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Circulating soluble receptor for advanced glycation end product (sRAGE) and left ventricular hypertrophy in patients with chronic kidney disease (CKD)

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Abstract *Background and Aim:* A decoy receptor for advanced glycation end product (soluble RAGE or sRAGE) is involved in left ventricular hypertrophy (LVH), and cardiomyopathy myocardial damage in experimental models and observational studies in patients with heart failure support the hypothesis that sRAGE attenuates the progression of heart disease and prevents death. Since sRAGE accumulates in patients with chronic kidney disease (CKD) we studied the relationship between plasma sRAGE with LVH in CKD patients.

Methods and results: We enrolled 142 patients with an average estimated glomerular filtration rate (eGFR) of 32 ml/min/1.73 m^2 and 49 healthy control individuals matched for age and gender.

Plasma sRAGE was significantly higher in CKD patients than in healthy controls. Significant inverse relationships were found between sRAGE with left ventricular mass index (LVMI) and mean wall thickness (MWT) but no such associations were found in controls. A bootstrap resampling validation study confirmed the estimates of the link between sRAGE and these variables. On covariance analysis, the slopes of LVMI and MWT to sRAGE were significantly steeper in CKD patients than in the controls. On logistic regression analysis 1 log unit increase in sRAGE was associated with a 82% decrease in the odds for LVH in CKD patients.

Conclusions: sRAGE is an inverse marker of LVH in CKD patients. This association generates the hypothesis that the RAGE pathway could be a causal risk factor for LVH in this population and that blockade of this pathway by the endogenous decoy receptor sRAGE could attenuate LVH in the same population.

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Introduction

The Receptor for Advanced Glycation End Products (RAGE) is a member of the immunoglobulin super family which is located at the cell surface of several cell species including endothelial cell, vascular smooth cells and the myocardiocyte [1]. Besides mediating the effects of advanced glycation end products (AGEs), RAGE has a broad repertoire of ligands that share the propensity to accumulate in tissues during aging, chronic degenerative diseases, inflammation and the host immune response [2]. AGE and other RAGE ligands accumulate in renal failure [3,4] and these compounds are currently considered as likely players in atherosclerosis in these patients (CKD) [5]. RAGE exists in several variants. A C-truncated variant, the soluble RAGE (sRAGE), could be a naturally occurring inhibitor of the ligand–RAGE interaction [6]. sRAGE attenuates atherosclerosis in experimental models in animals [7] and a vasculoprotective effect of sRAGE has been inferred also in studies in diabetic [8] and non-diabetic individuals [9]. The relevance of RAGE in human diseases goes beyond diabetes and we have recently reported a robust, inverse link between sRAGE and the plaque burden in patients with chronic kidney disease (CKD) [10] implicating the RAGE pathway in vascular damage in this population.

Atherosclerosis apart, evidence is accruing that the RAGE pathway may be implicated in a direct manner in heart disease. AGE concentration and RAGE expression is increased in the heart of Sprague–Dawley rats with streptozocin-induced diabetes while treatment with drugs that break the AGEs-connective tissue cross-links attenuate left ventricular hypertrophy (LVH) and cardiomyopathy in this model [11]. In a previous study in patients with end stage renal disease (ESRD) we observed that high levels of a major AGE, pentosidine, are associated with LVH [12]. LVH occurs early in CKD and its prevalence increases as renal function deteriorates [13,14]. LVH is a strong risk factor for cardiovascular sequelae in CKD, particularly in ESRD where this alteration is considered as the strongest predictor of death [15]. Since sRAGE is an inverse marker of the severity of atherosclerosis in CKD and since AGEs and the RAGE pathway are involved in a causal manner in LVH in experimental models, an analysis of the link between sRAGE and LV mass and function in CKD may provide useful information for exploring the hypothesis that the RAGE pathway is implicated in LVH in patients with CKD. With this background in mind we investigated the relationship between circulating sRAGE and LV mass in the same series of patients with CKD where we had studied the association between sRAGE and atherosclerosis.

Methods

The study protocol was in conformity with the ethical guidelines of our institution, and informed consent was obtained from each participant.

Patients

We studied 142 patients with CKD. All patients were in steady state condition and without inter-current inflammatory diseases. Their mean age was 56 ± 13 years (80 M and 62 F) and their estimated glomerular filtration rate (eGFR) ranged from 5 to 83 ml/min/1.73 m^2 [eGFR (mean ± SD): 32 ± 15 ml/min/1.73 m^2]. Thirty-six patients out of 142 were diabetic (type-1 diabetes: 64%; type-2 diabetes: 36%). The main demographic, somatometric, clinical and biochemical characteristics of patients included in the study are detailed in Table 1.

Control group

As control group we selected 49 healthy subjects (HS) accurately matched to CKD patients as for age (CKD patients: 56 ± 13 years versus Controls: 55 ± 11 years) and gender (CKD patients, Males: 56% versus Controls, Males: 57%). To be selected, HS had to have no alteration at an extensive clinical and biochemical work-up (including absence of albuminuria), and had to have normal urine analysis and eGFR equal or greater than 60 ml/min/1.73 m^2, which is the recommended threshold for the diagnosis of CKD [16].

Laboratory measurements

Blood sampling was performed after 20–30 min of quiet resting in a semi-recumbent position. After an overnight fasting, blood samples for serum lipids, creatinine, albumin, calcium and phosphate, and haemoglobin were obtained from all patients. GFR was estimated by using the simplified modification diet of renal disease study (MDRD) formula [eGFR (mL/min/1.73 m^2) = 186 × $(Scr)^{-1.154}$ × $(Age)^{-0.203}$ × (0.742 if female) × (1.212 if African American) (conventional units)] derived by Levey et al. [17]. Plasma samples were stored at −80 °C until the analysis. Plasma sRAGE levels were determined by an ELISA kit (DuoSet ELISA Development kit, R&D systems, Minneapolis, Minnesota, USA) containing the basic components required for the development of a double sandwich ELISA. Intra-assay and inter-assay coefficients of variation values were 5.9% and 8.2%, respectively. The lower limit of detection of sRAGE was 21.5 pg/mL. Serum C-Reactive Protein (CRP) was measured by a high-sensitivity immuno-turbidimetric method (hsCRP-Dade Behring, Marburg, Germany).

Echocardiography

All echocardiographic measurements were carried out according to the recommendations of the American Society of Echocardiography by an observer unaware of biochemical results. Left ventricular mass (LVM) was calculated according to the Devereux formula and indexed to $heigh^{2.7}$ (LVMI) [18]. LVH was defined as a LVMI of over 47 $g/m^{2.7}$ in women or over 50 $g/m^{2.7}$ in men. Mean wall thickness (MWT) was calculated by the standard formula [MWT = (posterior wall thickness + inter-ventricular septum thickness)/2]. The relative wall thickness (RWT: 2*posterior wall thickness/left ventricular end diastolic diameter) was also calculated, as an index of the left ventricular geometric pattern.

Eco-colour doppler of carotid arteries

Both in patients and controls, ultrasonographic studies on common carotid arteries were performed bilaterally by

Table 1 Main demographic, somatometric, clinical and biochemical data in CKD patients and in controls.

	CKD patients (n = 142)	Controls (n = 49)	P value
Age (years)	56 ± 13	55 ± 11	0.57
BMI (kg/m2)	27.8 ± 4.6	27.4 ± 3.8	0.58
Male sex n. (%)	80(56%)	28 (57%)	1.00
Smokers n. (%)	75(53%)	21 (43%)	0.18
Diabetic patients n. (%)	36(25%)	0(%)	—
On anti-hypertensive treatment n. (%)	129(91%)	0(%)	—
Previous myocardial infarction/stroke n. (%)	13(9%)	0(%)	—
Systolic blood pressure (mmHg)	132 ± 20	123 ± 10	<0.001
Diastolic blood pressure (mmHg)	79 ± 12	78 ± 8	0.87
Heart rate (beats/min)	72 ± 10	73 ± 10	0.38
Haemoglobin (g/dL)	12.6 ± 1.8	13.6 ± 1.6	<0.001
Total cholesterol (mg/dL)	171 ± 39	186 ± 33	0.01
Triglycerides (mg/dL)	150 ± 77	100 ± 46	<0.001
Albumin (g/dL)	4.0 ± 0.6	3.9 ± 0.5	0.27
Glucose (mg/dL)	110 ± 44	98 ± 12	0.008
Calcium (mMol/L)	2.27 ± 0.17	2.21 ± 0.48	0.33
Phosphate (mMol/L)	1.94 ± 0.37	1.59 ± 0.27	<0.001
Creatinine (mg/dL)	2.5 ± 1.4	0.87 ± 0.17	<0.001
eGFR (ml/min/1.73 m^2)	32 ± 15	89 ± 17	<0.001
CRP (mg/L)	1.67 (0.73—5.18)	1.50 (0.65—3.30)	0.31
Left ventricular mass index (g/$m^{2.7}$)	54.5 ± 16.9	41.3 ± 12.1	<0.001
Mean wall thickness (cm)	1.06 ± 0.16	0.93 ± 0.14	<0.001
Left ventricular end diastolic diameter (cm)	4.9 ± 0.7	4.7 ± 0.6	0.02
Total number of plaques (n)	2(0—11)	0.5(0—5)	<0.001
sRAGE (pg/mL)	1780 (1152—2662)	1072(460—1816)	<0.001

Data are expressed as mean ± SD, median and inter-quartile or as percent frequency, as appropriate. For the total number of plaques data are summarized as mean and range.

a single observer blinded as to the clinical and biochemical data. All studies were performed after echocardiography with a GE-Vingmed System 5 using a 10 MHz high-resolution probe. The number of atherosclerotic plaques [either as faint grey echoes (soft plaques) or bright white echoes (calcified plaque) protruding into the lumen] detected in the bulbar area (from 2 cm below to 2 cm above the bifurcation) of the carotid arteries was recorded on both sides and summed up.

Statistical analysis

Data are expressed as mean ± SD, median and inter-quartile range (IQR) or as percent frequency, and comparisons between groups were made by T-test, Mann—Whitney Test or Chi Square Test, as appropriate. The relationship between two variables was tested by Pearson correlation coefficient. When required, variables showing a positively-skewed distribution were log-transformed (lg_{10}) before the correlation analysis. Multi-factorial hypotheses were tested by multiple linear regression analysis and data were expressed as standardized regression coefficient (β) and P value.

The independent correlates of left ventricular hypertrophy in CKD patients were identified by multiple linear and logistic regression analyses. Multivariate models adjusting for all variables that meet criteria to be confounders [19], (that is variables 1) which were related (with $P \leq 0.20$) with both exposures [sRAGE and sRAGE × subject status (0 = HS; 1 = CKD patients) interaction term] and study outcomes (LVMI and MWT); 2) which were not an effect of exposure and 3) which were not in the

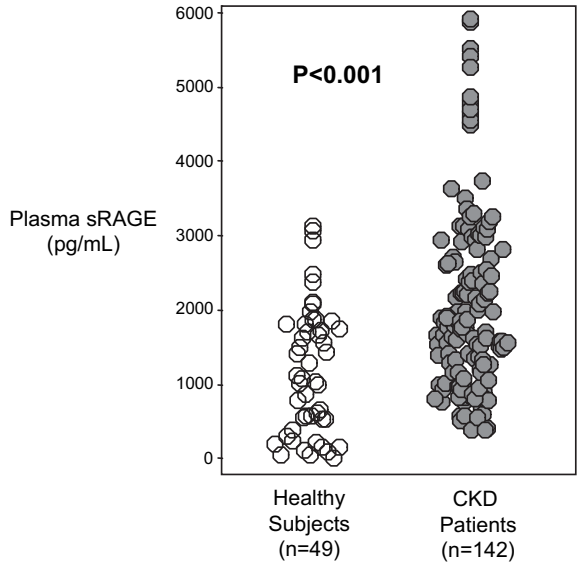

Figure 1 Plasma levels of sRAGE in CKD patients (grey circles) and in health subjects (open circles).

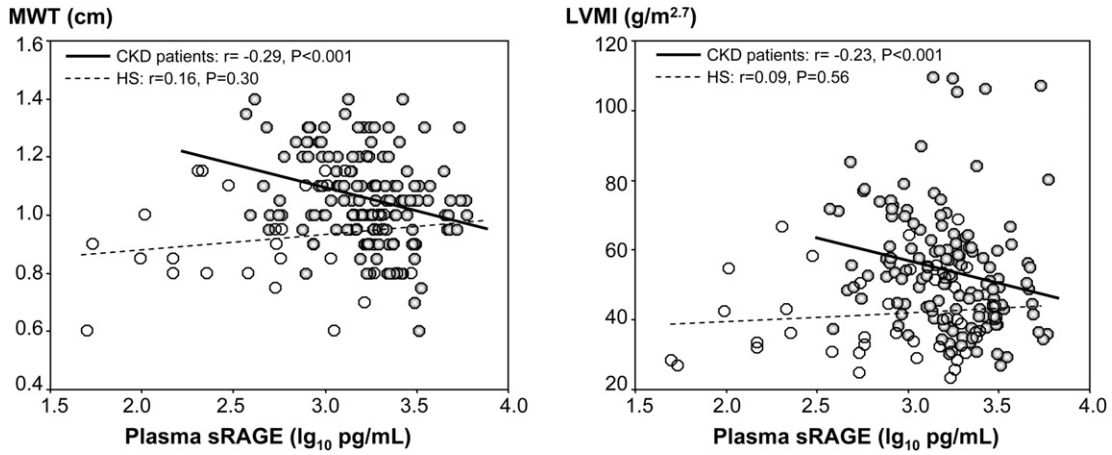

Figure 2 Relationships of plasma sRAGE with MWT and LVMI in CKD patients (grey circles) and in health subjects (open circles). Symbols are: _____ Regression line in CKD patients ———————— Regression line in health subjects.

causal pathway between the exposure and study outcomes [19]. Tested covariates included Framingham risk factors, anti-hypertensive treatment, previous myocardial infarction/stroke, body mass index (BMI), factors peculiar to CKD (haemoglobin, albumin, calcium and phosphate), eGFR/serum creatinine, serum CRP and total number of atherosclerotic plaques. Both crude and adjusted analysis of covariance (ANCOVA) was used to compare the slopes of regression lines between plasma sRAGE and echocardiographic data in CKD patients and in HS. To internally validate the independent link between plasma sRAGE with echocardiographic data in CKD patients and in controls, a bootstrap re-sampling technique of 1000 samples was performed. Data are expressed as regression coefficients, standardized regression coefficients (β), odds ratio (OR) and 95% confidence intervals and *P* values. All calculations were made using standard statistical packages (SPSS for Windows Version 9.0.1, 11 Mar-1999, Chicago, Illinois — USA; STATA/SE 9 for Windows, StataCorp LP, TX, USA).

Results

The plasma concentration of sRAGE was significantly higher ($P < 0.001$) in CKD patients (median: 1780 pg/mL, IQR: 1152—2662 pg/mL) than in HS (median: 1072 pg/mL, IQR: 460—1816 pg/mL) (Fig. 1). Thirty-six out of 142 CKD patients (25%) were diabetic, 129 (91%) were on anti-hypertensive treatment and 13 (9%) had a history of major cardiovascular complications (myocardial infarction/stroke). CKD patients had higher systolic arterial pressure, triglycerides, glucose, cholesterol, phosphate and lower haemoglobin than healthy controls while CRP did not significantly differ between the two groups.

Echocardiographic data in CKD patients and in HS

LVMI was markedly higher (+32%) in CKD patients than in healthy subjects (Table 1). Analysis of the individual components defining the geometry of the LV showed that both the muscular component (MWT) and the cavitary one (LV end diastolic diameter) were substantially elevated in CKD patients (Table 1). Eighty patients out of 142 (56%) displayed LVH at echocardiography and LVH was of the concentric type in 37% of cases and eccentric in other cases. The total number of atherosclerotic plaques was higher in CKD patients than in controls.

sRAGE and echocardiographic data: univariate and multivariate analyses

Plasma sRAGE was significantly lower ($P = 0.004$) in diabetic (median: 1411 pg/ml, inter-quartile range: 851—1815 pg/mL) than in non-diabetic (1900 pg/ml, inter-quartile range: 1175—2804 pg/mL) CKD patients. In CKD patients, plasma sRAGE was related inversely with LVMI ($r = -0.23$, $P < 0.001$) and MWT ($r = -0.29$, $P < 0.001$) while no such relationships were found in HS (sRAGE vs MWT: $r = 0.16$, $P = 0.30$; sRAGE vs LVMI: $r = 0.09$,

Table 2 Multiple linear regression analyses of LVMI (a) and MWT (b).

	Beta	*P*
a) Dependent variable: LVMI		
CRP (lg_{10})	0.19	0.02
Haemoglobin	−0.17	0.03
sRAGE (lg_{10})	−0.17	0.039
Diabetes	0.16	0.06
Cardiovascular comorbidities	0.15	0.07
Albumin	−0.11	0.18
b) Dependent variable: MWT		
Diabetes	0.29	<0.001
sRAGE (lg_{10})	−0.16	0.045
CRP (lg_{10})	0.14	0.07
Cardiovascular comorbidities	0.14	0.07
Triglycerides	0.14	0.08
Haemoglobin	0.12	0.10

Data are expressed as standardized regression coefficients (beta) and *P* values. Each model includes all pertinent confounding variables (see Statistical Analysis for more details).

Table 3 Multiple logistic regression analysis of LVH.

	Units of increase	Odds ratio and 95% CI	*P*
Dependent variable: LVH			
CRP	1 log unit	2.44(1.16—5.15)	0.02
sRAGE	1 log unit	0.18(0.04—0.82)	0.03
Diabetes	0 = No; 1 = yes	2.05(0.77—5.46)	0.15
Albumin	1 g/dL	0.61(0.31—1.23)	0.17
Haemoglobin	1 g/dL	0.86(0.69—1.08)	0.19
Cardiovascular comorbidities	0 = No; 1 = yes	2.21(0.41—11.81)	0.36

Data are expressed as odds ratio and 95% CI. The logistic model includes the same set of confounding variables considered in the linear model of LVMI (see Table 2a).

$P = 0.56$) (Fig. 2). Both in CKD patients and in HS, plasma levels of sRAGE were unrelated to RWT and LV end diastolic diameter (P = NS). In multiple linear regression models, adjusting for potential confounders, plasma sRAGE resulted to be an independent predictor of LVMI and MWT in CKD patients (Table 2). In a multiple logistic regression analysis (Table 3), plasma sRAGE was confirmed as an inverse and independent predictor of LVH in CKD patients in a model including cardiovascular comorbidities, haemoglobin, albumin, diabetes and serum CRP (i.e. the full set of potential confounders for the sRAGE-LVH link). Further data adjustment for systolic pressure did not materially affect these results (data not shown). In this model, for each unitary increase in log sRAGE the odds of LVH reduced by 82% (OR: 0.18, 95% CI: 0.04—0.82, $P = 0.03$).

Plasma sRAGE and *LV* mass: analysis of covariance

On covariance analysis the slopes of MWT and LVMI relationships with plasma sRAGE were significantly steeper in CKD patients (estimated decrease in MWT for each unitary increase in log sRAGE: 0.16 cm vs 0.03 cm, $P = 0.001$; estimated decrease in LVMI for each unitary increase in log sRAGE: 14.0 g/m2.7 vs 1.1 g/m2.7, $P = 0.02$) than the corresponding relationships in HS. Such differences were unaffected by data adjustment for all potential confounders [namely, MWT model: smoking, diabetes, cardiovascular comorbidities, systolic pressure, triglycerides, phosphate, eGFR and total number of plaques; LVMI model: diabetes, cardiovascular comorbidities, systolic pressure, haemoglobin, cholesterol, triglycerides, phosphate, eGFR and total number of plaques]. In this analysis the estimated decrease in MWT for each unitary increase in log sRAGE: was 0.11 cm in CKD patients versus 0.03 mm in controls ($P = 0.02$) and the corresponding estimated decreases in LVMI in the two groups were 10.0 g/m2.7 and 0.9 g/m2.7 ($P = 0.05$), respectively.

Bootstrap analysis

A bootstrap re-sampling validation fully confirmed these previous estimates of the link between sRAGE and MWT and LVMI (estimated decrease in MWT for each unitary increase in log sRAGE: 0.13 cm versus 0.02 mm; estimated decrease in LVMI for each unitary increase in log sRAGE: 12 g/m2.7 versus 0.90 g/m2.7).

Discussion

This study shows that in patients with CKD circulating sRAGE is an inverse correlate of LV mass, particularly of the muscular component (MWT) of the left ventricle. These associations are largely independent of traditional and non traditional risk factors and appear peculiar to CKD because LVMI as well as MWT are largely unrelated with circulating sRAGE in healthy subjects. Overall these findings implicate the RAGE pathway in LVH in patients with renal insufficiency.

LVH in CKD

LVH is recognized as a fundamental component of the risk for death and cardiovascular complications in CKD and accumulation of connective tissue in the myocardium is considered as a distinguishing feature of LVH in these patients [20]. The prevalence of this alteration increases as the GFR declines and as much as 78% of stage 5D CKD patients are eventually affected by LVH15. The mechanisms underlying LVH involve after-load (arterial pressure and compliance), preload (intravascular volume and anaemia) and a variety of other factors, including sympathetic overactivity, accumulation of the endogenous inhibitor of NO synthase ADMA, hyperparathyroidism, insulin resistance and other factors. However, collectively these risk factors only in part explain the variability in LV mass in patients with CKD [21].

AGEs in CKD

Advanced glycation end products (AGEs) are a heterogeneous group of bioactive molecules resulting by non-enzymatic glycation and oxidation of proteins and lipids. RAGE, a 35kD trans-membrane receptor of the immunoglobulin super family, is the main receptor mediating the effects of AGEs at cell level. Besides AGEs, RAGE is also able to bind other ligands and it is therefore classified as a classical pattern recognition receptor. RAGE stimulation potently activates several pro-inflammatory genes and represents a fundamental element of a pathway implicated in a variety of inflammatory disorders spanning from diabetes to cancer [2]. In diabetes, RAGE stimulation translates into LV hypertrophy and increased cardiac fibrosis in an experimental model in the rat [11]. The possibility that RAGE has

a role in cardiomyopathy in this condition is indicated by the fact that agents that reduce AGE accumulation also mitigate cardiomyopathy in the same model [11].

High AGE levels in patients with ESRD have been associated with concentric LVH [12], a geometric pattern which accompanies arterial stiffness in this population [22,23] but other studies in patients with moderate to severe CKD failed to show an independent relationship between AGEs and LVH [24]. The interpretation of the link between AGEs and LVH in CKD is complex because these compounds are affected by inflammation and wrong diet [25], i.e. two factors also associated with LVH. Furthermore, the RAGE signalling pathway may convey not only AGEs but also other inflammatory ligands that are increased or amplified in CKD.

sRAGE: cardiovasculoprotective properties in animal models and in CKD

The soluble form of RAGE is a decoy receptor shedded from cell surface which effectively bind circulating AGEs. By binding circulating AGEs, sRAGE attenuate RAGE signalling at cell level [26] thereby exerting a protective action on various organ systems. A protective role of high sRAGE has coherently emerged in various experimental studies where sRAGE administration either prevented or attenuated tissue damage triggered by RAGE stimulation2 including micro and macro-vascular disease and ischemia reperfusion injury [27] in murine models. Of note, in animal models of chronic diseases sRAGE suppresses chronic cellular activation and dysfunction more markedly than full abrogation of RAGE receptors [28–30]. This finding implies that circulating RAGE ligands exert actions also via other receptors [31]. A protective role of circulating sRAGE in humans is suggested by the observation that the plasma concentration of these receptors is lower in patients with coronary artery disease than in age-matched healthy controls [32] and that carotid atherosclerosis is inversely associated with sRAGE in diabetic [8] and non-diabetic patients [33]. In keeping with these observations, in our study plasma levels of sRAGE were significantly lower in diabetic than in non-diabetic CKD patients. Independently of diabetes, sRAGE accumulate in CKD, particularly in ESRD patients [34–36] and in this population low sRAGE predict a higher risk for incident cardiovascular complications [37]. We have recently reported an inverse association between sRAGE and the severity of atherosclerosis in patients with CKD [10]. We also demonstrated that in CKD the western blot profile of plasma sRAGE does not differ from that in healthy subjects suggesting that sRAGE in CKD does not undergo proteolysis or other major structural rearrangements which may affect its biological action [10].

sRAGE and LVH in CKD

RAGE is expressed in a variety of tissues including cardiac myocytes and this pathway is involved in LVH and cardiomyopathy in diabetic rats [11]. In a recent study on mouse type-2 diabetes model, blockade of RAGE signalling prevented the reduction in systolic function and development of increased LV diastolic chamber stiffness and these effects were associated with reduced cardiac expression of collagen [38]. Beyond diabetes, the potential implication of the RAGE pathway in cardiomyopathy in humans is suggested by the fact that serum levels of pentosidine, an AGE compound, is associated with the severity of heart failure [39] and predicts progression of heart failure and death, an observation mirrored by the inverse link between the sRAGE that blocks AGE effects, and the same outcomes [40].

Our study is the first analyzing the link between LVH and sRAGE in CKD patients. Studying this link in this population is a relevant question for two reasons, first because sRAGE has already been described as a putative protective factor for atherosclerosis in CKD [10] and secondly because accumulation of connective tissue in the context of the myocardium21, a phenomenon which is modified by sRAGE and RAGE blockade [11], is a peculiar feature in LVH in CKD patients. In line with the hypothesis that high sRAGE levels may limit myocardial hypertrophy in CKD we observed coherent inverse relationships between sRAGE and the MWT, a measure of the muscular component of the LV, as well as with LVMI. The LV mass-sRAGE link was independent of other risk factors including the GFR, CRP and the severity of atherosclerosis. Independence from indicators of carotid atherosclerosis suggests that this association underlies an effect of sRAGE at myocardial level rather than a phenomenon secondary to the protective effect of sRAGE at arterial level, a possibility in line with observations in animal models [11]. In our study circulating levels of CRP did not significantly differ between CKD patients and healthy subjects, a finding due to the fact that a substantial proportion of our patients had a GFR $>$45 ml/min, the threshold below which the CRP starts increasing [41].

Limitations

This study has several limitations. The first limitation pertains to design. Ours is a survey and therefore it merely generate a hypothesis. The second limitation is that we did not quantify the fibrotic component of the LV, a measure which can now be obtained by ultrasound myocardial tissue analysis and nuclear magnetic resonance studies. The third limitation is the fact that we did not measure AGEs in this study. However, evidences that these compounds are raised in CKD are quite consistent [3,4]. A strength of our study is the large number of traditional and non traditional risk factors considered as potential confounders and the fact that the relationship between LV mass and sRAGE materialized only in CKD patients, a population were the RAGE pathway is activated by AGEs accumulation and other stimuli but not in healthy subjects where RAGE signalling is not disturbed. Anther strength of our study is that sRAGE, the biomarker we used to explore the involvement of the RAGE pathway in LVH, is considered as a better indicator of the involvement of AGEs in disease states than the measurement of individual AGEs because it captures the combined effect of these compounds on the RAGE pathway and on pathways mediated by other receptors as well. Finally we validated the estimates of the links between sRAGE and MWT and LVMI by a bootstrap analysis.

In conclusion, the sRAGE, is an inverse marker of the LV mass in CKD patients. This finding generates the hypothesis that the RAGE pathway is a causal risk factor for LVH in this

population and that blockade of this pathway by the endogenous decoy receptor sRAGE attenuates LVH in the same population. However, the cross-sectional nature of our study precludes the possibility to draw definitive conclusions about the nature (causal/non causal) of these relationships. New interventions are being tested for attenuating RAGE signalling in human diseases and, when available, these interventions may allow full scale testing of the hypothesis.

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