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ORIGINAL RESEARCH ARTICLE

# Patients with moderate chronic kidney disease without heart disease have reduced coronary flow velocity reserve

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## Abstract

**Aims** The overall aim was to identify sub-clinical cardiac abnormalities by echocardiography in patients with chronic kidney disease (CKD) stages 3 and 4 and to investigate underlying mechanisms.

**Methods and results** Ninety-one patients with CKD stages 3 and 4, without a diagnosis of heart disease, and 41 healthy matched controls were included in this cross-sectional study. Cardiac morphology and function were analysed with Doppler echocardiography and coronary flow velocity reserve (CFVR) in response to adenosine was measured in the left anterior descendent artery to detect coronary microvascular dysfunction (CMD). All study subjects had a left ventricular (LV) ejection fraction $>$ 50%. Patients with CKD showed statistically significant increases in left atrial volume index and transmitral and pulmonary vein flow velocities during atrial contraction and prolonged LV isovolumetric relaxation time. Patients with CKD had significantly reduced CFVR vs. controls (2.74 ± 0.86 vs. 3.40 ± 0.89, $P < 0.001$), and 43% of patients were classified as having CMD compared with 9% of controls ($P = 0.001$).

**Conclusions** Patients with CKD stages 3 and 4, without a diagnosis of heart disease, showed early abnormalities in LV diastolic function that did not fulfil the criteria for LV dysfunction according to current guidelines. A large proportion of CKD patients had CMD, suggesting that microvascular abnormalities may have a pathogenic role in the development of heart failure in this patient group.

**Keywords** Chronic kidney disease; Diastolic dysfunction; Left ventricular hypertrophy; Cardio-renal syndrome; Left atrial volume; Coronary flow velocity reserve

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## Introduction

Chronic kidney disease (CKD) has an estimated prevalence of ~10% globally, and cardiovascular diseases are the major cause of morbidity and mortality in this patient group.[1] In addition, sudden cardiac death is the most common cause of death among patients with end-stage renal disease, comprising ~25% of all mortality.[2] The pathophysiology in patients with CKD includes a bidirectional interaction between the kidneys and the heart, which has been termed *cardio-renal syndrome*. Cardio-renal syndrome type 4, also known as chronic renocardiac syndrome, refers to cardiac abnormalities in patients with primary CKD.[3] Already when the glomerular filtration rate (GFR) falls <60 mL/min/1.73 m$^2$, there is an inverse relationship between GFR and cardiovascular risk,[4] and the incidence and prevalence of heart failure (HF) increase substantially as CKD progresses.[5–7] In addition, reduced GFR increases the risk for death and hospitalization in patients with HF.[8]

In a large community-based cohort,[9] it was shown that CKD was a risk factor for new onset of HF with preserved left ventricular (LV) ejection fraction (EF) (HFpEF), but not for HF with reduced EF (HFrEF). Recent studies suggest that 30–50% of patients with HF have HFpEF and that the mortality rate in these patients is as high as in those with HFrEF.[10] Notably, data from the Swedish Heart Failure Registry demonstrate that CKD increases the mortality risk in HF patients regardless of EF.[11] In a subset of participants of the Chronic Renal Insufficiency Cohort, who underwent repeated echocardiograms, it was shown that EF declined significantly as kidney function deteriorated and patients became dialysis dependent.[12] Hence, it is feasible to hypothesize that CKD patients initially develop HFpEF and that EF eventually may decline as kidney failure progresses. To prevent, or treat, HFpEF in patients with CKD, it is crucial to know more about the underlying pathophysiological mechanisms during the early stages of CKD. It has been suggested that coronary microvascular dysfunction (CMD) might be important in the pathogenesis of HFpEF.[13] Interestingly, coronary microvascular function can be assessed non-invasively by measuring coronary flow velocity reserve (CFVR) by Doppler echocardiography.[14] We hypothesized that CKD patients without symptomatic heart disease could have CMD and that this abnormality might contribute to the development of HFpEF.

The overall aim of this explorative, hypothesis-generating study was to identify early, sub-clinical abnormalities in cardiac morphology and function by echocardiography in patients with moderate CKD and to investigate underlying mechanisms. For this purpose, we performed detailed echocardiographic examinations, including analysis of CFVR, in patients with CKD stages 3 and 4 who did not have a diagnosis of heart disease.

# Methods

## Subjects and protocol

Study subjects were derived from a cohort of patients with CKD stages 3 and 4 who were recruited from the Nephrology Outpatient Clinic at the Sahlgrenska University Hospital, Gothenburg, Sweden, between February 2009 and December 2011. Newly referred patients or patients with planned follow-up within 1 month were offered to participate, and 122 individuals were included. Inclusion criteria were $>$18 years of age and an estimated GFR of 15 to 59 mL/min/1.73 $m^2$ according to the Modification of Diet in Renal Disease formula since at least 3 months (i.e. CKD stages 3 and 4). Exclusion criteria were previous organ transplantation, ongoing immunosuppressive medication, inflammatory systemic disease, endocrine disease aside from diabetes mellitus or substituted hypothyroidism, expected survival of $<$12 months, expected need of renal replacement therapy within 12 months, and pregnancy or current breastfeeding. In addition, healthy subjects were recruited through an advertisement in local newspapers; and 47 individuals, matched for age and gender, were included as controls. Of the 122 patients with CKD, 24 had a diagnosis of heart disease (15 patients had ischaemic heart disease, five atrial fibrillation, one HFpEF, two both ischaemic heart disease and HF, and one both atrial fibrillation and ischaemic heart disease) and were excluded. Of the remaining 98 CKD patients, 91 accepted to undergo echocardiographic examinations and were included in the present study. Forty-one healthy controls approved of echocardiographic examinations and were included.

In this cross-sectional study, a detailed medical history was gathered, and the following tests were performed: anthropometric measurements, urine and blood biochemistry, haemodynamic assessments, echocardiography, and plasma $^{51}$Cr-EDTA clearance to determine GFR. Subjects were considered as having hypertension if they had a prior diagnosis of hypertension or if they were prescribed antihypertensive drugs. The Ethics Committee of the University of Gothenburg approved the study. The research was conducted in accordance with the Declaration of Helsinki. All study subjects gave informed written consent to participate.

## Plasma analyses and measurement of glomerular filtration rate

Fasting blood samples were drawn from study subjects in a supine position and processed locally for routine analyses by standard laboratory methods at the Department of Clinical Chemistry at Sahlgrenska University Hospital (SWEDAC approved according to European norm 45001). For non-routine analyses, serum was stored at $-$70°C until analysis. The coefficient of variation (CV), calculated based on 12 repeated analyses on three consecutive days on the Roche COBAS methods were, creatinine, 2.1%; N-terminal pro-brain natriuretic peptide (NT-proBNP), 4.9%; and troponin T (TnT), 3.3%.[15] The CV calculated based on 12 repeated analyses on three consecutive days were for copeptin 4.1% (Brahms) and high-sensitivity troponin I 6.9% (Abbot).[15] Plasma concentrations of free metanephrine and normetanephrine were measured by liquid chromatography–mass spectrometry. Fibroblast growth factor-23 (FGF-23) was measured by ELISA (Kainos Laboratories Inc., Tokyo, Japan). The intra-assay CV was $<$3%, and the inter-assay CV was $<$4%. Measurements were performed in duplicates for FGF-23, and values were averaged. Urinary albumin-to-creatinine ratio (U-ACR, mg/mmol) was determined on urine collected for 24 h.

Plasma clearance of $^{51}$Cr-EDTA was used to measure GFR at the Department of Clinical Physiology at Sahlgrenska University Hospital according to clinical routines.

## Haemodynamic assessments

Ambulatory blood pressure (ABP) was recorded during 24 h (Spacelabs Healthcare, Model 90217). Blood pressure was measured three times per hour between 6 a.m. and 10 p.m. and twice per hour from 10 p.m. to 6 a.m. Daytime blood pressure was based on recordings between 6 a.m. and 9 p.m. and night-time blood pressure between 12 a.m. and 6 a.m. Nocturnal dipping of ambulatory systolic blood pressure (ASBP) was calculated as (night-time ASBP − daytime ASBP)/daytime ASBP and expressed in per cent.

A trained research nurse measured carotid–femoral pulse wave velocity (cfPWV), digital reactive hyperaemia, and ankle–brachial index under standardized conditions in the morning after an overnight fast, with the study participant in a supine position. By using applanation tonometry during simultaneous electrocardiogram (ECG) monitoring, the duration between the R-wave and the subsequent pressure wave was determined with SphygmoCor software (version 8, AtCor Medical, Sydney, Australia). cfPWV, an indirect measure of aortic stiffness, was derived by measuring the distance between the femoral and carotid pulses, using the suprasternal notch as reference measure point, divided by the pulse transit time between the two locations.

Digital reactive hyperaemia was analysed to assess endothelial function using the Endo-PAT2000 (Itamar Medical Ltd., Caesarea, Israel) as previously described.[16] Reactive hyperaemic index (RHI) was calculated as the mean flow response post-occlusion using the non-occluded arm as a reference. Ankle–brachial index was measured using a Doppler probe and a sphygmomanometer. The mean of the indices for the posterior tibial artery and dorsalis pedis artery for each foot was calculated, and the average value of the left and right feet was determined.

## Echocardiography

Examinations were performed by an experienced physician (C. W. G.) using Acuson Sequoia C256 (Siemens, Mountain view, CA, USA) or Philips IE 33 (Philips Electronics, Eindhoven, The Netherlands) in accordance with the recommendations of the American Society of Echocardiography (ASE).[17,18] Examinations were made in the morning, and study participants were instructed not to take their morning medication prior to the investigation. The offline echocardiographic analyses were performed using the software EchoPac (General Electric, Fairfield, CT, USA) by an investigator (C. W. G.) blinded to all other clinical data.

Study subjects who gave their consent (75 patients with CKD and 39 controls) received the echo contrast agent SonoVue® (Bracco, Milan, Italy) for LV opacification. SonoVue® was administered through an infusion pump at a rate of ~0.7 mL/min, and the rate was adjusted according to image quality. LV volumes were derived during contrast enhancement according to the modified biplane Simpson's method in the apical four-chamber view and if possible also in the two-chamber view. LVEF was rendered from the same Simpson's rule, or visually estimated, according to what was feasible. Stroke volume (SV) was determined by pulsed Doppler combined with calculated area of the LV outflow tract. Maximal ventricular septal wall thickness was measured in the four-chamber view in diastole during contrast enhancement. LV mass (LVM) was calculated by the cube formula[19] using parasternal 2D images and was indexed to body surface area (BSA) measured by the DuBois formula [LVM index (LVMI), g/m$^2$]. Relative wall thickness (RWT) was calculated by the following formula: [(2 × diastolic posterior wall thickness)/diastolic LV internal diameter]. LV hypertrophy (LVH) was defined as LVMI $>$ 115 g/m$^2$ in men or $>$95 g/m$^2$ in women.[19] LVH was further classified as either concentric (RWT $>$ 0.42) or eccentric (RWT $\leq$ 0.42).[19] Subjects with normal LVMI but RWT $>$ 0.42 were considered to have concentric remodelling. Transmitral peak flow velocities in early diastole (E) and at atrial contraction (A) were recorded at the tips of the mitral valve leaflets. Peak tissue velocities were derived by pulsed tissue Doppler analysis at the septal and lateral margin of the mitral annulus during systole (s′) and during early (e′) and late (a′) diastole. Mean values of the septal and lateral sites are presented. In the RV, tissue velocities s′, e′, and a′ were recorded at the free wall tricuspid annulus. The E/A ratio, the E deceleration time, and the ratio E/e′ were calculated. Left atrial (LA) volume index (LAVI), as computed by the area–length formula, was obtained from the apical four-chamber view at the end of systole and was indexed for BSA.[17] LA area and right atrial (RA) area were measured, and the intra-individual difference in atrial area size (LA–RA area) was calculated. Moreover, in case of a detectable tricuspid regurgitation (TR), the maximum flow velocity (m/s) was measured with continuous-wave Doppler, and the pressure gradient calculated. A standardized value of 5 mmHg was then added to estimate pulmonary artery systolic pressure (PASP). Calcification of the mitral and aortic valves was scored separately using a semi-quantitative scale from 0 to 3 and then added to generate a total score ranging from 0 to 6.

In subjects with normal EF, LV diastolic dysfunction was evaluated according to the guidelines by the ASE and were based on the following variables and cut-offs: E/e′ $>$ 14, septal e′ velocity $<$ 7 cm/s or lateral e′ $<$ 10 cm/s, TR peak velocity $>$ 2.8 m/s, and LAVI $>$ 34 mL/m$^2$.[18]

To assess CFVR, the mid to distal parts of the left anterior descending artery (LAD) was visualized with 3.5 MHz colour Doppler, and pulsed Doppler was used to sample flow velocity signals at rest (baseline) and during adenosine infusion (140 μg/min/kg) over ~5 min as previously described.[20] Measurements were performed under continuous ECG monitoring. CFVR was determined by the ratio of maximal diastolic flow velocity during adenosine-induced hyperaemia to that

during baseline. Measurements of CFVR were carried out on 49 CKD patients and 33 healthy controls who accepted to receive adenosine. Doppler echocardiography for assessment of CFVR has been validated against positron emission tomography (PET)-based measurements.[21] A CFVR $< 2.5$ was considered abnormal and compatible with CMD on the basis of prior studies.[14,22]

### Statistics

Statistical analyses were performed using the SPSS Statistics Data Editor (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA). Presented values are means and standard deviations (SDs) for continuous data and proportions (%) for categorical variables. Statistical significance was set at the level of $P < 0.05$. Correlations between continuous data were calculated using Pearson's or Spearman's test, when appropriate. The Mann–Whitney *U*-test was used for comparing differences in continuous data between groups. Differences in frequencies were analysed using Fisher's exact test. Univariate multiple regression analyses were designed on patients with CKD to evaluate the relationship between clinical characteristics and measures of cardiac function. Only continuous variables that showed a statistically significant correlation with the dependent variable were included in regression models. Similarly, only categorical variables that were significantly different in the dependent variable were included in the regression models.

## Results

### General characteristics of study population

The primary cause of CKD was glomerulonephritis in 32% of patients, diabetic kidney disease in 20%, hypertension in 14%, autosomal dominant polycystic kidney disease in 9%, renovascular disease in 6%, and other causes in 19%. Patients with CKD were somewhat younger than controls, and ≈87% had hypertension. The use of antihypertensive drugs in the CKD group was for angiotensin-converting enzyme (ACE) inhibitors/angiotensin receptor blockers (ARBs) 84%, diuretics 59%, calcium channel blockers 42%, beta-blockers 35%, and alpha-blockers 7%. Statins were used by 49% of CKD patients. No patients were on mineralocorticoid receptor antagonists or long-acting nitrates (*Table* 1).

Body mass index was significantly increased in patients with CKD. Patients with CKD, compared with controls, had an average GFR of 34.9 ± 13.9 mL/min/1.73 $m^2$ and significantly elevated serum levels of phosphate, FGF-23, parathyroid hormone, high-sensitivity C-reactive protein, NT-proBNP, TnT, and copeptin. In addition, blood haemoglobin count was significantly reduced, and U-ACR elevated, in CKD patients vs. controls. There were no statistically significant differences between groups in serum ionized calcium (data not shown), plasma metanephrine (0.18 ± 0.09 vs. 0.17 ± 0.10 nmol/L in CKD patients and controls, respectively, $P = 0.80$), or normetanephrine (0.43 ± 0.22 vs. 0.44 ± 0.15 nmol/L in CKD patients and controls, respectively, $P = 0.79$).

### Haemodynamic variables

There were no statistically significant differences between groups in ABP during 24 h, daytime, or night-time. However, nocturnal dipping of ASBP and ankle–brachial index were significantly reduced in CKD patients. There were no statistically significant differences between groups in digital RHI or cfPWV (*Table* 2).

### Left ventricular morphology and function by echocardiography

Patients with CKD, compared with controls, had significantly increased LV RWT and maximal ventricular septal wall thickness. Although there was no statistically significant difference between groups in LVMI, 10 CKD patients (11%), but no controls, met the criteria for LVH ($P = 0.027$ between groups). In the CKD group, seven patients had eccentric LVH and three had concentric LVH. In addition, 10 CKD patients (11%) had concentric LV remodelling. Patients with CKD had a significantly elevated LV SV even after correction for BSA. However, there was no statistically significant difference between groups in cardiac output or cardiac index. All patients with CKD, and controls, had an LVEF $> 50\%$. There was no statistically significant difference between groups in PASP [28 ± 6 vs. 26 ± 4 mmHg, in CKD patients ($n = 43$) and controls ($n = 25$), respectively, $P = 0.078$]. Physiological or minor regurgitations and/or aortic sclerosis were present in some individuals, but no study subject had a haemodynamically significant valvular lesion (*Table* 3).

In a regression model, to predict maximal ventricular septal wall thickness in patients with CKD, we included variables gender, diagnosis of hypertension, 24 h urinary sodium excretion, night-time ASBP, cfPWV, and BSA. Night-time ASBP ($B = 0.067$, $P = 0.001$), cfPWV ($B = 0.241$, $P = 0.01$), and BSA ($B = 2.947$, $P = 0.047$) showed independent associations with maximal septal wall thickness.

### Doppler measures and indices of diastolic function

Left ventricular tissue velocities e′, a′, and s′ were significantly elevated in CKD patients vs. controls. There was no

**Table 1** General characteristics, kidney function, and cardiac biomarkers

| | CKD $n = 91$ | Controls $n = 41$ | *P*-value |
|---|---|---|---|
| Age, years | 60 ± 12 | 64 ± 10 | 0.014 |
| Men (%) | 60 (65.9) | 28 (68.3) | 0.844 |
| Smoking history (%) | 41 (45.6) | 11 (26.8) | 0.054 |
| Body mass index, kg/m$^2$ | 26.7 ± 3.9 | 25.2 ± 3.2 | 0.023 |
| Body surface area (BSA), m$^2$ | 1.97 ± 0.22 | 1.90 ± 0.2 | 0.057 |
| Cerebrovascular disease (%) | 5 (5.5) | 0 | — |
| Diabetes (%) | 24 (26.4) | 0 | — |
| Hypertension (%) | 79 (86.8) | 0 | — |
| GFR, mL/min/1.73 m$^2$ | 34.9 ± 13.9 | 85.6 ± 13.3 | <0.001 |
| Serum creatinine, μmol/L | 185 ± 71 | 76 ± 11 | <0.001 |
| U-ACR, mg/mmol | 55 ± 84 | 1.5 ± 3.4 | <0.001 |
| Serum FGF-23, pg/mL | 176 ± 149 | 70 ± 19 | <0.001 |
| Serum phosphate, mmol/L | 1.1 ± 0.2 | 1.0 ± 0.2 | 0.021 |
| Serum PTH, ng/L | 118 ± 70 | 53 ± 16 | <0.001 |
| Blood haemoglobin count, g/L | 126 ± 14 | 137 ± 10 | <0.001 |
| Serum hs C-reactive protein, mg/L | 3.02 ± 3.82 | 1.39 ± 1.81 | 0.001 |
| Urinary Na excretion, mmol/24 hr | 148 ± 60 | 145 ± 61 | 0.437 |
| Serum NT-proBNP, ng/L | 237 ± 300 | 80 ± 72 | <0.001 |
| Serum troponin T, ng/L | 16.1 ± 14.1 | 6.9 ± 7.6 | <0.001 |
| Serum hs troponin I, ng/L | 5.7 ± 17.6 | 4.1 ± 7.5 | 0.497 |
| Serum copeptin, pmol/L | 30.0 ± 18.1 | 8.6 ± 6.1 | <0.001 |

Abbreviations: CKD, chronic kidney disease; GFR, glomerular filtration rate (measured by $^{51}$Cr-EDTA clearance); U-ACR, urine albumin-to-creatinine ratio; FGF-23, fibroblast growth factor-23; PTH, parathyroid hormone; hs, high sensitivity; and NT-proBNP, N-terminal pro-brain natriuretic peptide.
Values are proportions (%) or means ± SD.

**Table 2** Arterial blood pressures and haemodynamics

| | CKD $n = 83$–$91$ | Controls $n = 41$ | *P*-value |
|---|---|---|---|
| 24 h ASBP, mmHg | 122 ± 14 | 122 ± 11 | 0.767 |
| 24 h ADBP, mmHg | 74 ± 10 | 73 ± 7 | 0.542 |
| Daytime ASBP, mmHg | 128 ± 15 | 129 ± 13 | 0.701 |
| Daytime ADBP, mmHg | 78 ± 11 | 78 ± 7 | 0.812 |
| Night-time ASBP, mmHg | 113 ± 15 | 109 ± 11 | 0.232 |
| Night-time ADBP, mmHg | 66 ± 10 | 64 ± 7 | 0.157 |
| ASBP nocturnal dipping, % | −11.7 ± 7.1 | −14.8 ± 6.7 | 0.018 |
| Ankle–brachial index | 1.14 ± 0.12 | 1.20 ± 0.08 | 0.001 |
| Digital RHI | 2.58 ± 1.08 | 2.56 ± 0.56 | 0.751 |
| cfPWV | 9.52 ± 2.92 | 8.88 ± 2.05 | 0.256 |

Abbreviations: CKD, chronic kidney disease; ASBP, ambulatory systolic blood pressure; ADBP, ambulatory diastolic blood pressure; RHI, reactive hyperaemia index; cfPWV, carotid–femoral pulse wave velocity.
Values are means ± SD.

statistically significant difference between groups in E, E/e′, or E/A. However, A wave velocity was significantly elevated in CKD patients. In addition, pulmonary vein flow velocity during atrial contraction was significantly elevated in patients with CKD (0.39 ± 0.09 vs. 0.33 ± 0.07 m/s, $P = 0.001$). LV isovolumetric relaxation time (IVRT) was prolonged, and LAVI and the difference in area size between the LA and RA (LA–RA area) were significantly elevated in the CKD group. There was no statistically significant difference between groups in E deceleration time (214 ± 55 vs. 207 ± 41 ms, in CKD patients and controls, respectively, $P = 0.826$). Right ventricular tissue velocities e′ and s′ were significantly elevated in patients with CKD. Only one patient with CKD, and no control, met the criteria for LV diastolic dysfunction according to the ASE guidelines criteria. Twenty-six CKD patients (29%) had two abnormal variables out of four for identifying LV diastolic dysfunction and were classified as indeterminate according to ASE guidelines (*Table* 4).

In a regression model to predict LAVI in CKD patients, we included variables gender, medication with beta-blockers or diuretics, age, NT-proBNP, 24 h ASBP, LVMI, and LV diastolic volume/BSA. NT-proBNP ($B = 0.013$, $P = 0.001$) and LV diastolic volume/BSA ($B = 0.367$, $P < 0.001$) were independently associated with LAVI.

To investigate variables predicting LV e′, a regression model was created with age, NT-proBNP, 24 h urinary Na excretion, CFVR, and LVMI. Only age ($B = -0.130$, $P < 0.001$) and LVMI ($B = -0.029$, $P = 0.026$) showed independent associations with LV e′.

## Left anterior descending artery blood flow velocity and coronary flow velocity reserve

Baseline LAD flow velocity was significantly elevated in CKD patients vs. controls (0.37 ± 0.12 vs. 0.29 ± 0.07 m/s, $P < 0.001$, *Figure* 1A). In CKD patients, baseline LAD flow velocity correlated with 24 h ASBP ($r = 0.353$, $P = 0.014$). Baseline LAD flow velocity in patients with CKD did not correlate significantly with age ($r = 0.050$, $P = 0.733$), GFR ($r = -0.068$, $P = 0.645$), LVMI ($r = 0.193$, $P = 0.184$), cardiac index ($r = 0.240$, $P = 0.101$), or NT-proBNP ($r = 0.236$,

**Table 3** Left ventricular morphology and function

| | CKD ($n$ = 85–91) | Controls ($n$ = 39–41) | $P$-value |
|---|---|---|---|
| LV mass index, g/m$^2$ | 82.5 ± 21.7 | 74.9 ± 16.4 | 0.068 |
| LV RWT | 0.37 ± 0.06 | 0.32 ± 0.05 | 0.001 |
| Maximal septal wall thickness, mm | 8.6 ± 2.7 ($n$ = 75) | 7.5 ± 1.8 ($n$ = 39) | 0.047 |
| Valvular calcification score (0–6) | 0.5 ± 0.87 | 0.4 ± 0.8 | 0.359 |
| Ascending aortic diameter, mm | 31.9 ± 4 | 31.8 ± 3.1 | 0.993 |
| LV ejection fraction (estimated), % | 64 ± 5 | 63 ± 4 | 0.359 |
| LV ejection fraction (Simpson), % | 64 ± 7 ($n$ = 68) | 65 ± 5 ($n$ = 39) | 0.433 |
| LV diastolic volume/BSA, mL/m$^2$ | 68.3 ± 15.3 ($n$ = 70) | 63.1 ± 8.9 ($n$ = 39) | 0.140 |
| LV stroke volume, mL | 85.4 ± 16.9 | 76.9 ± 13.3 | 0.015 |
| LV stroke volume/BSA, mL/m$^2$ | 43.4 ± 6.5 | 40.5 ± 5.3 | 0.029 |
| Heart rate, beats/min | 66 ± 12 | 66 ± 10 | 0.947 |
| Cardiac output, L/min | 5.62 ± 1.24 | 5.05 ± 1.02 | 0.053 |
| Cardiac index, L/min/m$^2$ | 2.9 ± 0.6 | 2.7 ± 0.5 | 0.239 |

Abbreviations: CKD, chronic kidney disease; LV, left ventricle; RWT, relative wall thickness; BSA, body surface area.
Values are means ± SD. Left ventricular ejection fraction according to Simpson, LV diastolic volume, and maximal septal wall thickness were measured in a subset of patients using contrast enhancement (see Methods).

**Table 4** Doppler measures and indices of diastolic function

| | CKD ($n$ = 82–91) | Controls ($n$ = 38–41) | $P$-value |
|---|---|---|---|
| LV s′ mean, cm/s | 9.3 ± 1.8 | 8.1 ± 1.3 | <0.001 |
| LV e′ mean, cm/s | 9.7 ± 2.5 | 8.7 ± 2.1 | 0.019 |
| LV a′ mean, cm/s | 11.1 ± 2.4 | 9.4 ± 2.1 | <0.001 |
| E, m/s | 0.68 ± 0.15 | 0.65 ± 0.14 | 0.296 |
| A, m/s | 0.72 ± 0.18 | 0.61 ± 0.13 | 0.001 |
| E/e′ | 7.5 ± 2.6 | 7.8 ± 2.4 | 0.225 |
| E/A | 1.00 ± 0.32 | 1.12 ± 0.38 | 0.133 |
| LV IVRT, ms | 85 ± 16 | 78 ± 15 | 0.011 |
| LAVI, mL/m$^2$ | 36.3 ± 9.7 | 33.2 ± 9.7 | 0.013 |
| LA–RA area, cm$^2$ | 4.0 ± 2.5 | 1.8 ± 1.9 | <0.001 |
| RV s′ mean, cm/s | 13.9 ± 2.8 | 12.0 ± 2.1 | <0.001 |
| RV e′ mean, cm/s | 12.8 ± 3.2 | 10.6 ± 2.5 | <0.001 |
| RV a′ mean, cm/s | 15.2 ± 4.2 | 14.0 ± 3.8 | 0.133 |

Abbreviations: CKD, chronic kidney disease; LV, left ventricle; s′, systolic tissue velocity; e′, early diastolic tissue velocity; a′, late (atrial) diastolic tissue velocity; E, early diastolic transmitral flow velocity; A, late (atrial) diastolic transmitral flow velocity; IVRT, isovolumetric relaxation time; LAVI, left atrial volume index; LA, left atrium; RA, right atrium; RV, right ventricle; LAD, left anterior descending coronary artery.
Values are means ± SD. In the LV, tissue velocities were measured at the mitral annulus, and in the RV, at the free wall tricuspid annulus.

$P$ = 0.103). There were no statistically significant effects of gender, diabetes, smoking history, statin treatment, or antihypertensive drug class on baseline LAD flow velocity in CKD patients (data not shown).

Peak hyperaemic LAD flow velocity following adenosine did not differ significantly between groups ($P$ = 0.684, *Figure* 1A). However, CFVR was significantly reduced in CKD patients vs. controls (2.74 ± 0.86 vs. 3.40 ± 0.89, $P < 0.001$, *Figure* 1A and B). In CKD patients, CFVR correlated significantly with age, 24 h ASBP, LVMI, TnT, and NT-proBNP (*Figure* 2). In patients with CKD, CFVR did not correlate significantly with GFR ($r$ = 0.188, $P$ = 0.20), digital RHI ($r$ = −0.057, $P$ = 0.696), or cardiac index ($r$ = −0.147, $P$ = 0.318). There were no statistically significant effects of gender, diabetes, smoking history, statin treatment, or antihypertensive drug class, on CFVR in patients with CKD (data not shown). Age ($B$ = −0.024, $P$ = 0.028) and 24 h ASBP ($B$ = −0.022, $P$ = 0.025) were independently associated with CFVR in CKD patients when applying a regression model that also included LVMI, TnT, and NT-proBNP. The number of subjects with a CFVR < 2.5, indicating CMD, were three controls (9%) and 21 CKD patients (43%) ($P$ = 0.001). The characteristics of CKD patients with a CFVR ≥ 2.5 or <2.5 are shown in *Table* 5. Notably, CKD patients with CFVR < 2.5 had a significantly reduced hyperaemic LAD flow velocity than had patients with CFVR ≥ 2.5 (*Table* 5).

## Discussion

The main findings of the present study were that patients with CKD stages 3 and 4, without a diagnosis of heart disease and with preserved EF, showed abnormalities in LV diastolic function without fulfilling formal criteria for diastolic dysfunction and had reduced CFVR, indicating CMD. We hypothesize that CMD might play a role in the pathogenesis of HF in patients with CKD.

The majority of CKD patients in the present study had a diagnosis of hypertension. Hence, an increase in maximal interventricular septal wall thickness was expected, although we did not detect any significant difference between groups in 24 h ABP. Patients with CKD had an average 24 h ABP of 122/74 mmHg, clearly indicating good blood pressure control in our cohort. This and the fact that 84% of patients were on ACE inhibitors or ARBs are likely explanations for the relatively minor echocardiographic abnormalities detected. In a multiple regression model, we identified that cfPWV had an independent, positive association with interventricular septal

**Figure 1** Left anterior descending artery (LAD) flow velocities and LAD flow velocity reserve. Flow velocities in the LAD at baseline (open bars) and during adenosine infusion (filled bars) in patients with chronic kidney disease (CKD) and controls (A). (B) LAD flow velocity reserve. Flow velocities in LAD were measured by Doppler echocardiography (see Methods). Values are means ± SD. *$P < 0.01$ vs. controls, #$P < 0.01$ within group vs. baseline.

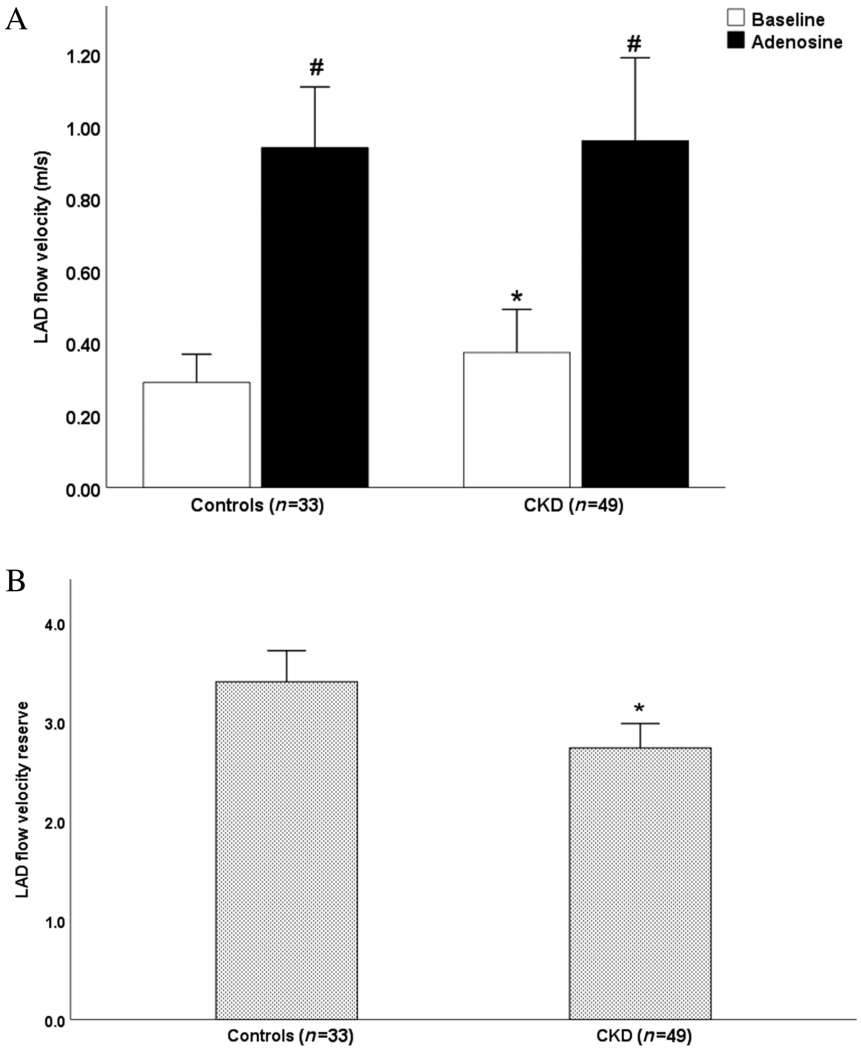


**Figure 2** Correlations to left anterior descending artery (LAD) flow velocity reserve in chronic kidney disease (CKD) patients. Correlations between age, serum troponin T, serum N-terminal pro-brain natriuretic peptide (NT-proBNP), 24 h ambulatory systolic blood pressure (ASBP), left ventricular mass index (LVMI), and flow velocity reserve measured by Doppler echocardiography in the left anterior descending coronary artery (LAD). *r*, Spearman's correlation coefficient; *n* = 49.

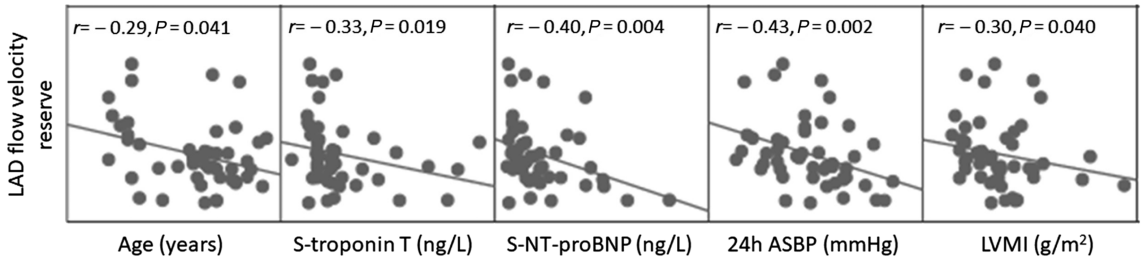

**Table 5** Characteristics for chronic kidney disease patients with coronary flow velocity reserve ≥ 2.5 or <2.5 measured in left anterior descending coronary artery

| | CFVR ≥ 2.5 $n = 28$ | CFVR < 2.5 $n = 21$ | *P*-value |
|---|---|---|---|
| Age, years | 58 ± 13 | 64 ± 9 | 0.063 |
| Men, (%) | 20 (71.4) | 16 (76.2) | 0.755 |
| Diabetes, (%) | 7 (25.0) | 6 (28.6) | 1.000 |
| History of smoking, (%) | 13 (46.4) | 10 (47.6) | 1.000 |
| Body mass index, kg/m$^2$ | 26.3 ± 4.3 | 26.8 ± 3.8 | 0.585 |
| GFR, mL/min/1.73 m$^2$ | 37.1 ± 15.6 | 31.3 ± 11.9 | 0.220 |
| U-ACR, mg/mmol | 44 ± 82 | 70 ± 88 | 0.404 |
| Serum NT-proBNP, ng/L | 146 ± 135 | 308 ± 267 | 0.014 |
| Serum troponin T, ng/L | 13.92 ± 13.02 | 21.94 ± 16.27 | 0.021 |
| Serum copeptin, pmol/L | 25.8 ± 16.6 | 32.0 ± 16.9 | 0.172 |
| 24 h ASBP, mmHg | 117 ± 13 | 130 ± 13 | 0.001 |
| Digital RHI | 2.44 ± 1.26 | 2.56 ± 0.84 | 0.337 |
| cfPWV, m/s | 8.96 ± 2.39 | 9.98 ± 2.41 | 0.070 |
| Ankle–brachial index | 1.19 ± 0.11 | 1.12 ± 0.11 | 0.051 |
| LV mass index, g/m$^2$ | 76.5 ± 20.2 | 88.7 ± 22.8 | 0.026 |
| Cardiac index, L/min/m$^2$ | 2.8 ± 0.5 | 2.9 ± 0.7 | 0.850 |
| Baseline LAD flow velocity, m/s | 0.33 ± 0.09 | 0.44 ± 0.12 | 0.002 |
| Hyperaemic LAD flow velocity, m/s | 1.03 ± 0.23 | 0.87 ± 0.21 | 0.015 |
| Coronary flow velocity reserve (LAD) | 3.26 ± 0.76 | 2.05 ± 0.33 | <0.001 |

Abbreviations: CKD, chronic kidney disease; GFR, glomerular filtration rate (measured by $^{51}$Cr-EDTA clearance); U-ACR, urine albumin-to-creatinine ratio; NT-proBNP, N-terminal pro-brain natriuretic peptide; ASBP, ambulatory systolic blood pressure; RHI, reactive hyperaemia index; cfPWV, carotid-femoral pulse wave velocity.
Values are means ± SD or proportions (%).

wall thickness in CKD patients. cfPWV is a surrogate marker for aortic stiffness,[23] and increased stiffness may elevate LV afterload even when brachial ABP is within the normal range.

In our cohort, LAVI and LA–RA area were both significantly increased in CKD patients. In addition, 53 patients (58%) had a LAVI > 34 mL/m$^2$, that is, above the cut-off value used to diagnose LV diastolic dysfunction.[18] Increased LAVI is an established marker of LV diastolic dysfunction and is considered to reflect the cumulative effects of increased LV filling pressures over time.[18] In addition, increased LAVI, regardless of the underlying cause, has been shown to be an independent risk factor for cardiovascular morbidity and mortality in different populations[24,25] including in patients with CKD stages 3 and 4.[26] In addition to LA enlargement, patients with CKD displayed also other alterations suggestive of impairments in LV diastolic function. LV IVRT was significantly prolonged, indicating an impaired LV relaxation during the early, active phase. Furthermore, transmitral flow velocity and pulmonary vein flow velocity were both significantly elevated in CKD patients during atrial contraction. The latter findings indicate increased dependence on filling through atrial contraction. It is reasonable to believe that the increase in A wave velocity might be a compensation for decreased LV filling during early diastole. Hence, it is feasible to interpret our findings as if patients with CKD had early signs of abnormalities in LV diastolic function, although these did not fulfil the ASE criteria for diastolic dysfunction.

The diagnosis of LV diastolic dysfunction is difficult and based on four echocardiographic variables: e' velocity, E/e' ratio, LAVI, and peak TR velocity.[18] If more than half of these variables are abnormal, diastolic dysfunction is present. Somewhat surprisingly, we found that CKD patients, with a high prevalence of hypertension, had elevated LV e' velocities than had healthy controls, indicating preserved, or even enhanced, LV relaxation during early diastole. The cause of increased LV e' velocities in CKD patients in the present study is difficult to determine. The fact that e' velocities were elevated also in the RV of CKD patients suggest that systemic factors such as hypervolaemia with increased preload, which is common in patients with renal impairment, could be involved. Previous well-controlled experimental studies have demonstrated that e' is preload dependent.[27] Additional findings supporting the notion that preload was elevated in CKD patients were that s' velocities were increased in both the LV and RV. Irrespective of its cause, elevated e' velocities in CKD patients are likely to reduce the ability to detect early stages of LV diastolic dysfunction using the current diagnostic criteria.

Patients with CKD had a significantly reduced CFVR, and 43% had CMD defined as CFVR < 2.5. This is an interesting finding in view of recent results indicating that CMD may be a cause of HFpEF.[13] In addition, reduced coronary flow reserve (CFR) has been shown to be a strong, independent, predictor of cardiovascular mortality in different patient groups[28] including in CKD.[29] Using myocardial perfusion PET, Charytan *et al.*[29] investigated CFR in a cohort of 3946 individuals with a high proportion of CKD. CFR was significantly reduced in patients with CKD stages 3–5 vs. individuals with preserved kidney function, and worsening CKD stage was independently associated with reduced CFR after multiple

adjustments. Thus, our results using Doppler echocardiography are in good agreement with earlier findings using myocardial PET. The mechanisms causing a reduced CFVR in CKD patients in our study need to be investigated further. Patients with CKD had an elevated LAD flow velocity at baseline vs. controls, presumably owing to an increased metabolic demand, and this might have diminished the capacity to increase flow velocity further in response to adenosine. However, we found that CKD patients with a CFVR $< 2.5$ had significantly reduced peak hyperaemic LAD flow velocities than had patients with a CFVR $\geq 2.5$ (*Table* 5). This finding clearly indicates a reduced vasodilatory capacity in patients with reduced CFVR and the presence of CMD. Chronic kidney disease is associated with inflammation, dyslipidaemia, and increased oxidative stress, and all these mechanisms can impair endothelial function[30] and thereby contribute to CMD. Digital reactive hyperaemia, an index of peripheral microvascular endothelial function, was not significantly correlated with either peak hyperaemic LAD flow velocity or CFVR in CKD patients. This suggests that microvascular dysfunction in patients with CKD was not generalized and that the coronary microcirculation is more susceptible. This difference between vascular beds is not surprising considering that central haemodynamics is altered in CKD and that LV workload increased as a consequence of hypervolaemia, hypertension, and elevated aortic stiffness.[31] A limitation of the present study was the relatively small sample size. This reduced our power to detect smaller differences between groups and less clear associations. Also, larger prospective studies are needed to determine the clinical implications of our findings.

In conclusion, patients with CKD stages 3 and 4, without heart disease, displayed abnormalities in LV diastolic function, albeit not fulfilling the criteria for LV diastolic dysfunction. We hypothesize that the observed increase in LV e′ velocity in CKD patients might reduce the ability to detect early stages of LV diastolic dysfunction and lead to underdiagnosis of HFpEF. Interestingly, patients with CKD had a reduced CFVR indicating CMD. This finding raises the possibility that CMD may be involved in the pathogenesis of HF in patients with CKD.

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## Conflict of Interest

None declared. The results presented in this paper have not been published previously in whole or in part. Maria K. Svensson is currently employed by Amgen AB, Sweden.

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## Ethics Statement

The experiments were approved by the regional ethics committee in Gothenburg, Sweden, and the research was conducted in accordance with the Declaration of Helsinki.

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