

# Skin Autofluorescence and the Association with Renal and Cardiovascular Risk Factors in Chronic Kidney Disease Stage 3

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## Summary

**Background and objectives** Tissue advanced glycation end products (AGE) accumulation is a measure of cumulative metabolic stress. Assessment of tissue AGE by skin autofluorescence (SAF) correlates well with cardiovascular (CV) outcomes in diabetic, transplant, and dialysis patients, and may be a useful marker of CV risk in earlier stages of chronic kidney disease (CKD).

**Design, setting, participants, & measurements** 1707 patients with estimated GFR 59 to 30ml/min per 1.73 m<sup>2</sup> were recruited from primary care practices for the Renal Risk In Derby (RRID) study. Detailed medical history was obtained, and each participant underwent clinical assessment as well as urine and serum biochemistry tests. SAF was assessed (mean of three readings) as a measure of skin AGE deposition using a cutaneous AF device (AGE Reader<sup>™</sup>, DiagnOptics, Groningen, The Netherlands).

**Results** Univariate analysis revealed significant correlations between AF readings and several potential risk factors for cardiovascular disease (CVD) and progression of CKD. SAF readings (arbitrary units) were also significantly higher among males ( $2.8 \pm 0.7$  versus  $2.7 \pm 0.6$ ), diabetics ( $3.0 \pm 0.7$  versus  $2.7 \pm 0.6$ ), patients with evidence of self-reported CVD ( $2.9 \pm 0.7$  versus  $2.7 \pm 0.6$ ), and those with no formal educational qualifications ( $2.8 \pm 0.6$  versus  $2.6 \pm 0.6$ ;  $P < 0.01$  for all). Multivariable linear regression analysis identified hemoglobin, diabetes, age, and eGFR as the most significant independent determinants of higher SAF (standardized coefficients  $-0.16, 0.13, 0.12,$  and  $-0.10,$  respectively;  $R^2 = 0.17$  for equation).

**Conclusion** Increased SAF is independently associated with multiple CV and renal risk factors in CKD 3. Long-term follow-up will assess the value of SAF as a predictor of CV and renal risk in this population.

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## Introduction

Cardiovascular disease (CVD) is the most important cause of morbidity and mortality in patients with chronic kidney disease (CKD), and the risk of cardiovascular events (CVE) is higher at all stages of CKD than in the general population (1,2). The mechanisms for this association remain the focus of intense investigation. Whereas the role of traditional cardiovascular risk factors such as hypertension, diabetes, dyslipidemia, and smoking has been well described, a growing number of nontraditional risk factors, unique to CKD, are becoming appreciated (3,4). One of these novel risk factors is accumulation of advanced glycation end products (AGE), a heterogeneous group of compounds formed by the reaction of free amino groups on proteins, lipids, and nucleic acids with reactive carbonyl groups on reducing sugars (5). AGE may accumulate by at least four mechanisms. First, they may be formed nonenzymatically over time (the Maillard reaction). Second, they may be produced more rapidly by reactive carbonyl products

(dicarbonyl stress), resulting from oxidative stress (6). Third, AGE may accumulate due to exogenous factors, including AGE in ingested food (7) and AGE formation by smoking (8). AGE form in food during processing at high temperatures, and about 10% of ingested AGE are absorbed (9). Fourth, AGE are normally excreted by the kidneys, and in the presence of decreased renal function, accumulation occurs. AGE accumulation during CKD may be exacerbated by increased formation, as dicarbonyl and oxidative stress are increased with reduced renal function (10,11). AGE formation therefore results from a variety of metabolic processes and has been proposed to be a marker of “cumulative metabolic stress” (3,12).

Modification of proteins, nucleic acids, and lipids by AGE results in cross-linking and changes in both structure and function. Mitochondrial glycation may also enhance oxidative stress, provoking a vicious circle (13). AGE bind to cell membrane receptors, in particular, the cell receptor for AGE (RAGE). This, in turn, may lead to activation of intracellular pathways,

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releasing cytokines, and may induce endothelial dysfunction and other deleterious vascular effects (14). RAGE has been implicated in the pathogenesis of both CKD (15) and CVD (16). AGE accumulation has also been associated with arterial stiffness (17), a critical determinant of cardiovascular outcomes in patients with advanced CKD (18).

Measuring the levels of AGE in blood requires isotope dilution tandem mass spectrometry or enzyme linked immunoassay (ELISA), which are relatively complex and expensive to perform, and assessment of tissue AGE has previously required a skin biopsy with immunohistochemistry. However, the development of the AGE Reader™ (Diagnoptics Technologies, Groningen, The Netherlands), which measures skin autofluorescence (SAF), has allowed for simple, noninvasive assessment of tissue AGE deposition by exploiting the close correlation between collagen linked fluorescence and AGE content observed in skin biopsies (19). Validation of SAF against levels of specific AGE molecules has been reported in healthy controls as well as patients with diabetes or CKD. In these studies, increased SAF was associated with the accumulation of AGE and the progression of chronic complications of both diabetes and end-stage kidney disease (ESKD) (3,20–24). Elevated SAF has been identified as an indicator of widespread atherosclerosis (25) and an independent predictor of cardiovascular death in patients with diabetes and on hemodialysis (3). One recent systematic review has identified seven studies that all reported positive associations between SAF readings and complications of diabetes (excluding retinopathy) (26). Although the relationship between SAF, renal function, and CVD has been reported in patients with more advanced CKD (both in dialysis and transplant patients) (3,27,28), and, more recently, in a hospital-based study of 304 predialysis patients (29), it has not previously been described in a community-based population of patients with mild to moderately reduced kidney function. In this cross-sectional analysis, we aimed to identify associations between SAF and renal, as well as cardiovascular, risk factors in a community-based cohort of patients with CKD stage 3.

## Materials and Methods

### Participants and Recruitment

Participants were recruited as part of the Renal Risk in Derby (RRID) study, a prospective cohort study, running over 10 years with the aim of studying renal and cardiovascular risk factors in patients with CKD stage 3 in a primary care setting. Eligible participants were 18 years or over, met the Kidney Disease Outcomes Quality Initiative criteria for CKD stage 3 (estimated GFR [eGFR] of between 30 to 59 ml/min per 1.73 m<sup>2</sup> on two or more occasions at least 3 months apart), were able to give informed consent, and were able to attend their general practitioner (GP) surgery for assessments. People who had previously had a solid organ transplant or who were terminally ill (expected survival <1 years) were excluded. The RRID study is conducted by a single nephrology department, but participants were recruited directly from 32 GP surgeries. Eligible patients were invited to participate via a letter sent by their GP and telephoned the coordinating center to schedule a study visit. Study visits were conducted at participating GP surgeries by the researchers.

### Data Collection

First study visits were conducted from August 2008 to March 2010. Screening and baseline visits were combined due to the large proportion of elderly participants and the logistical challenges associated with conducting study visits in multiple primary care centers. Participants were sent a medical and dietary questionnaire as well as three urine specimen bottles, and were asked not to eat cooked meat for at least 12 hours before the assessment (30). Socioeconomic status was assessed using the Indices of Multiple Deprivation score (IMD) (31). This is a social deprivation score comprising a composite measure of seven domains. It is widely used in public health departments in the United Kingdom and has demonstrated a strong relationship to health in all geographical locations. A higher IMD score indicates more social deprivation, and a score of 21.67 (range 2.66 to 80.62) represents average socioeconomic status in England (32).

At the assessment, information on questionnaires was checked, anthropomorphic measurements were taken, and urinalysis was performed. Blood specimens were taken and urine specimens were submitted for biochemical analysis. Blood pressure (BP), SAF, and pulse wave velocity (PWV) were also measured.

Diabetes was defined by having a previous clinical diagnosis in line with World Health Organization (WHO) criteria (33). Previous cardiovascular event (CVE) was defined as subject-reported myocardial infarction, stroke, transient ischemic attack, revascularization, or amputation due to peripheral vascular disease, or aortic aneurysm. Anemia was defined as hemoglobin <11.5 g/dl for women and <13.5 g/dl for men. Central fat distribution was defined as a waist to hip ratio of  $\geq 0.9$  for men or  $\geq 0.8$  for women (34).

The study was approved by the Nottingham Research Ethics Committee 1 and abided by the principles of the Declaration of Helsinki. All participants provided written consent. The study was included on the National Institute for Health Research (NIHR) Clinical Research Portfolio (NIHR Study ID:6632) and was independently audited by QED Clinical Services in November 2009.

**BP.** BP was measured after a minimum of 5 minutes of rest in the sitting position, using a validated oscillometric device, recommended by the British Hypertension Society. The same device was used for all readings. Measurements were taken until three readings that were within 10% of each other were obtained. BP was calculated as the mean of these three readings. Hypertension was defined as a systolic BP  $\geq 140$  mmHg, diastolic BP  $\geq 90$  mmHg, or current antihypertensive medication (35).

**PWV.** Carotid to femoral PWV was measured as a marker of arterial stiffness, a critical determinant of cardiovascular outcomes in CKD (18). Measurements were performed using a Vicorder™ device (Skidmore Medical Ltd., Bristol, UK) and were done in the semiprone position (at approximately 30°) to prevent venous contamination of the arterial signal (36).

**SAF.** SAF, a measure of skin AGE deposition, was assessed on the left forearm using an AGE Reader™ device (DiagnOptics, Groningen, The Netherlands). Three readings were taken and the average calculated. Care was

taken to avoid areas of skin that were tattooed or colored with cosmetics (37), heavily freckled, or had vessels near to the surface of the skin. It was not possible to conduct SAF readings on very dark or black skin. According to the manufacturers, the AGE Reader and its software have been validated in patients with skin reflection >6% (Fitzpatrick class 1 to 4). In patients with darker skin color (Fitzpatrick class 5 to 6, dark brown or black), a correction is made to the SAF value if the ultraviolet reflectance is between 6 and 8%. If the ultraviolet reflectance is below 6%, the AGE reader gives a warning that the signal is too low for valid results. SAF measurement is nonoperator dependent. Values are expressed in arbitrary units (AU). Coefficient of variation for 10 SAF readings obtained on a single patient by a single operator was 7%. Ten readings performed by 10 different operators yielded a coefficient of variation of 8%.

**Albuminuria.** Albuminuria was assessed by measuring the urine albumin to creatinine ratio (ACR) on three consecutive early morning urine specimens collected before the clinic visit and stored in a refrigerator. Microalbuminuria was defined as a mean urine ACR of >2.5 mg/mmol in males or >3.5 mg/mmol in females (30).

**Estimation of GFR.** Biochemical assessments were performed by autoanalyser in a single laboratory. The creatinine assay has been standardized against an isotope dilution mass spectrometry (IDMS) method and the modified four-variable MDRD equation was used to estimate GFR.

### Statistical Analysis

Results presented are a cross-sectional analysis of data from the first study visit. Variables are reported as the mean and SD, if normally distributed, or the median and interquartile range, if not. A *t* test was used to compare groups where variables were normally distributed, and a Mann-Whitney test used, if not. SPSS version 15.0 was used for analysis, and *P* < 0.05 was considered statistically significant. Multivariable linear regression analysis, using

the stepwise method, was used to determine independent determinants of higher SAF.

### Results

The RRID study cohort included 1741 participants, but 34 participants were excluded because SAF readings could not be obtained due to dark skin color (*n* = 17) or technical failure (*n* = 17). Thus, 1707 participants are included in the present analysis. Table 1 shows the baseline characteristics of the cohort and of subgroups with and without diabetes. For the total cohort, the mean age was 72.9 ± 9 years, 61% were female, and 98.5% were Caucasian. Almost one quarter of participants were anemic. Twenty-two percent had a history of previous cardiovascular events, and almost 55% had no formal educational qualification. Mean SAF reading was 2.7 ± 0.6 AU.

Table 2 shows previously reported mean SAF values in control participants (38) versus the RRID study cohort, according to age. In both groups, SAF increased with age, but values are higher in those with CKD. Differences in SAF between participants with CKD stage 3 and control values were greater in younger age groups. Using data derived from published studies (38), the weighted mean for SAF for a hypothetical population of control participants of a similar age composition to the RRID cohort is 2.55 AU versus a mean of 2.7 AU in our study population.

Table 3 shows the significant correlations with SAF. No significant correlations were observed with body mass index, systolic BP, serum total protein, calcium or phosphate, or treatment with renin-angiotensin aldosterone system inhibitors (RAASi). Comparison of potentially important subgroups showed significantly higher SAF values in males, diabetic participants, and those with a past history of CVE, no formal educational qualification, CKD stage 3B, and anemia (Table 4).

**Table 1. Baseline characteristics**

	Total Cohort ( <i>n</i> = 1707)	Diabetes Mellitus ( <i>n</i> = 284)	No Diabetes ( <i>n</i> = 1423)
Age (years)	72.9 ± 9	73.5 ± 8 <sup>a</sup>	72.8 ± 9
Female gender ( <i>n</i> [%])	1036 (61)	158 (56)	878 (62)
eGFR (ml/min per 1.73 m <sup>2</sup> )	52.5 ± 10.4	49.3 ± 10 <sup>a</sup>	53.1 ± 10.4
PWV (m/s)	9.9 ± 2	10.4 ± 2 <sup>a</sup>	9.8 ± 2
Skin AF (AU)	2.7 ± 0.6	3.0 ± 0.7 <sup>a</sup>	2.7 ± 0.6
Waist to hip ratio	0.91 ± 0.09	0.94 ± 0.09 <sup>a</sup>	0.9 ± 0.09
Hemoglobin (g/dl)	13.2 ± 1.4	12.7 ± 1.4 <sup>a</sup>	13.3 ± 1.4
Anemia ( <i>n</i> [%])	390 (22.8)	102 (35.9) <sup>a</sup>	288 (20.2)
Cholesterol (mmol/L)	4.8 ± 1.2	4.0 ± 0.8 <sup>a</sup>	4.9 ± 1.2
Albumin (g/L)	40.7 ± 3.2	40.5 ± 3.4	40.7 ± 3.1
Previous CVE ( <i>n</i> [%])	379 (22.2)	84 (29.6) <sup>a</sup>	295 (20.7)
RAASi use ( <i>n</i> [%])	1101 (64.5)	237 (83.5) <sup>a</sup>	864 (60.7)
Diastolic BP (mmHg)	73 ± 11	69 ± 10 <sup>a</sup>	74 ± 11
Total pack years	1.00 (0 to 18.75)	4.75 (0 to 21) <sup>a</sup>	0.5 (0 to 17.5)
Urine ACR (mg/mmol)	0.33 (0 to 1.5)	0.97 (0.13 to 3.7) <sup>a</sup>	0.3 (0 to 1.2)
No qualifications ( <i>n</i> [%])	935 (54.8)	161 (56.7)	774 (54.4)

Data are mean ± SD, median (interquartile range), or number (%). eGFR, estimated GFR; PWV, pulse wave velocity; AF, autofluorescence; CVE, cardiovascular event; ACR, albumin to creatinine ratio.

<sup>a</sup>*P* < 0.05 when compared with nondiabetic participants.

**Table 2. AF reference values (AU) in control participants versus RRID participants**

Age (years)	Control Participants <sup>a</sup>		RRID CKD Stage 3	
	AF ± SD	n	AF ± SD	n
30 to 39.9	1.73 ± 0.42	86	2.27 ± 0.62	5
40 to 49.9	1.81 ± 0.36	72	2.36 ± 0.51	23
50 to 59.9	2.09 ± 0.36	64	2.43 ± 0.51	97
60 to 69.9	2.46 ± 0.57	45	2.62 ± 0.59	439
70 to 79.9	2.73 ± 0.55	27	2.77 ± 0.60	738
80+	2.71 ± 0.44	5	2.87 ± 0.63	405

Autofluorescence (AF) values are expressed as the mean ± 1 SD. RRID, Renal Risk In Derby; CKD, chronic kidney disease. <sup>a</sup>Reference [38].

SAF was significantly higher in diabetic versus nondiabetic participants. Diabetic participants also evidenced significantly lower eGFR and higher levels of albuminuria. Diabetes was associated with lower diastolic BP and higher PWV, indicators of increased arterial stiffness. Previous CVE and anemia were also significantly more prevalent in the participants with diabetes.

Independent determinants of higher SAF are shown in Table 5. As indicated by the standardized coefficients, hemoglobin, diabetes, age, and eGFR were the strongest determinants in the total cohort, with smoking history, magnitude of proteinuria, previous CVE, having no formal educational qualifications, male gender, and deprivation score also significant. For diabetic participants, hemoglobin and gender were the strongest determinants, with magnitude of proteinuria, diastolic BP, and PWV also significant. Among nondiabetic participants

**Table 4. Skin autofluorescence readings (AU) in potentially significant subgroups**

	Yes	No
Male	2.8 ± 0.7 <sup>a</sup>	2.7 ± 0.6
Diabetes	3.0 ± 0.7 <sup>a</sup>	2.7 ± 0.6
No formal qualifications	2.8 ± 0.6 <sup>a</sup>	2.6 ± 0.6
Previous cardiovascular event	2.9 ± 0.7 <sup>a</sup>	2.7 ± 0.6
CKD stage 3B	3.0 ± 0.6 <sup>a</sup>	2.7 ± 0.6
Anemia	3.0 ± 0.7 <sup>a</sup>	2.7 ± 0.6

eGFR 45 to 59.9 ml/min per 1.73 m<sup>2</sup>; CKD Stage 3B, chronic kidney disease stage 3B.  
<sup>a</sup>P < 0.001 versus comparator group.

age, smoking history, eGFR, and hemoglobin were the strongest determinants, with previous CVE also significant.

**Discussion**

Our observations confirm previous observations in a relatively small cohort of hospital-based Japanese patients that chronological age, diabetes, eGFR, and past history of CVE are independent determinants of SAF in participants with CKD stage 3 (29). In addition, we found that hemoglobin (negative association), past history of smoking, proteinuria, and social deprivation were also determinants of SAF in CKD stage 3.

**Hemoglobin as a Determinant of Higher SAF**

We have identified lower hemoglobin as an independent determinant of SAF across all subgroups in this cohort of participants with CKD stage 3. One previous study (29)

**Table 3. Significant Correlations with SAF**

	Total Cohort		DM		No DM	
	r	P-value	r	P-value	r	P-value
eGFR (ml/min per 1.73m <sup>2</sup> )	-0.228	<0.01	-0.189	0.01	-0.210	<0.01
Hemoglobin (g/dl)	-0.228	<0.01	-0.331	<0.01	-0.172	<0.01
Age (years)	0.213	<0.01	0.150	0.01	0.225	<0.01
Total cholesterol (mmol/L)	-0.150	<0.01	-0.132	0.03	-0.089	<0.01
Smoking history (pack years)	0.174	<0.01	0.134	0.02	0.174	<0.01
Urine PCR <sup>a</sup>	0.173	<0.01	0.218	<0.01	0.124	<0.01
Urine ACR <sup>a</sup>	0.129	<0.01	0.193	0.01	0.078	<0.01
Diastolic BP (mmHg)	-0.149	<0.01	-0.171	<0.01	-0.107	<0.01
Albumin (mmol/L)	-0.125	<0.01	-0.144	<0.01	-0.117	<0.01
PWV (m/s)	0.120	<0.01	0.120	0.05	0.099	<0.01
HS CRP (units) <sup>a</sup>	0.115	<0.01	0.053	0.37 <sup>b</sup>	0.126	<0.01
Waist:hip ratio	0.111	<0.01	0.181	0.002	0.063	<0.01
Uric acid (μmol/L)	0.071	<0.01	-0.038	0.53 <sup>b</sup>	0.076	<0.01
IMD score	0.069	<0.01	0.011	0.86 <sup>b</sup>	0.082	<0.01
Bicarbonate (mmol/L)	-0.063	<0.01	-0.101	0.09 <sup>b</sup>	-0.028	0.29 <sup>b</sup>
HDL cholesterol (mmol/L)	-0.057	0.02	-0.118	0.05	-0.010	0.70 <sup>b</sup>

SAF, skin autofluorescence; DM, diabetes mellitus; ACR, albumin to creatinine ratio; eGFR, estimated GFR; hsCRP, high-sensitivity C-reactive protein; IMD, indices of multiple deprivation; PCR, protein to creatinine ratio; PWV, pulse wave velocity.

<sup>a</sup>Not statistically significant.

<sup>b</sup>Log-transformed data.

Table 5. Independent determinants of higher SAF

	Total Cohort Adjusted $R^2 = 0.17$			DM Adjusted $R^2 = 0.22$			No DM Adjusted $R^2 = 0.11$		
	B (95% CI)	$\beta$	P-value	B (95% CI)	$\beta$	P-value	B (95% CI)	$\beta$	P-value
Hemoglobin (g/dl)	-0.07 (-0.09 to -0.05)	-0.16	<0.01	-0.15 (-0.20 to -0.10)	-0.32	<0.01	-0.05 (-0.07 to -0.03)	-0.12	<0.01
Diabetes	0.22 (0.15 to 0.30)	0.13	<0.01	-	-	-	-	-	-
Age (10 years)	0.08 (0.05 to 0.11)	0.12	<0.01	-	-	-	0.10 (0.06 to 0.13)	0.15	<0.01
Smoking (10 pack years)	0.03 (0.01 to 0.05)	0.09	<0.01	-	-	-	0.05 (0.03 to 0.06)	0.15	<0.01
eGFR (10 ml/min per 1.73 m <sup>2</sup> )	-0.06 (-0.09 to -0.03)	-0.10	<0.01	-	-	-	-0.07 (-0.10 to -0.04)	-0.13	<0.01
Urine PCR <sup>a</sup>	0.16 (0.06 to 0.26)	0.08	<0.01	0.33 (0.13 to 0.54)	0.17	<0.01	-	-	-
Previous CVE	0.10 (0.04 to 0.17)	0.07	<0.01	-	-	-	0.11 (0.03 to 0.18)	0.08	<0.01
No formal qualifications	0.08 (0.02 to 0.14)	0.06	<0.01	-	-	-	-	-	-
Female gender	-0.09 (-0.15 to -0.02)	-0.07	<0.01	-0.28 (-0.43 to -0.14)	-0.21	<0.01	-	-	-
IMD score	0.003 (0.01 to 0.006)	-	<0.01	-	-	-	-	-	-
Ever smoked	0.07 (0.01 to 0.13)	0.06	<0.01	-	-	-	-	-	-
Diastolic BP (5 mmHg)	-	-	-	-0.05 (-0.09 to -0.01)	-0.16	<0.01	-	-	-
PWV (1 m/s)	-	-	-	0.04 (0.006 to 0.08)	0.13	<0.01	-	-	-

B, unstandardized coefficient (95% confidence intervals [CIs]);  $\beta$ , standardized coefficient; eGFR, estimated GFR; CVE, cardiovascular events; IMD, indices of multiple deprivation; PWV, pulse wave velocity.

<sup>a</sup>Log-transformed data.

reported a univariate correlation between hemoglobin and SAF, but hemoglobin did not enter the equation in the multivariable analysis. Hemoglobin was, however, an independent determinant of SAF in a subgroup of participants with diabetes in that study. The larger number of participants in our study likely afforded greater power to detect this association. The mechanisms whereby low hemoglobin may contribute to AGE formation and increased SAF remain to be elucidated (39,40). Hemoglobin binds reactive oxygen species, and anemia is therefore associated with increased oxidative stress, due to reduced tissue oxygen delivery (41) as well as reduced antioxidant effects. Furthermore, the membrane proteins of circulating erythrocytes are subject to AGE modification (42) that results in decreased red blood cell (RBC) deformability (43). This, in turn, may further reduce tissue oxygen delivery and contribute to a vicious circle of oxidative stress and AGE accumulation, as well as reducing RBC life span. Anemia is a common complication of CKD, and the effect of anemia on AGE accumulation in CKD may therefore be clinically important.

### Diabetes as a Determinant of Higher SAF

Hyperglycemia drives the nonenzymatic production of AGE by the Maillard reaction. Diabetes has been shown to be an independent determinant of higher SAF values in participants with and without CKD (3,22,29). We observed significantly higher SAF readings in those with diabetes compared with those without (Table 4), and confirmed diabetes as an important independent determinant of SAF in the multivariable analysis (Table 5). The importance of chronic hyperglycemia is illustrated by the observation that, among diabetic participants, chronological age, smoking history, and eGFR did not enter the equation as independent determinants. Since diabetes is the single most common cause of CKD in many countries, hyperglycemia is likely an important factor contributing to AGE formation in many patients with CKD.

### Age as a Determinant of Higher SAF

As expected, this cohort of patients with CKD stage 3 was predominantly elderly (mean age, 72.9 years). Chronological age has been shown, in many studies, to have a linear relationship with SAF until the age of 70 years (38,44–46). The very elderly may have a relatively lower SAF because higher SAF is associated with increased mortality (at least in patients with diabetes) and those surviving longer are therefore more likely to have a lower SAF (38). Age remained an independent determinant of increased SAF in our study and other studies of participants with CKD. These observations emphasize the importance of correcting all potential associations with SAF for age but also illustrate that CKD and factors associated with CKD contribute to AGE accumulation in addition to that observed with aging. Further studies will be required to evaluate the relationship between SAF, CKD, and age in younger populations.

### eGFR and Proteinuria as a Determinant of Higher SAF

Previous studies have demonstrated that as GFR declines, SAF increases (3,22,23,28), due largely to reduced

renal clearance of AGE (47) and increased production through dicarbonyl and oxidative stress. Nevertheless, much of the previous work was performed with participants who were already on renal replacement therapy or had developed macrovascular complications of diabetes. However, Tanaka *et al.* (29) found, in their cohort of pre-dialysis participants, that eGFR emerged as one of the strongest predictors of SAF and that SAF increased as CKD stage advanced. Even although all participants in our cohort had CKD stage 3, comparison of CKD stage 3A (eGFR = 45 to 59 ml/min per 1.73 m<sup>2</sup>) with CKD stage 3B (eGFR = 30 to 44 ml/min per 1.73 m<sup>2</sup>) showed significantly higher SAF in those with CKD stage 3B (3A = 2.7 AU versus 3B = 3.0 AU;  $P < 0.0001$ ), and eGFR remained an independent determinant of SAF in the multivariable analysis. In addition, we present a novel observation that proteinuria is an independent determinant of SAF. The mechanisms for this association remain unclear, but proteinuria has been identified as a powerful risk factor for CKD progression as well as CVE. Thus, it is possible that the mechanisms contributing to the development of proteinuria also provoke AGE formation and accumulation, or that AGE exacerbate proteinuria. A role for AGE in the pathogenesis of proteinuria is suggested by a study that observed a significant reduction in albuminuria after 2 weeks of a low AGE diet in 11 overweight and obese individuals (48).

#### Other Determinants of SAF in CKD

Enhanced statistical power resulting from the relatively large number of participants studied has afforded the opportunity to identify several other determinants of SAF in participants with CKD. Tobacco smoking has previously been shown to induce AGE formation and has been associated with SAF in participants without CKD (8). Measures of social deprivation (IMD score and educational status) have not previously been identified as determinants of SAF. We propose that dietary and other lifestyle factors, including smoking, that are associated with lower socioeconomic status may account for this association, but further investigation is required to explore this. These observations are important because factors such as diet and smoking are potentially modifiable. A recent study has reported a reduction in albuminuria and plasma levels of the proinflammatory cytokine monocyte chemoattractant protein-1 in overweight and obese participants after just 2 weeks of a low-AGE diet (48).

#### SAF as a Risk Factor in CKD

We have identified chronological age, hemoglobin, diabetes, and eGFR as the strongest independent determinants of SAF in participants with CKD stage 3. All of the independent determinants of SAF identified in this study have previously been identified as risk factors for CKD progression and/or future CVE. Thus, SAF may represent a composite risk factor reflecting the combined effect of multiple risk factors, and is attractive as a single, noninvasive measurement to predict renal and cardiovascular risk. In studies of patients with diabetes, SAF appears to represent such a risk factor and, after chronological age, was the best predictor of total and cardiovascular mortality. Fur-

thermore, SAF added predictive power to the UKPDS risk score for diabetic patients (23). The present analysis is limited by its cross-sectional nature, but the planned 10-year follow-up will allow comprehensive evaluation of SAF as a risk predictor. We concede that, although statistically significant, the magnitude of the contribution for each of the independent determinants of SAF was relatively modest. This suggests that multiple factors each make a small contribution to the accumulation of skin AGE. One limitation of current SAF measurement techniques is that they cannot be applied to participants with very dark skin or black skin; however, this applied only to the minority of participants (<1%). The manufacturers of the AGE Reader have advised that they are developing improvements to the software that will allow measurements in darker skin.

#### Conclusion

We have shown that tissue AGE, measured as SAF, is independently associated with multiple traditional and nontraditional risk factors for CKD progression and CVE in community-based participants with CKD stage 3. SAF measurement may therefore represent a clinically useful, noninvasive method for assessing renal and cardiovascular risk in participants with CKD. Planned follow-up for up to 10 years will evaluate this hypothesis in the RRID cohort.

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#### Disclosures

None.

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