β×A: r_s=0.03, P=0.80), BMI (β: r_s=0.02, P=0.89; β×A: r_s=0.04, P=0.69), or kidney size (β: r_s=0.17, P=0.13; β×A: r_s=0.17, P=0.13). There were significant negative correlations of the microbubble velocity (β) and the perfusion index (β×A) with kidney depth (β: r_s=−0.21, P=0.04; β×A: r_s=−0.25, P=0.02).

** Associations of CEUS-Derived Kidney Cortical Microvascular Perfusion Biomarkers with Kidney Function **

Table 3 shows the correlations of the microbubble velocity (β) and the perfusion index (β×A) with eGFR and CKD stage. The microbubble velocity (β) and the perfusion index (β×A) had significant positive correlations with eGFR and significant negative correlations with CKD stage (Figure 3). These associations were similar after excluding participants who had ESKD with an imputed eGFR value (Supplemental Table 1). These values were slightly stronger when restricted to participants who had a strong TIC model fit (R²>0.8). The microbubble velocity (β) and the perfusion index (β×A) remained significantly associated with eGFR in multivariable linear regression models adjusted for age, sex, race, BMI, diabetes, and ACEi or ARB use (Table 4).

**Discussion**

Chronic disturbances in kidney cortical microvascular perfusion may lead to the development and progression of CKD. In this study of individuals across the spectrum of kidney function, CEUS detected differences in kidney cortical microvascular perfusion among individuals with varying severity of kidney dysfunction compared with healthy volunteers. We found that individuals with lower kidney cortical microvascular perfusion, as assessed by CEUS, had lower eGFR. Importantly, the CEUS-derived kidney cortical microvascular perfusion biomarkers remained significantly associated with eGFR in multivariable models that adjusted for relevant covariates, which may influence kidney cortical microvascular perfusion. Our findings suggest that CEUS may serve as a tool to assess kidney cortical microvascular perfusion noninvasively, which warrants further investigation.

A few prior studies demonstrated the ability of CEUS to quantify kidney cortical microvascular perfusion. In animals, cortical blood flow, assessed by CEUS, correlated strongly with kidney blood flow measured by an ultrasonic flow probe placed directly over the renal artery (15–17). Confirmatory human data were generated using PAH clearance to measure effective renal plasma flow (ERPF) in two small studies in healthy volunteers (10,18) and a single small study in patients with CKD (19). Because GFR and ERPF are significantly correlated (21), our finding of moderate positive associations of CEUS-derived kidney cortical microvascular perfusion biomarkers with eGFR are consistent with the prior studies that incorporated gold-standard assessments of ERPF in humans. Although there are limited data in healthy volunteers (18,24), our results support additional studies to validate CEUS-derived kidney cortical

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**Table 4. Association of CEUS-derived kidney cortical microvascular perfusion biomarkers with eGFR**

| Model | β | 95% Confidence Interval | P | Model | β | 95% Confidence Interval | P |
|-------|---|------------------------|---|-------|---|------------------------|---|
| Unadjusted | | | | | | | |
| β mean | 0.32 | 0.12 to 0.52 | 0.002 | | | | |
| Adjusted | | | | | | | |
| β mean | 0.38 | 0.17 to 0.59 | <0.001 | | | | |
| Age, yr | −0.008 | −0.02 to 0.006 | 0.25 | | | | |
| Women | −0.05 | −0.46 to 0.37 | 0.83 | | | | |
| Race | −0.36 | −0.72 to −0.002 | 0.05 | | | | |
| BMI | 0.005 | −0.03 to 0.04 | 0.77 | | | | |
| Diabetes | −0.50 | −0.90 to −0.09 | 0.02 | | | | |
| ACEi/ARB | −0.11 | −0.51 to 0.29 | 0.59 | | | | |
| Adjusted | | | | | | | |
| β×A mean | 0.79 | 0.45 to 1.13 | <0.001 | | | | |
| Age, yr | −0.005 | −0.02 to 0.009 | 0.46 | | | | |
| Women | −0.13 | −0.52 to 0.26 | 0.50 | | | | |
| Race | −0.29 | −0.62 to 0.05 | 0.10 | | | | |
| BMI | 0.008 | −0.02 to 0.04 | 0.60 | | | | |
| Diabetes | −0.44 | −0.82 to −0.05 | 0.03 | | | | |
| ACEi/ARB | −0.07 | −0.45 to 0.32 | 0.73 | | | | |

β mean, β×A mean, and eGFR were natural log transformed. CEUS, contrast-enhanced ultrasound; BMI, body mass index; ACEi/ARB, angiotensin converting enzyme inhibitor or angiotensin II receptor blocker; CI, confidence interval.
microvascular perfusion biomarkers using detailed physiologic studies that measure ERPF through PAH clearance and to test differences in CEUS-derived kidney cortical microvascular perfusion after pharmacologic intervention to alter arteriolar tone across the spectrum of kidney function.

Prior studies that investigated the associations between CEUS-derived kidney cortical microvascular perfusion biomarkers and eGFR yielded inconsistent results. Our associations between kidney cortical microvascular perfusion biomarkers, as assessed by CEUS, with eGFR (r = 0.50) were similar in magnitude to a recent study (r = 0.54) (20). Although the investigators performed flash-replenishment sequences using a continuous infusion of microbubbles to calculate a perfusion index, which is similar to the perfusion index calculated in our study (β × ΔA), it is important to note that the study population was healthier, with only 18 participants who had CKD (eGFR 58 ± 28 ml/min per 1.73 m²) compared with our study population. In contrast, an earlier study (25) identified a weaker association between the microbubble velocity with eGFR (r = 0.28) than our study (r = 0.44). Although the latter study included 85 participants with CKD, which included 40 participants with eGFR < 30 ml/min per 1.73 m², the investigators used a bolus technique to generate their time-intensity curve. Although we were able to calculate both the microbubble velocity and perfusion index using the flash-replenishment sequences, which each had similar associations with eGFR, our results highlight the need to determine optimal microbubble contrast agent administration and imaging methods to quantify kidney cortical microvascular perfusion by CEUS. Another important difference between the prior studies and this study is that each used different image analysis software packages. The prior studies used commercially developed software packages (VUEBox [20] and MITANI WinROOF [25]), which were also different than our noncommercially developed software interface to perform image analysis. Our results should also stimulate additional research to compare software package platforms on the same study population to ensure reproducibility in future studies.

The chronic hypoxia hypothesis suggests that decreased tissue perfusion leads to chronic hypoxia, which prompts inflammatory cell infiltration and accumulation of extracellular matrix protein deposition with resultant fibrosis that further obliterates the microvasculature and propagates scarring and progressive loss of kidney function (1, 26). Our finding that CEUS-derived kidney cortical microvascular perfusion biomarkers are higher in individuals with preserved kidney function and lower in individuals with lower kidney function are consistent with recent data that found significant associations of lower CEUS-derived kidney cortical microvascular perfusion with more severe chronic histopathologic lesions and kidney function decline over time (27). Future studies should compare CEUS-derived kidney cortical microvascular perfusion against noninvasive tests of kidney cortical oxygenation and perfusion, as assessed by blood oxygen level–dependent and arterial spin labeling MRI, respectively, to investigate further the chronic hypoxia hypothesis (28, 29). Although eGFR and proteinuria improve the ability to risk stratify populations (4, 30), they may not be able to discern which individual patient with mild CKD will progress (31). Because arterial and arteriolar sclerosis are strongly associated with CKD progression independent of eGFR and proteinuria (2), noninvasive assessments of arterial and arteriolar sclerosis may identify individuals at risk of chronic tubulointerstitial hypoxia. Additional data are needed to determine whether CEUS-derived kidney cortical microvascular perfusion can identify more severe arterial and arteriolar sclerosis to provide complementary prognostic value to currently available CKD risk estimation markers.

Strengths of our study are the inclusion of participants across the spectrum of kidney function, the inclusion of a radiologist to aid in ROI placement, and the use of customized developed CEUS image analysis software on the basis of CEUS perfusion quantification as previously described (22). Our study has several limitations that warrant consideration as well. Although we included healthy volunteers and participants with ESKD to provide a full spectrum of kidney function, we had a limited sample size of healthy volunteers who were not age or sex matched. Although all CEUS images were obtained during a breath hold, we cannot exclude whether some images were influenced by breathing. We used hand-drawn ROIs, which may not be objective, and future studies should incorporate automated segmentation of images. Although a radiologist assisted in ROI placement, the radiologist evaluated each set flash-replenishment sequences separately. Our relatively high intraparticipant CVs may highlight potential issues with reproducibility related to the placement of the ROI or probe positioning by the ultrasonographer, which requires further investigation in follow-up studies. Because ultrasound has relatively low resolution, we cannot exclude the possibility that medullary perfusion influenced the CEUS-derived biomarkers, which may have led to more variability in the measurements (i.e., higher coefficients of variation) (32, 33). We used an average of values for each respective imaging biomarker, but the associations were similar when we used the first or second value. Although we used a custom-developed CEUS image analysis software, we did not test inter-reader variability, which should be performed in future studies. Although we imputed eGFR values for participants with ESKD and healthy volunteers who did not have eGFR values measured, the magnitude of our associations were similar with CKD stage. We did not control for dietary sodium intake or high protein meals, which could influence CEUS-derived kidney cortical microvascular perfusion (11, 20).

In conclusion, our study demonstrates that CEUS, a safe and noninvasive imaging modality, has the potential to quantify kidney cortical microvascular perfusion. Our results provide quantitative data regarding associations of kidney cortical microvascular perfusion with eGFR, which typically cannot be implemented easily in clinical practice due to impracticality of performing detailed physiologic studies. Our cross-sectional findings suggest the need for larger prospective studies to test CEUS as a novel noninvasive imaging tool to identify individuals at high risk of CKD progression. Confirmation of our findings in prospective studies may lead to the incorporation of CEUS as a tool to assess for kidney injury in real time, to provide
complementary prognostic information, or to monitor response to treatment in the setting of a clinical trial.

Disclosures

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Author Contributions

L.M.B. Burke, E.H. Chang, A. Sridharan, A. Srivastava, and R.W. Walmer were responsible for formal analysis; E.H. Chang, A. Sridharan, and A. Srivastava were responsible for conceptualization, data curation, and methodology, and wrote the original draft of the manuscript; E.H. Chang was responsible for funding acquisition, project administration, and supervision; and all authors were responsible for the investigation, reviewed and edited the manuscript, and contributed to critical revisions of the manuscript for important intellectual content.

Supplemental Material

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Supplemental Table 1. Correlation between contrast-enhanced ultrasound (CEUS)-derived kidney microvascular perfusion biomarkers and kidney function in individuals who did not have ESKD.

Supplemental Figure 1. Representative kidney CEUS images and time-intensity curves in healthy, CKD, and ESKD participants.

Supplemental Figure 2. CEUS-derived kidney microvascular perfusion biomarkers by semiquantitative assessments of proteinuria.

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