

Skin-autofluorescence, a Measure of Tissue Advanced Glycation End-products (AGEs), is Related to Diastolic Function in Dialysis Patients

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ABSTRACT

Background: Diastolic dysfunction is a frequent cause of heart failure, particularly in dialysis patients. Advanced glycation endproducts (AGEs) are increased in dialysis patients and are suggested to play a role in the development of diastolic dysfunction. The aim of our study was to assess whether AGE accumulation in dialysis patients is related to the presence of diastolic dysfunction.

Methods and Results: Data were analyzed from 43 dialysis patients, age 58 ± 15 years, of whom 65% were male. Diastolic function was assessed using tissue velocity imaging (TVI) on echocardiography. Tissue AGE accumulation was measured using a validated skin-autofluorescence (skin-AF) reader. Plasma N^ε-(carboxymethyl)lysine (CML) and N^ε-(carboxyethyl)lysine (CEL) were measured by stable-isotope-dilution tandem mass spectrometry. Plasma pentosidine was measured by high-performance liquid chromatography. Skin-AF correlated with mean E' ($r = -0.51, P < .001$), E/A ratio ($r = -0.39, P = .014$), and E/E' ($r = 0.38, P = .019$). Plasma AGEs were not significantly associated with diastolic function. Multivariable linear regression analysis revealed that 54% of the variance of average E' was explained by age ($P = .007$), dialysis type ($P = 0.016$), and skin-AF ($P = .013$).

Conclusions: Tissue AGEs measured as skin-AF, but not plasma AGE levels, were related to diastolic function in dialysis patients. Although this may support the concept that tissue AGEs explain part of the increased prevalence of diastolic dysfunction in these patients, the ambiguous relation between plasma and tissue AGEs needs further exploring. (*J Cardiac Fail* 2008;■:1–7)

Key Words: Heart failure, tissue velocity imaging, carboxymethyllysine.

Systolic dysfunction is commonly recognized as the main cause of heart failure. However, approximately 50% of the patients with chronic heart failure have a preserved

systolic function.¹ In most of these patients, heart failure is caused by diastolic dysfunction.² Recently, it became evident that the prognosis of patients with diastolic heart failure is nearly as poor as the prognosis of patients with systolic heart failure.^{2–4} Despite the magnitude of this problem, little is known about the pathophysiologic background of diastolic dysfunction.

Several mechanisms underlying diastolic dysfunction have been proposed.^{5–7} Diastolic dysfunction is generally associated with increased myocardial stiffness, which may be caused by modifications of collagen in the extracellular matrix. One important modification of collagen is increased cross-linking by the formation of advanced glycation endproducts (AGEs). These are carbohydrate and lipid dependent modifications of protein formed by oxidative or nonoxidative reactions.⁸

AGE accumulation occurs during life, but an increased level of AGEs has been found in patients with diabetes and renal failure. Particularly, dialysis patients are known for increased levels of AGEs, which were also

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independently associated with an impaired survival.⁹ Interestingly, a frequent echocardiographic finding in dialysis patients is diastolic dysfunction. The prevalence of diastolic dysfunction in dialysis patients varies from 25% to 87% depending on definitions used and the patients included.^{10,11} Diastolic dysfunction predisposes to the development of heart failure and is strongly related with outcome in dialysis patients.¹² We hypothesized that increased AGE accumulation in dialysis patients explains part of the increased prevalence of diastolic dysfunction; therefore, we analyzed the relation between tissue and plasma AGEs and diastolic function in dialysis patients.

Methods

Patients and Study Design

In this cross-sectional study, all patients receiving dialysis treatment at the Dialysis Center Groningen ≥ 18 years old were eligible to participate. Both hemodialysis and peritoneal dialysis patients were included. Exclusion criteria were a myocardial infarction within the last month, significant valvular disease, pacemaker use, sustained or accepted atrial fibrillation, active endocarditis, active myocarditis, active pericarditis, acute heart failure, heart transplant, and secondary (nonidiopathic) cardiomyopathies. Non-Caucasian patients were excluded from analysis because the autofluorescence reader was validated only in Caucasians. Study visits as well as blood collections were performed before hemodialysis therapy. In case of peritoneal dialysis, patients' blood was withdrawn at their study visit. Using standard laboratory techniques blood was analyzed for hemoglobin (Hb), HbA1c, total protein, albumin, calcium, phosphate, triglycerides, total cholesterol, and low-density lipoprotein (LDL). Furthermore, we analyzed the mean value of Kt/V per week (marker for dialysis quality based on urea clearance), which was expressed as a percentage of the Kt/V recommended by the Kidney Disease Outcomes Quality Initiative (K-DOQI) adequacy guidelines for dialysis therapy (Kt/V > 3.6 for hemodialysis patients; Kt/V > 2.0 for peritoneal dialysis patients). Clinical measurements were all performed on the same day as echocardiography, and included blood pressure and heart rate. In hemodialysis patients, blood pressure obtained before dialysis therapy was used for analysis. Current use of medication was extracted from medical records. History of cardiovascular disease (CVD) and family history of CVD were based on a documented or reported history of myocardial infarction, angina pectoris, cerebrovascular accident, transient ischemic attack, pulmonary embolus, venous thrombosis, or intermittent claudication in the medical history of the patient or first- and second-degree relatives, respectively. This study protocol complies with the Declaration of Helsinki, and was approved by the institutional review committee of the University Medical Center Groningen. All patients signed written informed consent.

Skin-autofluorescence

Tissue AGE accumulation was assessed using a validated skin-autofluorescence (skin-AF) reader (AGE-Reader; patent PCT/NL99/00607; DiagnOptics BV, Groningen, The Netherlands) described previously.^{9,13} In short, the AGE reader illuminates a skin surface of approximately 4 cm², guarded against surrounding light, with an excitation light source between 300 and 420 nm (peak excitation ~ 370 nm). Light from the skin is measured with a spectrometer in the 300- to 600-nm range, using 200- μ m glass

fiber. As a measure of skin-AF, the ratio between emission and excitation was calculated in arbitrary units (a.u.) by dividing the area under the curve between 420 and 600 nm by the area under the curve between 300 and 420 nm, and multiplying by 100. Skin-AF was measured at the volar side of the lower arm at approximately 10 to 15 cm below the elbow fold. Care was taken to perform the measurement at normal skin site (ie, without visible vessels, scars, lichenification, or other skin abnormalities). Intraobserver variation of repeated AFR measurements on 1 day was 6%.

Plasma N^ε-(carboxymethyl)lysine and N^ε-(carboxyethyl)lysine by LC-MS/MS

Plasma N^ε-(carboxymethyl)lysine (CML) and N^ε-(carboxyethyl)lysine (CEL) were determined by stable-isotope dilution tandem mass spectrometry (LC-MS/MS) as described previously.¹⁴ In short, CML and CEL were liberated from plasma proteins by acid hydrolysis after addition of deuterated CML and CEL as internal standards. Chromatographic separation was performed by gradient-elution reversed-phase chromatography with a mobile phase containing 5 μ mol/L nonafluoropentanoic acid as ion-pairing agent. Mass transitions of 205.1 $>$ 384.1 and 219.1 $>$ 384.1 for CML and CEL, respectively, and 209.1 $>$ 388.1 and 223.1 $>$ 388.1 for their respective internal standards were monitored in positive-ion mode. CML and CEL were separated by baseline resolution with a total analysis time of 21 minutes. Within-day and between-day coefficients of variation were $< 4.4\%$ and $< 3.2\%$ for CML, and $< 6.8\%$ and $< 7.3\%$ for CEL.

Pentosidine by High-performance Liquid Chromatography

Pentosidine levels were measured by high-performance liquid chromatography as described previously by Izuwara et al.¹⁵ Briefly, a 50- μ L solution of acid hydrolysate of plasma was injected into an high-performance liquid chromatography system and separated on a C18 reverse-phase column (Waters, Tokyo, Japan). The effluent was monitored using a fluorescence detector (RF-10A; Shimadzu, Kyoto, Japan) at an excitation-emission wavelength of 335/385 nm. Synthetic pentosidine was used to obtain a standard curve. The limit of detection was 5 pmol of pentosidine per milliliter of plasma. Normal values in 4 healthy subjects averaged 0.114 ± 0.011 μ mol/L, with a coefficient of variation of $5.48\% \pm 0.81\%$ on 4 different days.

Echocardiography

Patients underwent 2-dimensional echocardiography, including color flow mapping 2-dimensional-guided M-mode, blood pool, and tissue Doppler echocardiography. Echocardiography was performed by experienced cardiac technicians using a General Electric VIVID 7 system with a 2.5-MHz probe. Measurements included left ventricular and atrial dimensions, the peak early (E) and late (A) diastolic filling velocities, isovolumetric relaxation time, deceleration time (slope) of the early peak filling. Furthermore, using tissue velocity imaging (TVI), early diastolic velocity (E') was measured on the lateral, septal, anterior, and inferior wall areas, and subsequently averaged. E/E' was calculated by dividing the peak early diastolic filling (E) by the average E' measured using TVI. Systolic dysfunction was defined as an estimated left ventricular ejection fraction (LVEF) $\leq 45\%$. Diastolic dysfunction was defined as an E' < 8 cm/s. E' values could not

be assessed in 4 patients. LVEF could not be estimated in 6 patients.

Statistical Analyses

Data were analyzed using SPSS version 12.01 (SPSS Inc, Chicago, Illinois). Data are reported as mean \pm SD for parametric variables, as median (25%-75%, interquartile range) for nonparametric variables, and in case of nominal variables as n(%). Correlations coefficients were calculated using Pearson correlations. Linear regression analysis was used to assess the determinants of skin-AF and average E'. All variables included in Table 1 were analyzed by univariable linear regression. Although several echocardiographic parameters may correlate with diastolic

function, by forehand, these were excluded from linear regression analysis because these would not be of interest from a pathophysiologic perspective. Only the variables that showed a trend for a relation with skin-AF or average E' ($P \leq .10$) were included in multivariable linear regression. Variables that did not retain significance in this multivariable analysis were subsequently removed from the model (backward selection). Next, biologically plausible but excluded variables were reintroduced in the final model to see whether their potential influences were overlooked. To test whether the model is appropriate and whether the assumptions for linear regression are met, the model was tested for overall regression, collinearity, interaction terms, and lack-of-fit with analysis of variance. Residuals were tested for normality of distribution. $P \leq .05$ (2-sided) was considered statistically significant.

Results

Patient characteristics are summarized in Table 1. Data were analyzed from 43 patients, age 58 ± 15 years, of whom 65% were male. Mean skin-AF was 3.7 ± 0.7 a.u., which is markedly higher ($P < .001$) than in a normal control group ($2.0 [1.7-2.4]$ a.u.) previously described by our group.¹⁶ CML (7.2 ± 2.5 vs. 2.8 ± 0.4 $\mu\text{mol/L}$; $P < .001$), CEL (2.5 ± 0.6 vs. 0.8 ± 0.2 $\mu\text{mol/L}$; $P < .001$), and pentosidine (1.9 ± 1.0 vs. 0.11 ± 0.01 $\mu\text{mol/L}$; $P < .001$) levels were also significantly higher in patients compared with normal controls.^{14,15} The plasma AGEs CML, CEL, and pentosidine showed high correlation with each other (Table 2); however, they did not correlate with age and HbA1c. Dialysis quality (Kt/V) showed a strong association with plasma AGEs. Although a trend existed for CML to be related to skin-AF, no significant association existed between plasma AGEs and skin-AF.

Systolic dysfunction (LVEF $\leq 45\%$) was present in 9 (24%) patients. Diastolic dysfunction (mean E' < 8 cm/s) was present in 35 (81%) patients. Skin-AF was significantly correlated with measurements of diastolic filling (Table 3), including mean E' ($r = -0.51$; $P = .001$), E/A ratio ($r = -0.39$; $P = .014$), and E/E' ($r = 0.38$; $P = .019$). No correlations were found between LVEF, LV and atrial dimensions, and skin-AF. Plasma AGE levels were not significantly correlated with diastolic function. Plasma CEL ($r = 0.34$; $P = .029$) and pentosidine levels ($r = 0.31$; $P = .048$) did, however, correlate with LV septum diameters. Plasma CML levels showed a strong trend ($r = 0.30$; $P = .052$) for a correlation with LV septum diameters. Plasma CEL levels were additionally correlated to LV posterior wall diameters ($r = 0.36$; $P = .02$) and LA parasternal length ($r = 0.33$; $P = .04$).

Skin-AF showed a strong association with diastolic function; therefore, we performed additional analyses to determine factors that are associated with an increased skin-AF. Skin-AF was univariably associated with age, total protein, and albumin (Table 4). Additionally, skin-AF showed a trend ($P \leq .10$) for an association with diastolic blood pressure and CML levels. Multivariable linear regression analysis revealed that 37% of the variance of skin-AF was determined by age and CML levels. Next, several

Table 1. Patient Characteristics

Characteristic	Total (n = 43)
Age (y)	58 \pm 15
Gender (male; n, %)	28 (65)
Dialysis type (hemodialysis; n, %)	19 (44)
Duration of dialysis (y)	2.8 (1.3–5.3)
Kt/V per week (% of recommended Kt/V)	116 \pm 30
History of renal transplantation (n, %)	6 (14)
NYHA functional class	1.3 \pm 0.6
Cause of renal failure (n, %)	
Cystic renal disease	12 (28)
Hypertensive nephropathy	6 (14)
Glomerulonephritis	5 (12)
Nephrotic syndrome	3 (7)
Pyelonephritis/reflux	3 (7)
Diabetic nephropathy	2 (5)
Other or unknown	12 (30)
Risk factors for CVD (n, %)	
History of hypertension	29 (68)
Hypercholesterolemia	19 (44)
Smoking	13 (30)
Diabetes mellitus	3 (7)
History of CVD	12 (28)
Family history of CVD	22 (51)
Physical examination:	
Systolic blood pressure (mm Hg)	132 \pm 21
Diastolic blood pressure (mm Hg)	78 \pm 12
Heart rate (beats/min)	77 \pm 14
Body mass index (kg/m ²)	25 \pm 4
Laboratory assessments	
Hb (g/dL)	12.1 \pm 1.6
HbA1c (%)	6.3 \pm 1.1
Calcium-phosphate product (mmol ² /L ²)	3.7 \pm 1.0
Triglycerides (mg/dL)	168 \pm 89
Total cholesterol (mg/dL)	135 \pm 27
LDL cholesterol (mg/dL)	77 \pm 27
Total protein (g/L)	69 \pm 6
Albumin (g/L)	41 \pm 4
AGE accumulation	
Skin-AF (a.u.)	3.7 \pm 0.7
CML ($\mu\text{mol/L}$)	7.2 \pm 2.5
CEL ($\mu\text{mol/L}$)	2.5 \pm 0.6
Pentosidine ($\mu\text{mol/L}$)	1.9 \pm 1.0
Medication (n, %)	
ACE inhibitor	15 (35)
β -blockers	20 (47)
Ca-antagonists	9 (21)
Diuretics	8 (19)
Erythropoietin	36 (84)
Statins	18 (42)

NYHA, New York Heart Association; CVD, cardiovascular disease; Hb, hemoglobin; LDL, low-density lipoprotein; AGE, advanced glycation end-products; AF, autofluorescence; a.u., arbitrary units; CML, carboxymethyllysine; CEL, carboxymethyllysine; ACE, angiotensin-converting enzyme.

Table 2. Correlations between AGE Measurements, Age, HbA1c, and Dialysis Quality

	Skin-AF	CML	CEL	Pentosidine	Age	HbA1c	Kt/V
Skin-AF		$r = 0.27$ $P = .08$	$r = 0.02$ $P = .88$	$r = 0.24$ $P = .13$	$r = 0.46$ $P < .002$	$r = -0.13$ $P = .43$	$r = 0.12$ $P = .46$
CML	$r = 0.27$ $P = .08$		$r = 0.55$ $P < .001$	$r = 0.85$ $P < .001$	$r = -0.09$ $P = .59$	$r = -0.02$ $P = .93$	$r = -0.45$ $P = .003$
CEL	$r = 0.02$ $P = .88$	$r = 0.55$ $P < .001$		$r = 0.61$ $P < .001$	$r = -0.08$ $P = .60$	$r = -0.29$ $P = .06$	$r = -0.40$ $P = .009$
Pentosidine	$r = 0.24$ $P = .13$	$r = 0.85$ $P < .001$	$r = 0.61$ $P < .001$		$r = 0.05$ $P = .78$	$r = -0.11$ $P = .49$	$r = -0.49$ $P = .001$
Age	$r = 0.46$ $P = .002$	$r = -0.09$ $P = .59$	$r = -0.08$ $P = .60$	$r = 0.05$ $P = .78$		$r = -0.06$ $P = .70$	$r = 0.24$ $P = .12$
HbA1c	$r = -0.13$ $P = .43$	$r = -0.02$ $P = .93$	$r = -0.29$ $P = .06$	$r = -0.11$ $P = .49$	$r = -0.06$ $P = .70$		$r = 0.29$ $P = .07$
Kt/V	$r = 0.12$ $P = .46$	$r = -0.45$ $P = .003$	$r = -0.40$ $P = .009$	$r = -0.49$ $P = .001$	$r = 0.24$ $P = .12$	$r = 0.29$ $P = .07$	

AGE, AF, autofluorescence; CML, carboxymethyllysine; CEL, carboxymethyllysine.

biologically plausible variables were reintroduced in this model to see whether any possible relations were overlooked. Reintroducing gender, dialysis type, duration of dialysis, Kt/V, the presence of diabetes, smoking, history of hypertension, diastolic blood pressure, systolic blood pressure, HbA1c, total protein, albumin, CEL, pentosidine, statin use, and the use of angiotensin-converting enzyme inhibition showed that gender ($r = 0.34$, $P = .011$), albumin levels ($r = 0.28$, $P = .045$), and diastolic blood pressure ($r = -0.27$, $P = .045$) all individually determined an extra part of the variance of skin-AF levels. Further multivariable linear regression analysis led to the final model in which 56% of the variance of skin-AF was determined by age, gender, diastolic blood pressure, and CML levels.

Table 3. Correlations between Echocardiographic Parameters and Skin-AF

Parameter	Total (n = 43)	Skin-AF (a.u.)	
		<i>r</i>	<i>P</i> Value
Diameters			
LV septum (mm)	10 ± 2	0.11	.49
LV posterior wall (mm)	10 ± 2	0.13	.43
LVEDD (mm)	46 ± 6	−0.14	.37
LVEDS (mm)	29 ± 6	−0.13	.41
LA parasternal (mm)	38 ± 5	−0.03	.86
LA length (mm)	56 ± 7	−0.13	.41
LA cross (mm)	40 ± 6	−0.20	.20
Systolic function			
LVEF (%)	53 ± 8	0.09	.58
Diastolic function			
E (m/s)	0.74 ± 0.30	0.06	.73
A (m/s)	0.79 ± 0.31	0.33	.037
E/A ratio	0.98 ± 0.35	−0.39	.014
DCT (ms)	248 ± 78	0.29	.065
IVRT (ms)	90 ± 23	−0.05	.78
Lateral E' (cm/s)	7.4 ± 3.1	−0.49	.001
Septal E' (cm/s)	5.3 ± 2.0	−0.46	.003
Anterior E' (cm/s)	6.7 ± 2.8	−0.43	.006
Inferior E' (cm/s)	6.0 ± 2.6	−0.50	.002
Average E' (cm/s)	6.5 ± 2.3	−0.51	.001
E/E' (cm/s)	12.0 ± 4.6	0.38	.019

AF, autofluorescence; a.u., arbitrary units; LV, left ventricular; LVEDD, LV end-diastolic diameter; LVEDS, LV end-systolic diameter; LA, left atrial; LVEF, LV ejection fraction, DCT: deceleration time; IVRT, isovolumetric relaxation time.

Univariable linear regression analysis revealed that diastolic function (mean E') was associated with age and history of renal transplantation, hypertension, hypercholesterolemia, and skin-AF (Table 4). Furthermore, a trend ($P \leq .10$) for an association existed for glomerulonephritis as cause of renal failure, smoking, history of CVD, body mass index, albumin, and use of statins. Multivariable linear regression analysis showed that 45% of the variance of mean E' was determined by age and skin-AF. Reintroducing gender, dialysis type, duration of dialysis, Kt/V, the presence of diabetes, history of hypertension, HbA1c, systolic blood pressure, diastolic blood pressure, calcium-phosphate products, CML, CEL, pentosidine, and the use of angiotensin-converting enzyme inhibition showed that diastolic blood pressure ($r = -0.27$, $P = .036$) and dialysis type ($r = -0.29$, $P = .016$) both individually determined an extra part of the variance of diastolic function. Further multivariable linear regression analysis led to the final model in which 54% of the variance of diastolic function was determined by age, skin-AF, and dialysis type. However, diastolic blood pressure did show a nonsignificant contribution, thereby creating an alternative model explaining 61% of the variance of diastolic function by age ($\beta = -0.39$, $P = .009$), skin-AF ($\beta = -0.47$, $P = .003$), dialysis type ($\beta = -0.26$, $P = .034$), and diastolic blood pressure ($\beta = -0.22$, $P = .07$).

Discussion

The main result of our study is that AGE accumulation, measured as skin-AF, is independently associated with diastolic function in dialysis patients. Age, dialysis type, and skin-AF together explain 54% of the variance of diastolic function. Plasma AGE levels were not associated with diastolic function in our study. To our knowledge, this is the first study that demonstrates the relation between diastolic function and AGE accumulation in dialysis patients.

Diastolic dysfunction is a frequent finding in dialysis patients and is associated with an impaired survival.¹⁰⁻¹² We used TVI as a diagnostic tool to assess diastolic function.

Table 4. Determinants of Skin-AF and Diastolic Function in Linear Regression Analysis

Characteristic	Skin-AF				Diastolic Function			
	Univariable		Multivariable $r^2 = 0.56$		Univariable		Multivariable $r^2 = 0.54$	
	β	P	β	P	β	P	β	P
Age (y)	0.46	.002	0.57	<.001	−0.61	<.001	−0.41	.007
Gender (male)	0.25	.11	0.36	.006	−0.11	.50		
AGE accumulation								
Skin-AF (a.u.)*	—	—			−0.59	<.001	−0.41	.013
CML ($\mu\text{mol/L}$)	0.27	.08	0.35	.006	−0.22	.19		
CEL ($\mu\text{mol/L}$)	0.02	.88			0.05	.78		
Pentosidine ($\mu\text{mol/L}$)	0.24	.13			−0.20	.25		
Dialysis type (peritoneal)	−0.05	.77			−0.23	.17	−0.29	.016
Duration of dialysis (y)	0.17	.28			0.23	.15		
Kt/V per week (%)	0.12	.46			−0.18	.27		
History of renal transplantation	−0.01	.94			0.34	.033		
NYHA functional class	−0.04	.80			0.04	.81		
Cause of renal failure								
Cystic renal disease	−0.21	.17			0.07	.69		
Hypertensive nephropathy	0.18	.26			−0.23	.16		
Glomerulonephritis	−0.13	.43			0.27	.095		
Nephrotic syndrome	0.01	.95			−0.04	.81		
Pyelonephritis/reflux	−0.02	.89			0.05	.79		
Diabetic nephropathy	0.01	.93			−0.15	.37		
Other or unknown	0.16	.29			−0.03	.87		
Risk factors for CVD (n, %)								
History of hypertension	0.23	.14			−0.35	.031		
Hypercholesterolemia	−0.01	.94			0.35	.028		
Smoking	0.09	.55			0.28	.082		
Diabetes mellitus	0.15	.35			−0.17	.31		
History of CVD	0.12	.45			−0.27	.097		
Family history of CVD	0.01	.96			−0.22	.18		
Physical examination:								
Systolic blood pressure (mm Hg)	−0.06	.70			−0.15	.40		
Diastolic blood pressure (mm Hg)	−0.32	.054	−0.34	.008	−0.12	.50		
Heart rate (beats/min)	0.00	.99			0.05	.78		
Body mass index (kg/m^2)	0.13	.44			−0.30	.077		
Laboratory assessments								
Hb (g/dL)	0.20	.20			−0.18	.26		
HbA1c (%)	−0.13	.43			−0.08	.64		
Calcium-phosphate product	−0.08	.62			−0.08	.65		
Triglycerides (mg/dL)	0.20	.20			−0.16	.32		
Total cholesterol (mg/dL)	0.13	.40			−0.10	.56		
LDL cholesterol (mg/dL)	0.06	.69			0.05	.77		
Total protein (g/L)	0.42	.006			−0.26	.11		
Albumin (g/L)	0.40	.008			−0.30	.069		
Medication (n, %)								
ACE inhibitor	−0.20	.20			0.17	.30		
β -blockers	0.04	.78			−0.05	.77		
Ca-antagonists	0.08	.63			−0.11	.49		
Diuretics	−0.23	.14			−0.06	.73		
Erythropoietin	−0.20	.19			0.23	.16		
Statins	−0.01	.97			−0.30	.069		

Skin-AF and average E' were used as dependent variable. Only parameters that obtained at least a P value $\leq .10$ in univariable linear regression analysis were included in multivariable regression analysis.

AF, autofluorescence; a.u., arbitrary units; CML: carboxymethyllysine; CEL: carboxymethyllysine; NYHA, New York Heart Association; CVD, cardiovascular disease; Hb, hemoglobin; LDL, low-density lipoprotein; ACE, angiotensin-converting enzyme.

*Skin-AF (a.u.) was inversed and multiplied by -1 .

TVI is a noninvasive tissue Doppler echocardiographic technique that measures the myocardial tissue velocities of the mitral valve annulus during diastole. Although TVI measurement is less affected by changes in preload in opposition to conventional Doppler echocardiography, measurements are still preload-dependent. To prevent preload-dependent differences, all echocardiography measurements in hemodialysis patients were performed before dialysis therapy.

Diastolic function is strongly related to age in the general population. Although we did not use an age-dependent cut-off point to diagnose diastolic dysfunction, our results were adjusted for age. Hypertension is another well-known risk factor for diastolic dysfunction. However, we did not find a significant association between diastolic function and blood pressure, although we did find a modest association between diastolic function and a history of hypertension. Furthermore, we found that diastolic blood pressure,

although insignificant, can explain an additional 7% of the variance of diastolic function in multivariable analysis.

Skin-AF was used as a measure for tissue AGE accumulation. We previously demonstrated that skin-AF shows a strong correlation with collagen-linked fluorescence ($r = 0.71$, $P < .001$), pentosidine levels ($r = .75$, $P < .001$), and levels of the nonfluorescent AGEs CML ($r = 0.45$, $P = .01$) and CEL ($r = 0.45$, $P = .01$) measured in tissue biopsies obtained from dialysis patients.⁹ Furthermore, skin-AF levels were independently associated with an impaired prognosis of dialysis patients.⁹

In contrast with tissue AGE accumulation, plasma AGEs levels were not associated with diastolic function in the present study. Both the LC-MS/MS method and the high-performance liquid chromatography method used in our study to assess plasma AGEs are currently considered as the most accurate methods available. In our opinion, our findings may suggest that plasma AGE levels do not adequately reflect tissue AGE accumulation. Indeed, CML explains only a part of measured skin-AF in our study, whereas pentosidine and CEL have not shown an independent relationship with skin-AF. Plasma AGE levels may be further influenced by dialysis modalities and absorption from food and smoking. Indeed, we showed that plasma AGEs in these dialysis patients were strongly associated with dialysis quality, but not related to traditional determinants of AGE formation such as age and HbA1c. However, we cannot exclude the possibility that a power issue may explain the lack of correlation found between plasma AGEs and diastolic function. Additionally, it would seem reasonable to assume that tissue AGEs are more closely related to diastolic function, because they are intrinsically linked with the actual pathophysiologic effects of AGEs (ie, protein cross-linking).

Although our data limit us from drawing conclusions about causality, our results are in line with the hypothesis that AGEs play a pathophysiologic role in the development of diastolic dysfunction. AGEs may contribute to the development of diastolic dysfunction either via cross-linking of matrix molecules or via the interaction with AGE receptors. Cross-linking of extracellular matrix proteins is essentially a physiologic phenomenon. AGEs, however, can covalently bind other AGEs, and form additional cross-links between matrix proteins such as collagen, laminin, and elastin.⁸ Excessive cross-linking caused by AGE accumulation undermines the flexibility of matrix proteins. This increased rigidity may induce diastolic dysfunction in the heart. One of the receptor-mediated effects of AGEs is the induction of fibrosis via the upregulation of transforming growth factor- β .¹⁷ AGE receptor activation also influences calcium metabolism in cardiac myocytes, causing a significant delay in calcium reuptake and consequent increase in the duration of the repolarization phase.¹⁸

The role of AGEs in the development of diastolic dysfunction has been investigated extensively in animals.^{19–23} The effect of various AGE-lowering strategies used in these studies confirms an active role for AGEs in the

development of diastolic dysfunction. Only a limited number of human studies have been published, of which none are in dialysis patients.^{24–26} Berg et al²⁴ analyzed serum AGE levels and left ventricular diastolic function in 52 patients with type 1 diabetes. The authors found a positive correlation between serum AGEs and isovolumetric relaxation time and left ventricular end-diastolic diameter. No correlation was found between AGEs and other echocardiographic parameters for diastolic function. It should be noted, however, that tissue velocity or Doppler imaging was not used to determine diastolic function in the latter study.

Recently, we showed that plasma levels of CML correlate with NTpro-BNP and NYHA functional class and predicted outcome in patients with chronic heart failure.²⁷ The most convincing evidence for a role of AGEs in the development of cardiac dysfunction originates from 2 trials with the AGE cross-link breaker alagebrium (ALT-711) in patients with chronic heart failure. In the DIAMOND [Distensibility Improvement and Remodeling in Diastolic Heart Failure] trial, Little et al²⁵ treated 23 patients with stable diastolic heart failure open-label with ALT-711. After 16 weeks, left ventricular mass (measured by magnetic resonance imaging) was reduced and diastolic function (measured by tissue Doppler) had improved. Furthermore, the drug was well tolerated and had a positive effect on patients' quality of life. The PEDESTAL [Patients with Impaired Ejection Fraction and Diastolic Dysfunction: Efficacy and Safety Trial of Alagebrium] trial is an open-label study investigating the effects of ALT-711 on diastolic function and LV mass in 20 patients with systolic heart failure and diastolic dysfunction. Preliminary results confirm the findings of the DIAMOND trial.²⁶ Although the results of both trials should be interpreted with caution because of the open-label design, further exploration of the effects of AGE-lowering therapies is warranted because no therapy has as yet shown effectiveness in the treatment of diastolic heart failure. A prospective randomized, double-blind, placebo-controlled trial on the effects of alagebrium on exercise tolerance and diastolic function in 100 chronic heart failure patients is ongoing (BENEFICIAL trial [A double-blind placebo-controlled, randomized trial evaluating the efficacy and safety of Alagebrium (ALT-711) in patients with chronic heart failure] www.clinicaltrials.gov; NCT00516646).

Conclusion

Tissue AGEs measured as skin-AF, but not plasma AGE levels, were related to diastolic dysfunction in dialysis patients. Although this may support the concept that tissue AGEs explain part of the increased prevalence of diastolic dysfunction in these patients, the ambiguous relation between plasma and tissue AGEs needs further exploring. Moreover, the concept that AGEs are related to the development of diastolic dysfunction needs to be established in interventional studies using AGE lowering therapies.

References

- Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL, Redfield MM. Trends in prevalence and outcome of heart failure with preserved ejection fraction. *N Engl J Med* 2006;355:251–9.
- Persson H, Lonn E, Edner M, Baruch L, Lang CC, Morton JJ, et al. Diastolic dysfunction in heart failure with preserved systolic function: need for objective evidence: results from the CHARM Echocardiographic Substudy-CHARMES. *J Am Coll Cardiol* 2007;49:687–94.
- Aurigemma GP. Diastolic heart failure—a common and lethal condition by any name. *N Engl J Med* 2006;355:308–10.
- Bhatia RS, Tu JV, Lee DS, Austin PC, Fang J, Haouzi A, et al. Outcome of heart failure with preserved ejection fraction in a population-based study. *N Engl J Med* 2006;355:260–9.
- Hartog JW, Voors AA, Bakker SJ, Smit AJ, van Veldhuisen DJ. Advanced glycation end-products (AGEs) and heart failure: pathophysiology and clinical implications. *Eur J Heart Fail* 2007;9:1146–55.
- Zile MR, Brutsaert DL. New concepts in diastolic dysfunction and diastolic heart failure: part II: causal mechanisms and treatment. *Circulation* 2002;105:1503–8.
- Zieman SJ, Kass DA. Advanced glycation endproduct crosslinking in the cardiovascular system: potential therapeutic target for cardiovascular disease. *Drugs* 2004;64:459–70.
- Smit AJ, Lutgers HL. The clinical relevance of advanced glycation endproducts (AGE) and recent developments in pharmaceuticals to reduce AGE accumulation. *Curr Med Chem* 2004;11:2767–84.
- Meerwaldt R, Hartog JW, Graaff R, Huisman RJ, Links TP, den Hollander NC, et al. Skin autofluorescence, a measure of cumulative metabolic stress and advanced glycation end products, predicts mortality in hemodialysis patients. *J Am Soc Nephrol* 2005;16:3687–93.
- Virga G, Stomaci B, Munaro A, Mastrosimone S, Cara M, Artuso E, et al. Systolic and diastolic function in renal replacement therapy: a cross-sectional study. *J Nephrol* 2006;19:155–60.
- Alpert MA. Cardiac performance and morphology in end-stage renal disease. *Am J Med Sci* 2003;325:168–78.
- Rakhit DJ, Zhang XH, Leano R, Armstrong KA, Isbel NM, Marwick TH. Prognostic role of subclinical left ventricular abnormalities and impact of transplantation in chronic kidney disease. *Am Heart J* 2007;153:656–64.
- Meerwaldt R, Graaff R, Oomen PH, Links TP, Jager JJ, Alderson NL, et al. Simple non-invasive assessment of advanced glycation endproduct accumulation. *Diabetologia* 2004;47:1324–30.
- Teerlink T, Barto R, Ten Brink HJ, Schalkwijk CG. Measurement of Nepsilon-(carboxymethyl)lysine and Nepsilon-(carboxyethyl)lysine in human plasma protein by stable-isotope-dilution tandem mass spectrometry. *Clin Chem* 2004;50:1222–8.
- Izuhara Y, Miyata T, Saito K, Ishikawa N, Kakuta T, Nangaku M, et al. Ultrapure dialysate decreases plasma pentosidine, a marker of “carbonyl stress. *Am J Kidney Dis* 2004;43:1024–9.
- Hartog JW, de Vries AP, Lutgers HL, Meerwaldt R, Huisman RM, van Son WJ, et al. Accumulation of advanced glycation end products, measured as skin autofluorescence, in renal disease. *Ann N Y Acad Sci* 2005;1043:299–307.
- Striker LJ, Striker GE. Administration of AGEs in vivo induces extracellular matrix gene expression. *Nephrol Dial Transplant* 1996;11(Suppl 5):62–5.
- Petrova R, Yamamoto Y, Muraki K, Yonekura H, Sakurai S, Watanabe T, et al. Advanced glycation endproduct-induced calcium handling impairment in mouse cardiac myocytes. *J Mol Cell Cardiol* 2002;34:1425–31.
- Schafer S, Huber J, Wihler C, Rutten H, Busch AE, Linz W. Impaired left ventricular relaxation in type 2 diabetic rats is related to myocardial accumulation of N(epsilon)-(carboxymethyl) lysine. *Eur J Heart Fail* 2006;8:2–6.
- Norton GR, Candy G, Woodiwiss AJ. Aminoguanidine prevents the decreased myocardial compliance produced by streptozotocin-induced diabetes mellitus in rats. *Circulation* 1996;93:1905–12.
- Avendano GF, Agarwal RK, Bashey RI, Lyons MM, Soni BJ, Jyothirmayi GN, et al. Effects of glucose intolerance on myocardial function and collagen-linked glycation. *Diabetes* 1999;48:1443–7.
- Asif M, Egan J, Vasan S, Jyothirmayi GN, Masurekar MR, Lopez S, et al. An advanced glycation endproduct cross-link breaker can reverse age-related increases in myocardial stiffness. *Proc Natl Acad Sci U S A* 2000;97:2809–13.
- Vaitkevicius PV, Lane M, Spurgeon H, Ingram DK, Roth GS, Egan JJ, et al. A cross-link breaker has sustained effects on arterial and ventricular properties in older rhesus monkeys. *Proc Natl Acad Sci U S A* 2001;98:1171–5.
- Berg TJ, Snorgaard O, Faber J, Torjesen PA, Hildebrandt P, Mehlsen J, et al. Serum levels of advanced glycation end products are associated with left ventricular diastolic function in patients with type 1 diabetes. *Diabetes Care* 1999;22:1186–90.
- Little WC, Zile MR, Kitzman DW, Hundley WG, O’Brien TX, deGroof RC. The effect of alagebrium chloride (ALT-711), a novel glucose cross-link breaker, in the treatment of elderly patients with diastolic heart failure. *J Card Fail* 2005;11:191–5.
- Thohan V, Koerner MM, Pratt CM, Torre GA. Improvements in diastolic function among patients with advanced systolic heart failure utilizing alagebrium (an oral advanced glycation end-product cross-link breaker). *Circulation* 2005;112(Suppl 2):U620–47.
- Hartog JW, Voors AA, Schalkwijk CG, Scheijen J, Smilde TD, Damman K, Bakker SJ, Smit AJ, van Veldhuisen DJ. Clinical and prognostic value of advanced glycation end-products in chronic heart failure. *Eur Heart J* 2007;28:2879–85.