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Skin autofluorescence, a non-invasive biomarker of advanced glycation end products, and its relation to radiographic and MRI based osteoarthritis

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SUMMARY

Objectives: Accumulation of advanced glycation end products (AGEs) in articular cartilage during aging has been proposed as a mechanism involved in the development of osteoarthritis (OA). Therefore, we investigated a cross-sectional relationship between skin AGEs, a biomarker for systemic AGEs accumulation, and OA.

Methods: Skin AGEs were estimated with the AGE Reader™ as skin autofluorescence (SAF). Knee and hip X-rays were scored according to Kellgren and Lawrence (KL) system. KL-sum score of all four joints was calculated per participant to assess severity of overall radiographic OA (ROA) including or excluding those with prosthesis. Knee MRI of tibiofemoral joint (TF_{MRI}) was assessed for cartilage loss. Sex-stratified regression models were performed after testing interaction with SAF.

Results: 2,153 participants were included for this cross-sectional analysis. In women (n = 1,206) for one unit increase in SAF, the KL-sum score increased by 1.15 (95% confidence interval = 1.00–1.33) but excluding women with prosthesis, there was no KL-sum score increase [0.96 (0.83–1.11)]. SAF was associated with higher prevalence of prosthesis [Odds ratio, OR = 1.67 (1.10–2.54)] but not with ROA [OR = 0.83 (0.61–1.14)] when compared to women with no ROA. In men (n = 947), there was inconclusive association between SAF and KL sum score or prosthesis.

For TF_{MRI} (n = 103 women), SAF was associated with higher prevalence of cartilage loss, full-thickness [OR = 5.44 (1.27–23.38)] and partial-thickness [OR = 1.45 (0.38–5.54)], when compared to participants with no cartilage loss.

Conclusion: Higher SAF in women was associated with higher prosthesis prevalence and a trend towards higher cartilage loss on MRI. Our data presents inconclusive results between SAF and ROA in both sexes.

Introduction

Pathological changes in articular cartilage with older age play a pivotal role in progression of osteoarthritis (OA). The exact underlying mechanisms by which aging increases the predisposition to OA has, as of yet, not been elucidated. During the last decade, a key mediator identified in the pathogenesis of aging-related diseases, such as OA, is chronic low grade inflammation[1,2] which, among other effects, is thought to trigger the formation of advanced glycation end-products (AGEs) in multiple tissues[3]. AGEs are a diverse cluster of compounds formed non-enzymatically on proteins when reducing sugars react to amino acid residues and undergo a series of biochemical modifications[4]. AGEs accumulation on collagen in articular cartilage alters extracellular matrix composition leading to stiffness which makes it prone to damage[5–7]. In addition, binding of AGEs to the receptor for AGEs (RAGE) on

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chondrocytes has been shown to induce oxidative and inflammatory stress thereby promoting cartilage matrix degradation[8]. Altogether, AGEs accumulation in long-lived articular cartilage proteins may be involved in OA pathogenesis.

Previous studies have used AGEs levels in serum or urine, such as pentosidine (PEN), Methylglyoxal-derived hydroimidazolone (MG-H1) and n-6-Carboxymethyllysine (CML), to study a relationship with OA. Serum PEN levels were higher in OA ($n = 38$) participants when compared to controls but there was no correlation of either serum or synovial fluid PEN with radiographic severity of knee OA[9]. Urinary PEN levels did not predict progressive knee cartilage loss obtained from MRI's in 127 participants with already established OA[10]. Higher levels of urinary PEN were found in persons with erosive hand OA compared to non-erosive OA and in those with more hand pain and stiffness[11]. These studies were heterogeneous with respect to AGEs estimated in various body fluids such as serum, urine or synovial fluid and type of AGE determined such as CML, MG-H1 or PEN. Serum and urine AGE levels have been shown to fluctuate based on e.g., diet and renal clearance[12,13] which might have obscured the relationship between AGEs and OA.

AGEs measured in tissues might represent long-term AGEs burden due to long half-life of AGEs bound to proteins in tissues[14], although detecting tissue AGEs is often non-feasible due to its invasive nature. A non-invasive and reproducible technique has been used since the last decade to estimate skin (tissue) AGEs with the help of an AGE reader™[15]. As skin AGEs are predominantly bound to skin collagen which has been estimated to have a half-life of ~14 years[16], skin AGEs are assumed to remain quite stable over time. Additionally, studies using human cadavers have shown that skin and cartilage pentosidine levels per milligram of collagen are moderately correlated ($R = 0.473$) to each other [11]. Therefore, we used non-invasive skin AGEs measurements as a proxy of cartilage AGEs accumulation over an extended period of time in order to study its relationship with OA.

In the present study, we aimed to investigate a potential relationship of skin AGEs with hip and knee osteoarthritis based on X-rays and with cartilage loss on knee MRI in participants from a population-based cohort.

Methods

Study population

This study was embedded within the Rotterdam Study (RS), an ongoing prospective population-based cohort study of Dutch individuals living in the Ommoord district of Rotterdam in the Netherlands[17]. In short, participants were included at three different points in time, namely in 1990, 2000 and 2006 and named RS-I, RS-II (≥55 years) and RS-III (≥45 years) sub-cohorts based on year of inclusion, respectively. After the inclusion visit, participants visited the research center every 4–6 years for follow-up examinations. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC. All participants provided written informed consent.

For this cross-sectional analysis, we included 2,153 participants from RS-I (6th follow-up, $n = 629$ or 29%), RS-II (4th, follow-up, $n = 959$ or 45%) and RS-III (2nd baseline visit, $n = 565$ or 26%) unless specified otherwise. Briefly, 2,196 out of 3,001 participants with skin autofluorescence (SAF) had data on the outcome i.e., knee or hip X-rays. Afterwards, we excluded only 43 participants with missing data on covariates including effective glomerular filtration rate (eGFR), body mass index (BMI), smoking and diabetes status (Fig. 1). No imputation was performed for this small subset of participants ($n = 43$) with missing data on covariates.

Skin autofluorescence

Skin AGEs were estimated using an AGE reader™ (DiagnOptics Technologies B.V., Groningen, The Netherlands) as SAF[18]. Participants were advised not to apply lotions or creams on the dominant arm for 2 days preceding SAF measurement. Briefly, AGE Reader illuminates a skin surface of ~4 cm^2 with a black light source, guarded against surrounding light. The AGE Reader uses an excitation light source between 300 and 420 nm (peak excitation ~350 nm) for illumination and measures emission in the 420–600 nm range. The measure of autofluorescence we apply is the average light intensity per nanometer in the range between 420 and 600 nm divided by the average light intensity per nanometer in the range between 300 and 420 nm (in arbitrary units [a.u.])[19,20]. We have chosen this ratio to correct for the influence on autofluorescence by light absorption due to, for example, skin pigmentation. An automated software in the AGE reader ensured the incorporation of skin reflectance values between 6 and 10% (corresponding to Fitzpatrick type V) in SAF values and exclusion of participants with skin reflectance under 6%[21]. Values were defined as outliers in SAF and excluded from the analysis if it exceed the scope of mean + 4SD; based on this 8 participants were excluded.

Plain radiographic evaluation of osteoarthritis

Within the participants with SAF measurements, knee and hip radiographs were available for 2,153 participants. Participants from the cohort RS-I (29%) and RS-II (45%) underwent X-rays on average 4–5 years before SAF measurements. RS-III (26%) had cross-sectional measurements of both SAF and X-rays of knee and hip joint. Knee radiographs were taken with the knee and patella in an extended and central position respectively[22]. Radiographs of the pelvis including hips were obtained when both feet were rotated 10° inward and the X-ray beam was centred on the umbilicus[22].

We defined OA according to the original Kellgren and Lawrence (KL) system[23,24] graded scale (0–4) based on features including joint space narrowing (JSN), osteophytes and sclerosis. Briefly, trained individuals assessed X-rays and labeled them as no, possible, definite or marked JSN and osteophytes and reported KL score per joint (see Supplementary Table 1)[24]. KL score of 0 or 1 was defined as absence of radiographic osteoarthritis (no ROA) and a KL score of 2–4 or a KL score of 5 (total joint replacement) as presence of radiographic osteoarthritis (ROA). KL score of 5 was included to take the most severe form of OA into account. KL scores were subsequently summed for right and left joints to form the hip or knee KL sum score (0–10) and for all four joints together Hip + Knee KL sum score (0–20) per participant.

MRI evaluation for knee joint osteoarthritis

103 women from the cohort RS-III (2nd follow-up) had both SAF measurements and knee MRI imaging[25]. MRI was performed on the knee joint using multi-sequence MRI protocol on a 1.5-T MRI scanner (Sigma Excite 2, General Electric Healthcare, Milwaukee, Wisconsin, USA), as reported elsewhere[25]. Briefly, MRIs were scored with the semi-quantitative MRI Osteoarthritis Knee Score (MOAKS)[26]. Two experienced readers were extensively trained by an highly experienced musculoskeletal radiologist, as described in a short report[27]. In short, the knee was divided into 14 sub-regions (medial/lateral patella, medial/lateral trochlea, medial/lateral central femur, medial/lateral posterior femur and medial (anterior, central and posterior) and lateral (anterior, central and posterior) tibia for scoring articular cartilage and bone marrow lesions (BML). Articular cartilage lesions were scored as partial or full thickness loss based on: (1) size of any cartilage loss (including partial and

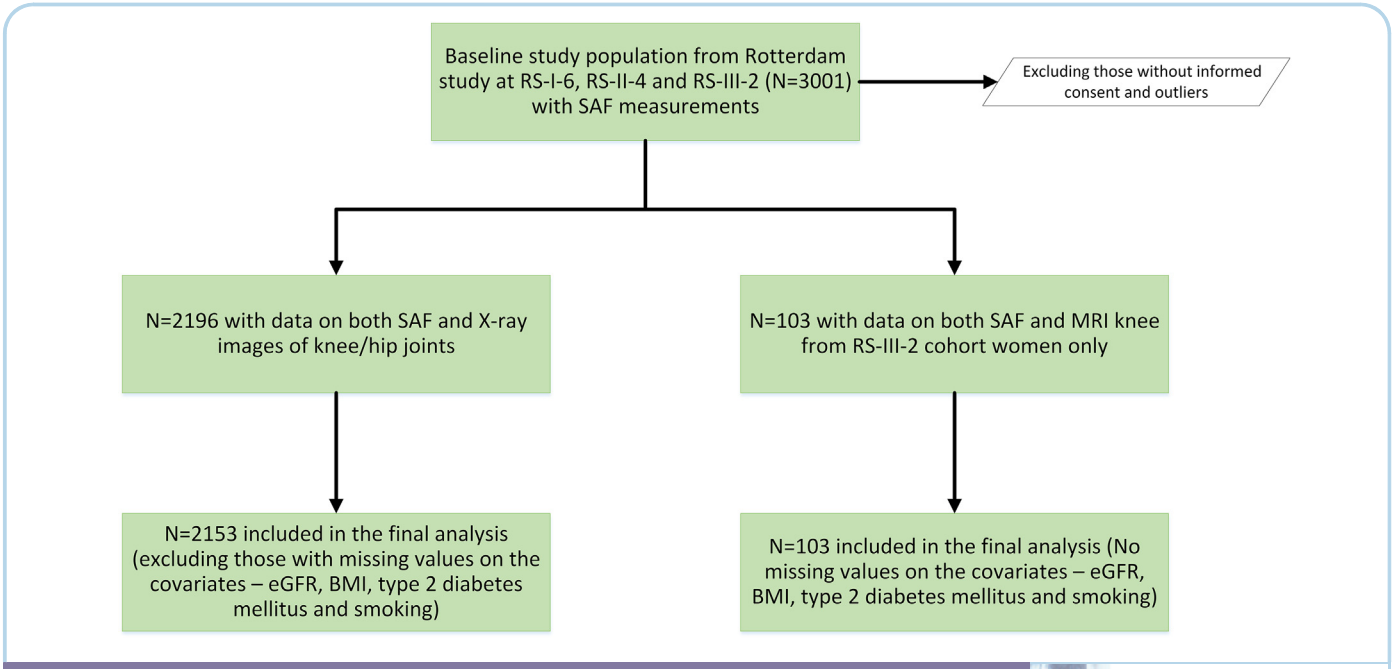

Fig. 1 Osteoarthritis and Cartilage

Flowchart of participant inclusion from the Rotterdam study. RS, Rotterdam study; SAF, skin autofluorescence; BMI, body mass index; MRI, magnetic resonance imaging; eGFR, effective glomerular filtration rate.

full-thickness loss) as a percentage of surface area as related to the size of each individual region; and (2) as a percentage full-thickness cartilage loss of the region (0 = none, 1 = <10% of region of cartilage surface area, 2 = 10–75% of region of cartilage surface area, 3 = >75% of region of cartilage surface area). Bone marrow lesions (BMLs) and cysts were scored based on the size relative to each subregion (0 = none, 1 = <33%, 2 = 33–66%, and 3 = >66%) and were considered to be present when grade ≥1. Osteophytes were scored based on size (0 = none, 1 = small, 2 = medium, 3 = large) in each of the twelve locations and were classified as definite when grade ≥2. For the present study we focus on the tibiofemoral joint as assessed in the conventional radiographs and therefore left out the patella and medial and lateral trochlear facet femur. The MRI definition for tibiofemoral OA ($TFOA_{MRI}$) was presence of a definite osteophyte and full thickness cartilage loss, or one of these features plus two of the following features: 1) subchondral BML or cyst 2) meniscal subluxation, maceration or degeneration 3) partial thickness cartilage loss 4) bone attrition[28].

Covariates

Height and weight were recorded in standing position at the research Centre without shoes and BMI was computed as weight in kilograms divided by height in meters squared. Smoking was obtained through self-reporting during home interviews and was classified as current, past or never smokers. Type 2 Diabetes Mellitus was defined by combining the information on antidiabetic medication use, fasting blood glucose levels and diagnosis in the GP registries. Serum creatinine and fasting glucose were measured through automated enzymatic method. Effective glomerular filtration rate (eGFR) was calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation using serum creatinine concentration, age and sex data[29].

Statistical analysis

We identified potential confounders in the relationship between exposure (SAF) and outcome (OA) based on literature evidence. We consistently adjusted for age, sex and RS cohorts in our model 1 and additionally for BMI[30], eGFR[31,32], smoking[33] and diabetes status[34] in our model 2 as covariates. During all the analyses, interaction terms were tested between SAF and diabetes status and sex in the multivariate fully adjusted models, and results were reported stratified where statistically significant (P for interaction ≤0.10 for each) interaction was found. Depending on the nature of outcome, different generalized linear models were used (1) KL sum score were not normally distributed (left-skewed with an overabundance of zeros as shown in Supplementary Fig. 1), we assessed its association with SAF using a zero-inflated negative binomial (ZINB) regression model, which can account for the non-normal distribution including overdispersion and zero-inflation (KL sum score: 0–10 for both knees or both hips and 0–20 per participant for combined hip + knee joints). We reported the results from the count model part of the ZINB in all our main analysis and from the zero-inflation model only in the supplementary section; (2) multinomial logistic regression when participants with no ROA (KL-score ≤1, coded as 0) were compared with those with ROA (KL-score ≥2 and ≤ 4, coded as 1) and those with prosthesis (coded as 2) assuming to represent the most severe OA cases; (3) Multinomial logistic regression was used to evaluate tibiofemoral knee MRI data (TF_{MRI}), when participants who had no cartilage loss (coded as 0) on TF_{MRI} were compared with those with partial (coded as 1) and full (coded as 2) thickness cartilage loss. Due to a small subgroup of women with MRI knee and relatively low number of events, we adjusted all the analyses on MRI data only for age and BMI in order to prevent overfitting of the model. For logistic regression, nonlinear association with OA was explored by performing

quintiles analyses and adding a quadratic term for SAF to the original model. However, for all analyses, a linear model had the best fit. All underlying assumptions were checked and met for the statistical models, whenever we proceeded with any analyses. All analyses were performed through IBM SPSS statistics 25 (version 25.0).

Results

Cohort profile based on availability of SAF and X-rays of hip and knee

Table I shows the demographic and radiographic characteristics of all included participants and also based on sex. Men had higher SAF values (2.55 ± 0.49 A.U. vs 2.31 ± 0.46 A.U.), lower mean KL score knee (1.19 ± 1.68 vs 1.61 ± 2.03), fewer participants with prevalent knee OA (18% vs 27.5%) and hip + knee prosthesis (6% vs 11%) than women. Age, BMI, eGFR, mean KL score hip and prevalence of hip OA did not differ between the two sexes. Supplementary Tables 2 and 3 shows the results of demographic and radiographic characteristics of study population based on age and sex adjusted SAF tertiles and based on no ROA, ROA and presence of prosthesis.

SAF and KL sum score knee and/or hip

First, we observed that higher KL sum score for hip + knee was associated with the higher values of SAF in all subjects [RM = 1.08 (0.97–1.20)] using ratio of means (RM) from the coefficients derived from the count model part of ZINB regression (Table II) with a *P*-value for interaction of 0.09 for SAF*sex. Therefore, we performed the rest of the analyses stratified in men and women (Supplementary Fig. 2 shows the distribution of SAF in men and women). One unit increase in SAF significantly increased mean hip + knee KL sum score with 15% [RM = 1.15 (1.00–1.33)] in women but no such increase was observed in men [RM = 1.00 (0.86–1.16)] in our adjusted models. This association between SAF and hip + knee KL sum score attenuated also in women [PR = 0.96 (0.83–1.11)] when participants with a hip or knee prosthesis were excluded. When we stratified the analysis according to knee and hip separately, we observed that for every unit increase in SAF, mean hip KL sum score increased by 31% [RM = 1.31 (0.97–1.77)] and mean knee KL sum score increased by 17% [RM = 1.17 (1.00–1.36)] in women. When participants with either a hip or knee prosthesis were excluded, the association between SAF and hip [RM = 0.90 (0.67–1.19)] or knee [PR = 1.02 (0.88–1.17)] KL sum score in women disappeared and become inconclusive. In men, no significant associations were found for hip or knee KL sum score (Table II). No coefficients from the zero-inflation model part of our ZINB model showed any relationship between increasing values of SAF and excess zero's for KL sum score i.e., no effect of excess zeroes on its relationship with SAF (Supplementary Table 4).

SAF and prevalent knee and/or hip prosthesis

Table III shows a comparison of participants without OA to those with ROA and those with a prosthesis (THP or TKP) with increasing values of SAF. In women, SAF was associated with higher prevalence of having a knee or hip prosthesis [Odds ratio, OR = 1.67 (1.10–2.54)] but not with presence of ROA [OR = 0.83 (0.61–1.14)] compared to those without OA. We repeated the same analysis separately for knee and hip joints. SAF showed a similar trend

	All	Men	Women	Women with MRI knee†
Cohort participants	2,153	947	1,206	103
Skin autofluorescence	2.43 ± 0.49	2.55 ± 0.49	2.31 ± 0.46$^{\#\#}$	2.17 ± 0.40**
BMI (kg/m^2)	27.6 ± 4.2	27.3 ± 3.4	27.5 ± 4.5	26.9 ± 3.9
Age (years)	74.9 ± 7.8	74.6 ± 7.4	74.5 ± 7.7	62.8 ± 2.48***
eGFR (ml/min/1.73m^2)	75.9 ± 14.2	75.7 ± 13.98	76.4 ± 13.6	86.7 ± 10.3**
Diabetes status (y/n)	297 (14%)	148 (16%)	149 (12%)$^{\#}$	5 (5%)**
Physical activity (n = 1,789)	42.9 (17–85)	42.0 (17.5–78)	43.8 (17–89)$^{\#}$	46.0 (53.7)*
Smoking status (y/n)				
Never	32%	19%	42%	34%
Past	55%	65%	47%	49%
Current	13%	15%	11%	17%
KL sum score hip + knee	2.39 ± 2.78	2.20 ± 2.56	2.53 ± 2.93$^{\#\#}$	NA
Hip or knee OA (y/n)	715 (33%)	276 (29%)	439 (36%)$^{\#\#\#}$	NA
Hip or knee prosthesis	185 (9%)	57 (6%)	128 (11%)$^{\#\#\#}$	NA
Hip KL sum score	0.96 ± 1.81	1.01 ± 1.71	0.93 ± 1.88	NA
Hip OA (y/n)	302 (14%)	133 (14%)	169 (14%)	NA
Hip prosthesis	112 (5%)	37 (4%)	75 (6%)$^{\#}$	NA
Knee KL sum score	1.42 ± 1.90	1.19 ± 1.68	1.61 ± 2.03	NA
Knee OA (y/n)	508 (23.5%)	174 (18%)	334 (27.5%)$^{\#}$	28 (27%)‡
Knee prosthesis	89 (4%)	25 (3%)	64 (5%)$^{\#}$	NA

SAF, skin autofluorescence; BMI, body mass index; MRI, magnetic resonance imaging; eGFR, effective glomerular filtration rate.
Depicted are mean ± standard deviation, median (interquartile range) and number (percentage).
$^{\#}$ or $^{*}P < 0.05$, $^{\#\#}$ or $^{**}P < 0.01$ and $^{\#\#\#}$ or $^{***}P < 0.001$. Significant differences between women with X-rays and MRI or men and women.
† Knee MRI's were only available for an all-female obese subgroup of the Rotterdam Study with SAF measurments (n = 103).
‡ Knee OA was based on Knee Osteoarthritis Scoring System (KOSS) on MRI tibiofemoral joint.

Table I

Osteoarthritis and Cartilage

General characteristics of Rotterdam study cohort with both skin autofluorescence (SAF) and hip and knee joint imaging data

KL sum scores	All		Men		Women	
	Estimate (95% confidence interval)	*P*-value	Estimate (95% confidence interval)	*P*-value	Estimate (95% confidence interval)	*P*-value
Including all participants *n* = 2,153						
HIP and KNEE	1.08 (0.97–1.20)	0.18	1.00 (0.86–1.16)	0.99	1.15 (1.00–1.33)	**0.05**
HIP ALONE	1.15 (0.96–1.38)	0.13	1.12 (0.86–1.46)	0.39	1.31 (0.97–1.77)	0.08
KNEE ALONE	1.08 (0.95–1.22)	0.25	0.89 (0.71–1.13)	0.35	1.17 (1.00–1.36)	**0.04**
Excluding participants with prosthesis *n* = 1,948						
HIP and KNEE	0.97 (0.87–1.08)	0.60	0.93 (0.79–1.09)	0.38	0.96 (0.83–1.11)	0.60
HIP ALONE	0.93 (0.77–1.12)	0.45	0.93 (0.73–1.19)	0.56	0.90 (0.67–1.19)	0.46
KNEE ALONE	0.98 (0.87–1.11)	0.79	0.86 (0.69–1.07)	0.18	1.02 (0.88–1.17)	0.83

Analyses were sex-stratified as *P*-value for interaction for SAF*sex = **0.09** for HIP and KNEE, **0.03** for HIP ALONE and **0.009** for KNEE ALONE.
All analyses were adjusted for age, sex, RS-cohorts, eGFR, BMI, diabetes and smoking status.
Note: Results for all zero-inflation model coefficients from ZINB did not show any relationship between increasing values of SAF and excess zeroes for KL sum score and are presented elsewhere (Supplementary Table 4).

Table II

Osteoarthritis and Cartilage

Zero-inflated negative binomial regression reporting count model coefficients for mean KL sum score hip and/or knee change with one unit increase in SAF values adjusting for potential confounders in participants from the Rotterdam study

Ternary outcome using participants with no ROA or prosthesis as reference		*N*	Men (*n* = 947)		Women (*n* = 1,206)	
			Estimate (95% confidence interval)	*P*-value	Estimate (95% confidence interval)	*P*-value
HIP and KNEE	No ROA	1,438	Reference		Reference	
	ROA	530	0.87 (0.62–1.22)	0.42	0.83 (0.61–1.14)	0.24
	THP + TKP	185	0.96 (0.53–1.71)	0.88	1.67 (1.10–2.54)	**0.02**
HIP ALONE	No ROA	1,850	Reference		Reference	
	ROA	191	0.97 (0.61–1.53)	0.90	0.88 (0.54–1.42)	0.59
	THP	112	1.28 (0.65–2.52)	0.48	1.39 (0.84–2.31)	0.20
KNEE ALONE	No ROA	1,648	Reference		Reference	
	ROA	416	0.85 (0.57–1.26)	0.40	0.92 (0.66–1.27)	0.59
	TKP	89	0.81 (0.33–2.06)	0.65	2.09 (1.19–2.67)	**0.01**

ROA, radiographic osteoarthritis; THP, total hip prosthesis; TKP, total knee prosthesis.

Table III

Osteoarthritis and Cartilage

Odds ratio of prevalent knee and/or hip prosthesis by an increase of one unit in SAF values adjusting for potential confounders in participants from the Rotterdam study

towards higher prevalence of having a hip prosthesis [OR = 1.39 (0.84–2.31)] and knee prosthesis [OR = 2.09 (1.19–2.67)] but not with presence of hip ROA [OR = 0.88 (0.54–1.42)] and knee ROA [OR = 0.92 (0.66–1.27)] in women. In men, there was no difference in the prevalence of ROA and hip and/or knee prosthesis with increasing values of SAF. Additional adjustment for physical activity and excluding T2D participants did not change the results (data not shown/Supplementary Table 5).

Cohort profile based on availability of SAF and MRI knee

If AGEs accumulation is causally related to increased risk of OA, a possible mechanism might be through crosslinking of AGEs between the collagen molecules in the extracellular matrix leading to stiffness of cartilage making it prone to degeneration[35,36]. Table I shows also the characteristic of 103 women with SAF and available knee MRI data. Subgroup of women with knee MRI were younger (62.8 ± 2.48 vs 74.5 ± 7.7 years), had lower SAF (2.17 ± 0.40 vs 2.31 ± 0.46 A.U.) and fewer participants with T2DM (5% vs 12%) as compared to women in total population (Supplementary Tables 6 and 7).

We observed that one unit increase in SAF was associated with higher prevalence of full thickness cartilage loss [OR = 5.44 (1.27–23.38)], but not with an increased prevalence of partial thickness cartilage loss [OR = 1.45 (0.38–5.54)] (Table IV). We also observed a trend towards higher prevalence of definite osteophytes

$n = 103$ with MRI knee data on TF joint	Odds ratio (95% Confidence interval)	*P*-value
No cartilage loss ($n = 65$ or 55.5%)	Reference	
Partial thickness cartilage loss ($n = 22$ or 29%)	1.45 (0.38–5.54)	0.58
Full thickness cartilage loss ($n = 16$ or 15.5%)	5.44 (1.27–23.38)	**0.02**
Other MRI abnormalities		
Definite osteophytes ($n = 24$ or 32%)	2.76 (0.92–8.23)	0.07
Tibio-femoral knee OA ($TFOA_{MRI}$)	2.12 (0.70–6.43)	0.18

All analyses were adjusted for age and BMI.

Table IV Osteoarthritis and Cartilage

Odds ratio of presence of individual MRI defects and tibio-femoral knee OA ($TFOA_{MRI}$) by an increase of one unit in SAF values adjusting for potential confounders in female participants from the Rotterdam study ($n = 103$)

[OR = 2.76 (0.92–8.23)] with each unit increase in SAF and a trend towards higher prevalence of $TFOA_{MRI}$ [OR = 2.12 (0.70–6.43)], but the associations were not significant, probably due to modest power.

Discussion

In this paper, we investigated the relationship of knee and hip X-ray based KL sum scores and knee MRI cartilage loss with SAF. High SAF in women was associated with higher prevalence of knee and hip prosthesis and with a trend towards higher prevalence of cartilage loss on MRI. High SAF was not associated with increasing severity and prevalence of knee and/or hip ROA in either sex when participants with prosthesis were excluded.

We observed an association between higher skin AGEs values and higher prevalence of knee or hip joint prosthesis, which is most likely equivalent to severe OA, in women only but we observed inconclusive results for SAF and severity or presence of ROA. In line with these findings, PEN, a prototype AGE, in skin biopsy of 300 human participants failed to predict progression of knee and hip OA over 5 years[37]. Cartilage AGEs in living participants have been assessed only in those undergoing surgery due to OA (equivalent to severe OA) in the absence of controls without OA or early stage OA which prevents us from understanding the course of AGEs accumulation in early stages of OA[38]. Pentosidine, an AGE also measured through SAF, has been shown to be present nearly 5 times higher in cartilage than in skin while there was a modest correlation between cartilage and skin PEN (R = 0.473) within paired macroscopically normal samples from cadavers[39]. This higher accumulation of AGEs in cartilage could be due to extremely low turnover rate of cartilage collagen[16]. This may partially explain the absence of relationship between SAF and OA severity as the rate of increase in cartilage AGEs might not be in proportion to increase in skin AGEs. However, our results suggest a relationship between SAF and risk of prosthesis although the possibility of reverse causation remains whereby a vicious cycle may start when chronic low grade inflammation in severe OA (prosthesis) leads to increase in AGEs formation and *vice versa*. Besides, low pain threshold is perhaps a risk factor for undergoing joint replacement but not for OA and whether accumulation of AGEs might be related to pain or other factors specific to OA that increase risk of prosthesis is unknown. Therefore, the relationship between SAF and presence of prosthesis could be confounded by the indication of joint replacement which has not been taken into account. Future studies measuring skin AGEs in early and late stage OA in a longitudinal fashion are needed to explore this further.

In a small subset of 103 women with available knee MRI, increasing SAF values were associated with a higher prevalence of cartilage loss although the confidence interval was very wide pointing towards an imprecise estimate. Increasing skin AGEs have been reported to be associated with greater JSN over 4 years on X-rays in men but not in women who were both at high risk of developing knee OA[40]. In contrast to these and our findings, no relationship was found between urinary PEN and any degree of cartilage loss on MRI knee during 30 months follow-up in 127 symptomatic OA participants at baseline[10]. It should be noted that urine AGEs represent a transient measurement which can fluctuate depending on diet, renal function or tissue turnover rate. In our study, increasing SAF values also showed a trend towards higher prevalence of definite osteophytes (moderate and severe) and $TFOA_{MRI}$. Whether this relationship between SAF and osteophytes on MRI is a consequence of overt cartilage loss needs to be further analyzed in a well powered population to study the relationship of skin AGEs with cartilage loss and osteophytes on MRI.

In our study, we found an association of increasing SAF with higher prevalence of hip or knee prosthesis in women but not in men and a trend towards increasing cartilage loss in a small subset of women with knee MRI. Women have been known to have a higher OA incidence especially after menopause[41] but sex differences in MRI based biomarkers and joint metabolism have rarely been studied and with varying results[42,43]. Absence of any association with SAF in men could partly be due to low power as they had significantly lower prevalence of OA and prostheses than women (Table I). Also, we had no knee MRI data on men to study cartilage loss. Our findings, however, may suggest a potential sex specific effect of AGEs on OA especially in women after menopause. This might be due to a state of chronic low-grade inflammation after menopause[44,45] which has potential for accelerated AGEs accumulation, however, a concrete mechanism still needs to be elucidated and our findings need to be replicated.

There are various mechanisms through which AGEs might deteriorate articular cartilage and increase predisposition to OA. *In vitro* studies showed both intra- and extracellular AGEs accumulation leads to endoplasmic reticulum stress and apoptosis in chondrocytes[46,47]. Immunostaining on cartilage sections illustrated increased expression of RAGE on chondrocytes from OA patients as compared to chondrocytes from young adults without OA[8]. Furthermore, young RAGE-knockout mice were shown to be partly protected from developing OA after knee destabilization surgery than controls[48]. AGEs lead to non-enzymatic cross-linking of collagen fibers in cartilage extracellular matrix and decreasing collagen degradability by the matrix metalloproteinases/cathepsin K either by masking the cleavage site or by preventing unfolding of collagen molecules due to an extra AGEs cross-link[49,50]. Both mechanisms could increase the risk of OA by stiffening cartilage and making it vulnerable to damage. In summary, there are several potential mechanisms by which AGE accumulation in articular cartilage could influence osteoarthritis development. Our data on knee MRI reinforces that the influence of AGEs on OA is primarily mediated through cartilage loss.

Our study has several strengths. It is a comprehensive study of relationship of tissue AGEs and several OA outcomes based on X-ray and MRI images in the two main joints affected by OA, the knees and hips. It is embedded in a large RS cohort study of the general population which allowed for adjustment for several potential confounders. Our study has also several limitations. As mentioned, AGE levels in skin may not adequately reflect AGEs in OA cartilage although human cadaver studies have shown that cartilage and skin PEN were correlated (R = 0.473) in macroscopically normal cartilage[51]. Although a cluster of AGE moieties are known, the AGE reader™ detects only fluorescent AGEs as SAF. Residual confounding could not be excluded despite adjusting for currently know most important risk factors for AGEs accumulation. In our study, broad confidence intervals of odds ratio between radiographic osteoarthritis and SAF in men (but also in women) were observed. This indicates that we still have uncertain knowledge about the true effect and that more information is needed. Cartilage thickness on MRI data and its relationship to SAF should be interpreted with caution due to small sample size and sparse prevalence of outcome.

In conclusion, skin AGEs showed an association with end stage OA, represented by the presence of a prosthesis, and a trend towards an association with full thickness cartilage loss on knee MRI in a small subset of women. Based on our findings, skin AGEs do not appear to have potential to be used as a marker of severity of ROA in hip and knee joints. This may be related to a different half-life of AGEs in cartilage than skin and a moderate correlation between the two. Longitudinal data in well-powered cohorts on skin AGEs and cartilage thickness are needed to confirm and expand our preliminary findings.

Data availability

Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided. Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Disclaimers

No.

Authors' roles

KW, IKS and MCZ designed the study. IKS, CJB, DS and JBM provided essential materials. KW assessed and IKS (statistically) analyzed the data. KW, IKS and MCZ interpreted the results. KW created the figures and tables. KW, IKS, CJB and MCZ drafted the manuscript. All authors provided contribution to intellectual content, acquisition and interpretation of data and critically revising the manuscript. All authors have read and revised the manuscript and approved the final submitted version.

Conflict of interest

All authors declare to have no disclosures and conflicts of interest relevant to the current study.

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Supplementary data

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