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Clinical Research Article

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Skin Autofluorescence, a Noninvasive Biomarker for Advanced Glycation End-products, Is Associated With Sarcopenia

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Abstract

Background: Accumulation of advanced glycation end-products (AGEs) in skeletal muscle has been implicated in development of sarcopenia.
Aim: To obtain further insight in the pathophysiology of sarcopenia, we studied its relationship with skin AGEs in the general population.
Methods: In a cross-sectional analysis, 2744 participants of northern European background, mean age 74.1 years, were included from the Rotterdam Study. Skin AGEs were measured as skin autofluorescence (SAF) using AGE Reader™, appendicular skeletal mass index (ASMI) using insight dual-energy X-ray absorptiometry, hand grip strength (HGS) using a hydraulic hand dynamometer, and, in a subgroup, gait speed (GS) measured on an electronic walkway (n = 2080). We defined probable sarcopenia (low HGS) and confirmed sarcopenia (low HGS and low ASMI) based on the European Working Group on Sarcopenia in Older People (EWGSOP2) revised criteria cutoffs. Multivariate linear and logistic regression were performed adjusting for age, sex, body fat percentage, height, renal function, diabetes, and smoking status.
Results: The prevalence of low ASMI was 7.7%; probable sarcopenia, 24%, slow GS, 3%; and confirmed sarcopenia, 3.5%. SAF was inversely associated with ASMI [β –0.062 (95% CI –0.092, –0.032)], HGS [β –0.051 (95% CI –0.075, –0.026)], and GS [β –0.074 (95% CI –0.116, –0.033)]. A 1-unit increase in SAF was associated with higher odds of probable sarcopenia [odds ratio (OR) 1.36 (95% CI 1.09, 1.68)] and confirmed sarcopenia [OR 2.01 (95% CI 1.33, 3.06)].
Conclusion: Higher skin AGEs are associated with higher sarcopenia prevalence. We call for future longitudinal studies to explore the role of SAF as a potential biomarker of sarcopenia.

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Primary or age-related sarcopenia has been officially recognized in 2016 as an independent disease (1). Advanced glycation end-products (AGEs) have been implicated in the pathogenesis of primary sarcopenia and other chronic diseases related to aging (2). AGEs constitute a diverse group of compounds formed spontaneously, nonenzymatically, and irreversibly on proteins after their initial glycation (3). Accumulation of AGEs in skeletal muscles has been shown to be associated with reduced skeletal muscle strength and mass in independent human studies (4-6). AGEs can form cross-links between collagen fibers in intramuscular connective tissue, which leads to increased stiffness and reduced elasticity (7). Additionally, binding of AGEs to receptor for advanced glycation end-products (RAGE) induces inflammation and causes endothelial dysfunction, leading to loss of myocytes and muscle regenerating satellite cells (8,9).

Previous studies have reported an inverse association between AGEs measured in serum and skeletal muscle mass, strength, or function. High levels of circulating carboxymethyl-lysine (CML), a noncross-linking AGE, have been associated with slow walking speed in 944 adults (10) and low hand grip strength (HGS) in moderate to severely disabled 559 community-dwelling women including only those ≥65years (11). Tanaka et al reported a significant negative correlation between circulating serum pentosidine, a cross-linking AGE, and SMI in 133 postmenopausal women with type 2 diabetes (T2DM) (12). Yet, these studies used small cohorts with specific population subgroups and serum AGEs whose levels could vary on daily basis depending on, for example, dietary intake or renal function.

In contrast to serum AGEs, tissue AGEs bound to proteins with longer half-life are assumed to represent the long-term burden of AGEs. A promising, reproducible, and noninvasive technique to estimate tissue AGEs is skin autofluorescence (SAF) measured with the help of an AGE Reader. The technique has been validated by comparing SAF to the total and independent AGE levels (CML and pentosidine) determined in skin biopsies (13). Additionally, AGEs have never been studied in relation to sarcopenia—a clinical entity combining those with reduced muscle mass and strength. Thus, using skin AGEs might be a reflection of muscle burden of AGEs and a potential biomarker of sarcopenia in elderly.

The aim of this study was to investigate a potential relationship between SAF and sarcopenia and with its individual components—skeletal muscle mass, HGS, and gait speed (GS)—in a Dutch cohort comprised of middle-age and elderly.

Methods

The Rotterdam Study (RS) was approved by the Medical Ethics Committee of the Erasmus Medical Centre and by the Ministry of Health, Welfare, and Sport of the Netherlands, implementing the Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study). Written informed consent has been obtained from all participants.

Study Population

For our cross-sectional analysis, we included participants from an ongoing prospective, large population-based cohort study: the RS. A detailed description of the design and methodology of this study has been described elsewhere (14). Participants aged ≥55 years residing in the Ommoord district of Rotterdam were invited in 1989-1990 for their first evaluation (RS-I). The cohort was extended in 2000-2001 by inviting new inhabitants aged ≥55years (R-II) and in 2006-07, with new residents aged ≥45 years (RS-III). All participants were invited for follow-up visits every 3to 4 years after their inclusion.

In RS, 3029 participants had measurements on SAF and skeletal muscle related parameters at the sixth follow-up visit of RS-I (2014-2016), the fourth follow-up visit of RS-II (2014-2016), and second follow-up visit of RS-III (2011-2013). We excluded individuals with no informed consent for follow-up; outliers of SAF values; and missing data on appendicular lean mass, HGS, creatinine value, smoking, and diabetes status [Supplementary Figure 2 (15)]. The present study comprised, thus, a total of 2744 participants for whom the included parameters were available from RS-I (n = 670), RS-II (n = 988), and RS-III (n = 1086) in the aforementioned visits. In a subset of total participants (n = 2080), GS was also assessed, which we use consistently for comparison and exploratory analysis.

Measurement of Skin Autofluorescence

SAF was measured using an AGE Reader^{TM} (DiagnOptics Technologies BV, Groningen, The Netherlands) as described elsewhere (13). Participants were advised not to apply lotions or creams on the dominant arm for 2 days preceding SAF measurement. A small area of forearm skin, approximately 4 cm^2, was illuminated with an excitation light source from the AGE Reader with a peak wavelength of 370 nm. The AGE Reader estimates AGEs based on the emission and reflection spectrum of skin converted through a software program into numerical values reported in

arbitrary units. In practice, the AGE Reader can be used up to skin color Fitzpatrick type V and is suitable for most racial groups, except part of subjects of sub-Saharan African or Afro-American descent. An automated software in the AGE Reader ensured the incorporation of skin reflectance values between 6% and 10% (corresponds to Fitzpatrick type V) in SAF values and exclusion of participants with skin reflectance under 6% (16).

Assessment of Skeletal Muscle Parameters

Muscle mass

Total body composition was analyzed by insight dual-energy X-ray absorptiometry total body fan-beam densitometer (GE Lunar Corp., Madison, WI, USA). enCORE software was employed to analyze the scans using an algorithm which divides the total body into regions of interest, such as trunk, arms, and legs. Appendicular lean mass is computed by summing up the lean mass from the arms and legs. Appendicular skeletal muscle index (ASMI) was defined by dividing appendicular lean mass by squared body height (kg/m^2). Low muscle mass was defined as ASMI < 7 kg/m^2 for males and < 5.5 kg/m^2 for females using European Working Group on Sarcopenia in Older People (EWGSOP2) revised definition (17).

Muscle strength

HGS was assessed using a hydraulic hand dynamometer (Fabrication Enterprises Inc., White Plains, NY, USA). Measurements were performed in the nondominant hand, and the maximum value out of 3 was recorded as HGS. Weak muscle strength was defined differentially across sexes as weak HGS < 27kg for men and <16 kg for women using EWGSOP2 criteria.

Muscle performance

Gait was evaluated using a 5.79-m long walkway (GAITRite Platinum; CIR systems, Sparta, NJ: 4.88-m active area; 120-Hz sampling rate). The reliability and validity of this device have been previously established (18,19). Low physical performance was defined as having a slow GS < 0.8 m/s for both men and women using EWGSOP2 criteria.

Assessment of Sarcopenia

Sarcopenia was categorized according to the EWGSOP2 revised definition (Fig. 1). Probable sarcopenia is defined as having only weak HGS, confirmed sarcopenia as having both weak HGS and low ASMI simultaneously, and severe sarcopenia as having weak HGS, low ASMI, and slow GS at the same time.

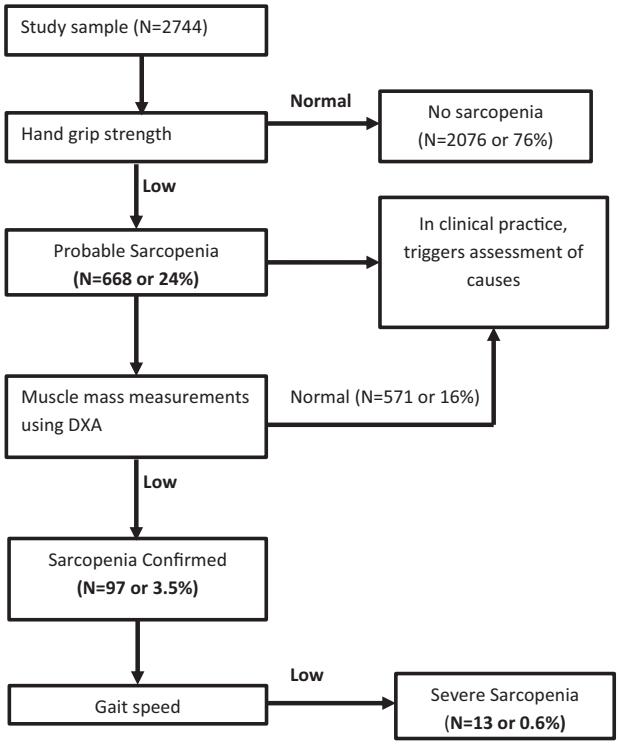

Figure 1. Flowchart of participants classification using algorithm proposed in European Working Group of Sarcopenia in Older People 2 (EWGSOP2) revised criteria.

Assessment of Covariates

Smoking status was obtained through self-reporting by the participants recorded during home interviews by the research staff. It was classified as current, past, or never-smokers. Height was recorded in standing position at the research center without shoes. An adaptive version of the LASA Study Physical Activity Questionnaire was used to calculate metabolic equivalent of task values as a representative of physical activity levels (20). T2DM was defined by combining the information on fasting blood glucose levels, antidiabetic medication use, or diagnosis in the general physician registries (21). Serum creatinine and serum fasting glucose were measured through an automated enzymatic method. Estimated glomerular filtration rate (eGFR) was calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation using serum creatinine concentration, age, and sex data (22).

Statistical Analysis

We performed statistical analysis using IBM SPSS Statistics 25 (version 25.0). Normality of the residuals of the exposure and predictors of interest was determined using histograms and Shapiro-Wilk test. Data are presented as mean ± SD in case of normal distribution and median (interquartile range) in case of nonnormal distribution.

Means of continuous variables among groups were compared by using independent samples *t* test or analysis of variance when the variable was normally distributed or Mann-Whitney Wilcoxon test when a nonnormal distribution was assumed. Chi-square test was adopted to compare the frequencies of categorical variables based on their distribution across groups.

Potential confounders were identified based on literature to assess the relationship between SAF (exposure) and skeletal muscle parameters (outcomes). Model 1 included age, sex, and RS cohorts, and Model 2 included Model 1 plus eGFR, smoking status, diabetes status, body fat percentage (total fat mass/body weight * 100), and height as covariates to study these relationships. We used body fat percentage instead of body mass index (BMI) as our outcome of appendicular lean mass is a component of BMI. Hence, we avoid potential collider bias caused by adjusting for BMI including lean mass.

Multiple linear regression analysis was performed to investigate the associations between SAF and continuous outcomes of ASMI, HGS, and GS including the aforementioned confounders. Multiple logistic regression was performed to investigate whether SAF was associated with probable sarcopenia, confirmed sarcopenia, or severe sarcopenia. A nonlinear association was explored by performing quintiles analyses and adding a quadratic term to the original model. However, for all analyses, a linear model had the best fit. Despite this, we also studied SAF in quartiles to allow for a comparison with the existing literature, especially with the large Japanese cohort using SAF. Participants were categorized by sex-stratified, age-adjusted SAF quartiles as SAF and sarcopenia are highly influenced by sex and increasing age (23). We calculated residuals for every individual from linear regression model in which SAF was the dependent variable and age was the independent variable. This was done separately for males and females to take potential sex differences into account.

We repeated all analyses in the subgroup with complete data on GS (n = 2080) and compared this to the results of the whole cohort (N = 2744) to evaluate any potential impact from missing data in GS. Physical activity is an important risk factor for development of sarcopenia. In a subgroup of the population with data on physical activity available, we further adjusted the associations for physical activity in addition to Model 2. In addition, SAF levels have been reported to be higher in subjects with T2DM (24), smokers, and in those with renal dysfunction. Therefore, interaction terms were included for testing the effect modification between SAF and diabetes, smoking status, eGFR, and sex in the multivariate models. Results were reported separately where statistically significant (*P* for interaction ≤ 0.10 for each) interaction was found and by diabetes status no matter a significant interaction present because it is the most important risk factor for AGEs accumulation.

Results

Demographic and skeletal muscle specific characteristics of our cohort are summarized in Table 1 and compared to the subgroup with available data on GS. In the total population, the median age on the date of SAF measurement was 74 years (interquartile range: 66.9-81.1) with 43.8% males and 13.4% subjects with T2DM. The mean (±SD) value of SAF for those 2744 participants was 2.38 ± 0.48 arbitrary units and mean BMI was 27.5 ± 4.2 kg/m^2. Figure 1 summarizes the application of EWGSOP2 algorithm on our study population, which is used in clinical practice to classify individuals in different sarcopenia groups as probable, confirmed, and severe. The prevalence (95% CI) of low ASMI was 7% (6.2%, 7.9%); of weak HGS, 24% (23%-26%);

Table 1. Demographic and skeletal muscle-specific characteristics of the total population and subgroup with complete data on gait speed

	Total population (N = 2744)	Subgroup with data on gait speed (n = 2080)
Age, years	74 (14.2)	73.4 (14.7)
Sex, males	1187 (44)	932 (45)
BMI, kg/m^2	27.5 ± 4.18	27.4 ± 4.03
Diabetes	363 (13)	271 (13)
SAF, AU	2.38 ± 0.48	2.37 ± 0.48
Physical activity, MET h/week	42.8 (67.1)	44.9 (67.9)
Smoking status		
Current	411 (15)	323(16)
Past	1444(53)	1093(53)
Never	855(31.5)	646 (31)
Total fat mass, kg	28.19 ± 8.560	27.96 ± 8.40
Total lean mass, kg	46.06 ± 9.159	46.38 ± 9.04
Total body BMD, g/cm^2	1.09 ± 0.14	1.07 ± 0.14
Hand grip strength, kg	26.59 ± 10.09	27.07 ± 10.09
Appendicular lean mass, kg	21.26 ± 4.87	21.46 ± 4.82
ASMI, kg/m2	7.37 ± 1.12	7.40 ± 1.10
Low ASMI	209 (8)	145 (7.0)
Weak HGS	668 (24)	479 (23)
Confirmed Sarcopenia	97 (3.5)	65 (3.1)
Gait speed, cm/s	NA	120.31 ± 18.94
Slow gait speed	NA	61 (2.9)
Severe sarcopenia	NA	13 (0.6)

Data are expressed as mean ± SD, median (interquartile range) or n (%).
Abbreviations: ASMI, appendicular skeletal muscle index; AU, arbitrary unit; BMD, bone mineral density; BMI, body mass index; HGS, hand grip strength; MET h/week, metabolic equivalent hours per weeks; NA, not applicable; SAF, skin autofluorescence.

and of confirmed sarcopenia, 3.5% (2.9%, 4.3%). In the subgroup (n = 2080) with data on the GS, there were no major differences in the demographic, clinical, and skeletal muscle parameters compared to the entire study population for this analysis. The prevalence of low GS was 2.9% (2.%3, 3.9%) and of severe sarcopenia 0.6% (0.3, 1.1%).

SAF values were significantly higher in males than females (2.50 ± 0.49 *vs* 2.30 ± 0.46, $P < 0.0001$). SAF and sarcopenia are be highly influenced by aging. Hence, we made sex-stratified, age-adjusted SAF quartiles, and Table 2 shows the characteristics of our study participants in these quartiles. There was a trend toward increasing number of subjects with T2DM ($P < 0.0001$) and current smokers ($P < 0.0001$) from the first to the fourth SAF quartile. The highest quartile of SAF (Q4) contained a significantly higher number of individuals with weak HGS, low ASMI, and confirmed sarcopenia in comparison with the lower quartiles. This trend was not significant for low GS and severe sarcopenia ($P = 0.14$ and $P = 0.16$, respectively).

Linear Regression Analysis Between Skin Autofluorescence and Skeletal Muscle-related Parameters

The multivariate associations between SAF and individual components that sum up to the confirmed or severe sarcopenia in our cohort are shown in Table 3. SAF was significantly and negatively associated with HGS after adjusting for age, sex, and RS cohorts in Model 1 [β −0.059 (95% CI −0.083, −0.035), $P = 3 \times 10^{-6}$], which attenuated slightly after adjusting for other confounders in Model 2 [ie, eGFR, smoking, diabetes status, body fat percentage, and height; Model 2: β −0.051 (95% CI −0.075, −0.026), $P = 2 \times 10^{-5}$]. Similarly, after adjusting for the potential confounders in Model 2, SAF had an inverse association with ASMI [β −0.062 (95% CI −0.092, −0.032), $P = 3 \times 10^{-5}$] and a negative association with GS [β −0.074 (95% CI −0.116, −0.033), $P = 3 \times 10^{-4}$].

Logistic regression analysis between SAF and confirmed sarcopenia, probable sarcopenia, low ASMI, and slow GS.

Table 3. Linear regression analysis showing associations of skin autofluorescence levels with hand grip strength, appendicular skeletal muscle index and gait speed

	Standardized coefficient, β (95% CI) [P-value]	
	Model 1[a]	**Model 2**[b]
Hand grip strength	−0.059 (−0.083, −0.035) [**3×10^{-6}**]	−0.051 (−0.075, −0.026) [**2×10^{-5}**]
Appendicular skeletal muscle index	−0.042 (−0.072, −0.011) [**0.003**]	−0.062 (−0.092, −0.032) [**3×10^{-5}**]
Gait speed	−0.106 (−0.149, −0.065) [**1×10^{-5}**]	−0.074 (−0.116, −0.033) [**3×10^{-4}**]

[a]Model 1: Skin autofluorescence plus age, sex, and Rotterdam Study cohorts.
[b]Model 2: Model 1 plus estimated glomerular filtration rate, smoking, diabetes status, body fat percentage, and height.

Table 2. Demographic and skeletal muscle specific characteristics of participants according to sex-stratified, age-adjusted skin autofluorescence quartiles

	Q1 (n = 688)	Q2 (n = 683)	Q3 (n = 688)	Q4 (n = 685)	*P*-value for trend
SAF, AU	1.88 ± 0.22	2.19 ± 0.18	2.48 ± 0.19	3.01 ± 0.36	NA
Age, years	74.8 (14.3)	72.6 (15.2)	72.9 (14.3)	74.6 (13.4)	**<0.01**
Males	302 (44)	296 (43)	300 (44)	299 (44)	NA
Smoking					**<0.0001**
Current	10	13.5	14	23	
Past	53	54	58	49	
Never	38	33	28	28	
Diabetes	49 (7)	62 (9)	92 (13)	127 (18.5)	**<0.0001**
Hand grip strength, kg	26.6 ± 9.8	27.5 ± 10.3	26.7 ± 10.4	25.5 ± 9.8	
Weak hand grip strength	19	17	19	22	**0.07**
Appendicular skeletal muscle index, kg/m^2	7.32 ± 1.04	7.45 ± 1.14	7.43 ± 1.17	7.27 ± 1.11	**0.009**
Low ASMI	7	7	6	10.5	**0.01**
Confirmed sarcopenia	3.1	3.0	2.6	5.5	**0.04**
Gait speed, cm/sec	120.7 ± 0.80	122.9 ± 0.81	120.7 ± 0.84	116.7 ± 0.86	**<0.001**
Slow Gait speed	3	2	3	4	0.14
Severe sarcopenia	0.7	0.4	0.2	1.2	0.16

Data are expressed as mean ± SD, median (interquartile range), n (%), or %.
Abbreviations: ASMI, appendicular skeletal muscle index; AU, arbitrary unit; BMD, bone mineral density; BMI, body mass index; HGS, hand grip strength; MET h/week, metabolic equivalent hours per weeks; N/A, not applicable; SAF, Skin autofluorescence;

Table 4 and Figure 2 show the results of logistic regression analysis depicting the association between SAF as a continuous variable as well as in sex-stratified, age-adjusted quartiles with confirmed sarcopenia and its components. Higher SAF value as continuous variable was associated with the presence of confirmed sarcopenia [OR 2.01 (95% CI 1.33, 3.06)]. Compared to the lowest quartile of SAF (Q1) as a reference, the highest SAF quartile (Q4) was significantly associated with higher prevalence of sarcopenia [OR 2.03 (95% CI 1.12, 3.70)] after adjusting for the previously mentioned confounders in Model 2. Considering the individual sarcopenia components, continuous SAF was significantly associated with weak HGS [OR 1.36 (95% CI 1.09, 1.68)], and the odds of weak HGS increased from the second to fourth quartile [Q2: OR 1.09 (95% CI 0.81, 1.48); Q3: OR 1.21 (95% CI 0.89, 1.63); Q4: OR 1.27 (95% CI 0.95, 1.71)] when the first quartile (Q1) was used as reference. We also found that a 1-unit increase in SAF was significantly associated with higher odds of low ASMI in Model 2 [OR (2.02, 95% CI 1.47, 2.76)] and participants in the highest SAF quartile (Q4) had significantly higher odds for low ASMI [OR 1.75 (95% CI 1.16, 2.63)] when compared to the Q1.

Severe sarcopenia could only be analyzed in individuals with data available on GS (n = 2080) (Table 5). Continuous SAF showed a nonsignificant association [OR 1.63 (95% CI 0.96, 2.79)] with slow GS (n = 61/2080, 2.9%) in Model 2. Similarly, there was a nonsignificant trend from the second to fourth quartile in terms of increasing odds for low GS [Q2: OR 1.72 (95% CI 0.31, 1.69); Q3: OR 1.14 (95% CI 1.54, 2.52),; Q4: OR 1.48 (0.73, 2.99)] compared to the first SAF quartile. Only 13/2080 (0.6%) fulfilled the criteria for severe sarcopenia based on EWGSOP2 definition, which was extremely low in our cohort. Table 5 shows logistic regression analysis between SAF, slow GS, and severe sarcopenia. We observed a nonsignificant relationship between SAF and severe sarcopenia [OR 2.47 (95% CI 0.86, 7.13), $P = 0.10$] when adjusted for age and sex, which attenuated further in Model 2 [OR 1.97 (95% CI 0.70, 5.52), $P = 0.20$].

Sensitivity Analysis

Subgroup analysis for association of SAF with confirmed sarcopenia in those with data on gait speed and physical activity

The OR of SAF for confirmed sarcopenia attenuated slightly from 2.01 (95% CI 1.33, 3.06) to 1.98 (95% CI 1.17, 3.35) when our Model 2 was compared between the whole cohort and the subset with complete data on GS [Supplementary Table 1A (15)].

Of the participants included from our cohort, 2448 have also data on physical activity [Supplementary Table 1B (15)]. We observed no difference in the effect size for the association between SAF and confirmed sarcopenia

Table 4. Binary logistic regression models using confirmed sarcopenia and its components as outcomes and skin autofluorescence as a predictor variable (n = 2744)

Skin autofluorescence (continuous)	Model 1[a]		Model 2[b]	
	Odds ratio (95% CI)	*P*-value	Odds ratio (95% CI)	*P*-value
Confirmed sarcopenia, n = 97	2.06 (1.3, 3.09)	**0.001**	2.01 (1.33, 3.06)	**0.001**
Weak hand grip strength (probable sarcopenia), n = 668	1.41 (1.15, 1.73)	**0.001**	1.36 (1.09, 1.68)	**0.005**
Low appendicular skeletal muscle index, n = 209	1.87 (1.40, 2.50)	**0.00005**	2.02 (1.47, 2.76)	**0.00001**

[a]Model 1: SAF plus age, sex, Rotterdam Study cohorts.
[b]Model 2: Model 1 plus estimated glomerular filtration rate, smoking, diabetes status, body fat percentage, and height.

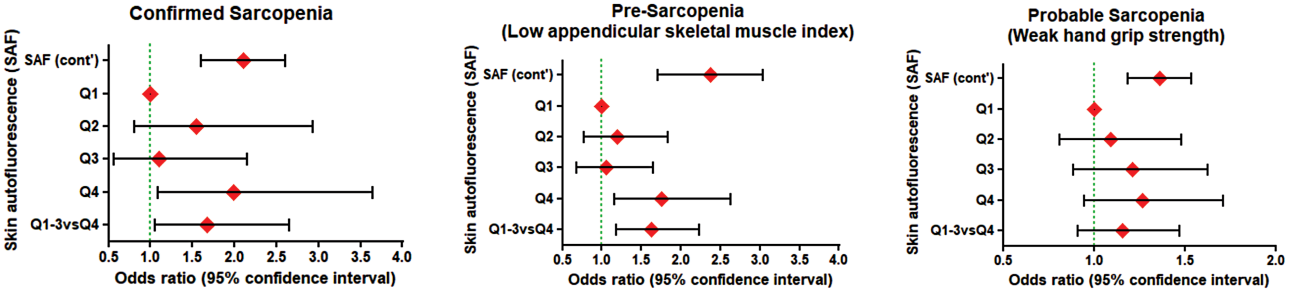

Figure 2. Odds ratio for confirmed sarcopenia and its components associated with SAF as a continuous variable (cont') and sex-stratified, age-adjusted SAF quartiles (Q). Abbreviations: SAF (cont') = SAF as a continuous variable; Q1-3 = lower 3 SAF quartiles; Q4 = highest SAF quartile.

with or without including physical activity in our Model 2 [1.77 (95% CI 1.14, 2.75) and 1.77 (95% CI 1.14, 2.75), respectively].

Stratified analysis based on diabetes, sex, and smoking status for association of SAF with skeletal muscle-related parameters

Subgroup analysis was further performed in predefined strata of T2DM and non-T2DM and if the *P*-value of interaction term was ≤0.20 for a particular interaction with SAF [Supplementary Figure 1 (15)]. For all skeletal muscle related parameters, SAF has a significant interaction with sex and smoking status (all $P < 0.15$). First, the association of SAF with confirmed sarcopenia was statistically significant only in males but not in females although there is an overlap between the CIs. Second, stratification by diabetes showed a statistically significant association only in individuals without T2DM between SAF and skeletal muscle parameters but again overlapping CIs with T2DM. However, HGS was significantly and negatively associated with SAF in both individuals with and without T2DM (data not shown). Lastly, stratification by smoking status showed a significant association between SAF and confirmed sarcopenia in current and ex-smokers but not in never-smokers. In a subgroup analysis after removing those with confirmed sarcopenia, the association of SAF with ASMI and HGS remained similar [Supplementary Figure 3 (15)].

Subgroup analysis using multinomial logistic regression analysis in subjects with complete data on all 3 muscle parameters

Table 6 shows the results of multinomial logistic regression analysis in 2080 subjects with complete data on all 3 muscle parameters depicting the association between sarcopenia components as the quaternary dependent variable and SAF as the continuous independent variable. For every 1-unit increase in SAF, ascending odds were observed starting off from the presence of any 2 component of sarcopenia [1.43 (95% CI 1.11, 1.85)], to any 2 components [2.07 (95% CI 1.24, 3.46)], and to all 3 components [2.50 (95% CI 0.88-7.14)].

Discussion

In this study, we observed an association between skin AGEs and confirmed sarcopenia, based on a clinically operated definition, independent of confounders including physical activity in our population based cohort. Skin AGEs showed an inverse association with individual components contributing to sarcopenia (ie, muscle mass, strength, and GS) after adjusting for potential confounders. Overall, our findings

Table 5. Binary logistic regression models using slow gait speed and severe sarcopenia as outcomes and SAF as a predictor variable in a subset of our cohort (n = 2080)

Skin autofluorescence (continuous)	Model 1[a]		Model 2[b]	
	Odds ratio (95% CI)	*P*-value	Odds ratio (95% CI)	*P*-value
Slow gait speed (n = 61)	1.65 (0.97, 2.79)	0.06	1.63 (0.96, 2.79)	0.07
Severe sarcopenia (n = 13)	2.47 (0.86, 7.13)	0.10	2.09 (0.73, 5.97)	0.17

[a]Model 1: Skin autofluorescence plus age, sex, and Rotterdam Study cohorts.
[b]Model 2: Model 1 plus estimated glomerular filtration rate, smoking, diabetes status, body fat percentage, and height.

Table 6. Multinomial logistic regression between skin autofluorescence as exposure and sarcopenia components (weak hand grip strength, slow gait speed, and low appendicular lean mass) as ordinal variable in outcome when compared to normal individuals (N = 2080)

Skin autofluorescence component(s)	n	Model 1[a]		Model 2[b]	
		OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Normal	1514	Reference		Reference	
Any 1 component	473	1.44 (1.12, 1.85)	**0.004**	1.43 (1.11, 1.85)	**0.006**
Any 2 components	80	1.98 (1.21, 3.25)	**0.006**	2.07 (1.24, 3.46)	**0.005**
All 3 components	13	3.07 (1.05, 8.99)	**0.04**	2.50 (0.88, 7.14)	*0.09*
Any of the 3 components	566	1.54 (1.26, 1.87)	**0.00002**	1.55 (1.26, 1.90)	**0.0003**

[a]Model 1: Skin autofluorescence plus age, sex, and Rotterdam Study cohorts.
[b]Model 2: Model 1 plus estimated glomerular filtration rate, smoking, diabetes status, body fat percentage, and height.

suggest that skin AGEs seem to be a good representative of muscle AGEs, and their role as a potential biomarker should be explored in longitudinal settings.

We observed a significant association of higher skin AGEs not only with lower skeletal muscle index but also with weaker HGS, thus, with sarcopenia at a preclinical stage in non-T2DM subjects. Even after removing those with confirmed sarcopenia, the association of SAF with independent skeletal muscle parameters remained similar, which points to a potential role of SAF at a preclinical stage (ie, even before sarcopenia develops) [Supplementary Figure 3 (15)]. Our findings are consistent with the findings of a large Japanese cohort (n = 9203) in which SAF was inversely associated with ASMI and, in a subpopulation (n = 1934), negatively with weak HGS (25). Of relevance, a few subtle differences between the 2 cohorts exist. First, we measured SAF on the dominant forearm using an AGE Reader whereas in the Japanese cohort, skin AGEs were measured on the finger of a dominant forearm using the AGE sensor RQ-AG01J. To the best of our knowledge, no studies have so far performed a comparison of these 2 AGE measurements. Second, we measured muscle mass using insight dual-energy X-ray absorptiometry and the Japanese study, by using bioelectrical impedance analyzer with similar cutoffs, but the prevalence of low SMI was much higher in Japanese (19%) than ours (7.7%) despite a lower mean age of 58 years in Japanese *vs* 74 years in our cohort. Despite these differences, a role of AGEs in muscle mass and strength appears consistent between the studies.

In a subset of our population, we observed a negative linear association between SAF and GS. In line with our findings, participants in the highest quartile of the serum CML were found at higher risk of slow walking speed (n = 944) (10)and severe walking disability (GS < 0.4 m/s; n = 394) (26) in adults aged ≥65 years. In contrast, the highest quartile of SAF was not significantly associated with slow GS in a Japanese cohort (n = 1934) with a mean 68 years of age (25). We did not observe an association between SAF and severe sarcopenia (defined as low ASMI, weak HGS, and slow GS), which has a prevalence of only 0.6% in our cohort. One of the reasons for this low prevalence could be missing data on GS. In a sensitivity analyses, participants with missing data on GS showed higher prevalence of low ASMI, weak HGS, and confirmed sarcopenia (data not shown) and thus might have a tendency to omit GS measurements. Alternatively, one could hypothesize that selective survival might also play a role in low sarcopenia prevalence in our cohort, as sarcopenia has been associated with increased hospitalization and mortality rates in the elderly community-dwelling individuals (27,28). Future longitudinal and intervention studies are needed to explore the potential relationship between AGEs and severity of sarcopenia.

We did not observe an association between skin AGEs and skeletal muscle mass in 363 subjects with T2DM, but of interest, the negative relation with muscle strength was significant. Our findings are in line with a recent study showing a higher proportion of subjects with T2DM having low HGS despite no difference in sarcopenia prevalence between subjects with or without T2DM (29). In contrast to our findings, a recent small-scale study in 166 Japanese subjects with T2DM (mean age 63 years) found a significant association between SAF and low SMI (30). Earlier, serum AGEs had also been negatively associated with reduced muscle mass in 133 postmenopausal women with T2DM (12). A close comparison of these studies also yielded differences in sarcopenia prevalence, mean SAF values, and ethnicity, which could be a potential explanation of not observing an association with muscle mass in our cohort. Second, whether skin AGEs are a representative of muscle AGEs in subjects with T2DM remains complex due to their bidirectional relationship; that is, skin AGEs relating to incident T2DM and T2DM increase skin AGEs formation. Lastly, Alt et al compared the levels of AGEs corrected for protein in muscle and skin of rats and showed that in diabetic rats there was a less robust relative increase in AGEs in myofibrils and total muscle proteins than in skin collagen when compared to age-matched nondiabetic rats (31). This might be an additional potential explanation of absence of the association between skin AGEs and muscle mass in subjects with T2DM. In the absence of data for direct comparison between skin and muscle AGEs in T2DM, future research on this topic is essential.

After stratification based on sex and smoking status, there was a significant relationship of SAF with confirmed sarcopenia in males and smokers and a similar trend in females and never-smokers, although statistically nonsignificant. This nonsignificant association could partly be explained by unexpected lower prevalence of confirmed sarcopenia in females than in males (1.5% *vs* 6%) and a lower number of never-smokers and thus low power, although the direction of associations were in the same direction in all subgroups. Additionally, it is worth mentioning that the prevalence of sarcopenia, using new EWGSOP2 criteria, is much lower than reported in other cohorts of European origin, especially in women (32, 33), and replication of our findings in such populations might increase strength of the associations. Future prospective studies are needed to investigate the potential sex- and smoking-based differences in skin AGEs accumulation in relation to muscle health within well-powered cohorts.

AGEs have been implicated in the pathophysiology of sarcopenia through several different mechanisms including

cross-linking between the collagen molecules (7); binding to the RAGE, which leads to activation of inflammatory and oxidative stress pathways; and endothelial cell dysfunction in muscle blood vessels (4,34). Furthermore, satellite cells (ie, quiescent cells responsible for regeneration of muscles) isolated from aged individuals showed higher expression of RAGE ligand, S100B, which has been known to inhibit myoblast differentiation and reduce muscle mass. AGE-modified proteins have been found to be resistant to degradation through ubiquitin-proteasome pathways leading to age-related functional decline in muscle function (35). Lastly, an intervention study in healthy mice fed on high AGE diet for 16 weeks showed higher CML accumulation in muscles and reduced skeletal muscle mass and strength than those fed a low-AGE diet (36). Taken together, our study combined with these findings point toward a potential clinically relevant role for AGEs as a risk factor for sarcopenia by reducing both muscle mass and strength independently.

Although sarcopenia has been recognized in 2016 as an independent diagnostic code in *International Statistical Classification of Diseases and Related Health Problems* (ICD-10-CM), the diagnosis of sarcopenia in geriatric population could not be established in a uniform manner (1,37). Thus, clinically there is now a need for the establishment of measurable risk factors predicting the development of sarcopenia. We presume that SAF is a long-term representative of body AGE burden based on long half-life of skin collagen–bound AGEs (38). Thus, SAF might be used as a potential predictor in addition to known risk factors such as age.

Our study has multiple strengths, one of which is a well-characterized, high-quality, and harmonized data collection in the RS cohorts. Limitations include cross-sectional nature of our analysis and inclusion of only Caucasian subjects. Although we cannot be sure that the amount of AGEs in skin is representative for AGEs in muscle or bone, it is has been found that skin collagen has a long half-life of 14.8 years (38), and collagen turnover is a major determinant of AGEs accumulation in tissues. This may points toward relatively stable values of skin AGEs over a longer period of time. We did not correct for multiple testing, but we had 1 clear primary hypothesis and an exploratory subgroup or component analysis were performed where individual comparison-wise error rate (α) was taken into account (39,40). We could neither exclude residual confounding despite inclusion of the most important biological confounders nor the potential impact of survival bias in our analyses in this aging population.

To conclude, this study demonstrates a positive association between skin AGEs and confirmed sarcopenia in middle-age and elderly RS cohort. We also found that higher SAF levels are independently and inversely associated with HGS, GS, and ASMI independently of the potential confounders. Future research with a longitudinal study design is important to explore the potential of SAF in predicting sarcopenia in a clinical setting. On a large scale such as in primary care, implementation of noninvasive SAF measurements can be an efficient solution to screen elderly for the risk of sarcopenia once a causal association and a good predictive capability has been established.

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References

1. Vellas B, Fielding RA, Bens C, et al. Implications of ICD-10 for Sarcopenia Clinical Practice and Clinical Trials: report by the International Conference on Frailty and Sarcopenia Research Task Force. *J Frailty Aging*. 2018;7(1):2-9.
2. Chen JH, Lin X, Bu C, Zhang X. Role of advanced glycation end products in mobility and considerations in possible dietary and nutritional intervention strategies. *Nutr Metab (Lond)*. 2018;**15**:72.
3. Singh R, Barden A, Mori T, Beilin L. Advanced glycation end-products: a review. *Diabetologia*. 2001;**44**(2):129-146.

4. Riuzzi F, Sorci G, Sagheddu R, Chiappalupi S, Salvadori L, Donato R. RAGE in the pathophysiology of skeletal muscle. *J Cachexia Sarcopenia Muscle*. 2018;**9**(7):1213-1234.
5. Riuzzi F, Sorci G, Beccafico S, Donato R. S100B engages RAGE or bFGF/FGFR1 in myoblasts depending on its own concentration and myoblast density: implications for muscle regeneration. *PLoS One*. 2012;**7**(1):e28700.
6. Riuzzi F, Sorci G, Donato R. The amphoterin (HMGB1)/receptor for advanced glycation end products (RAGE) pair modulates myoblast proliferation, apoptosis, adhesiveness, migration, and invasiveness. Functional inactivation of RAGE in L6 myoblasts results in tumor formation in vivo. *J Biol Chem*. 2006;**281**(12):8242-8253.
7. Haus JM, Carrithers JA, Trappe SW, Trappe TA. Collagen, cross-linking, and advanced glycation end products in aging human skeletal muscle. *J Appl Physiol (1985)*. 2007;**103**(6):2068-2076.
8. Chiu CY, Yang RS, Sheu ML, et al. Advanced glycation end-products induce skeletal muscle atrophy and dysfunction in diabetic mice via a RAGE-mediated, AMPK-down-regulated, Akt pathway. *J Pathol*. 2016;**238**(3):470-482.
9. Riuzzi F, Sorci G, Sagheddu R, Donato R. HMGB1-RAGE regulates muscle satellite cell homeostasis through p38-MAPK- and myogenin-dependent repression of Pax7 transcription. *J Cell Sci*. 2012;**125**(Pt 6):1440-1454.
10. Semba RD, Bandinelli S, Sun K, Guralnik JM, Ferrucci L. Relationship of an advanced glycation end product, plasma carboxymethyl-lysine, with slow walking speed in older adults: the InCHIANTI study. *Eur J Appl Physiol*. 2010;**108**(1):191-195.
11. Dalal M, Ferrucci L, Sun K, Beck J, Fried LP, Semba RD. Elevated serum advanced glycation end products and poor grip strength in older community-dwelling women. *J Gerontol A Biol Sci Med Sci*. 2009;**64**(1):132-137.
12. Tanaka K, Kanazawa I, Sugimoto T. Elevated serum pentosidine and decreased serum IGF-I levels are associated with loss of muscle mass in postmenopausal women with type 2 diabetes mellitus. *Exp Clin Endocrinol Diabetes*. 2016;**124**(3):163-166.
13. Meerwaldt R, Links T, Graaff R, et al. Simple noninvasive measurement of skin autofluorescence. *Ann N Y Acad Sci*. 2005;**1043**:290-298.
14. Ikram MA, Brusselle G, Ghanbari M, et al. Objectives, design and main findings until 2020 from the Rotterdam Study. *Eur J Epidemiol*. 2020;**35**(5):483-517.
15. Waqas K, Chen J, Trajanoska K, et al. Supplementary data for: "Skin autofluorescence, a non-invasive biomarker for advanced glycation end-products, is associated with sarcopenia. DANS: Easy. Uploaded May 7, 2021. https://doi.org/10.17026/dans-x54-kyfd
16. Koetsier M, Nur E, Chunmao H, et al. Skin color independent assessment of aging using skin autofluorescence. *Opt Express*. 2010;**18**(14):14416-14429.
17. Cruz-Jentoft AJ, Bahat G, Bauer J, et al. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing*. 2019;**48**(1):16-31.
18. Menz HB, Latt MD, Tiedemann A, Mun San Kwan M, Lord SR. Reliability of the GAITRite walkway system for the quantification of temporo-spatial parameters of gait in young and older people. *Gait Posture*. 2004;**20**(1):20-25.
19. Webster KE, Wittwer JE, Feller JA. Validity of the GAITRite walkway system for the measurement of averaged and individual step parameters of gait. *Gait Posture*. 2005;**22**(4):317-321.
20. Stel VS, Smit JH, Pluijm SM, Visser M, Deeg DJ, Lips P. Comparison of the LASA Physical Activity Questionnaire with a 7-day diary and pedometer. *J Clin Epidemiol*. 2004;**57**(3):252-258.
21. Ligthart S, van Herpt TT, Leening MJ, et al. Lifetime risk of developing impaired glucose metabolism and eventual progression from prediabetes to type 2 diabetes: a prospective cohort study. *Lancet Diabetes Endocrinol*. 2016;**4**(1):44-51.
22. Hofman A, Brusselle GG, Darwish Murad S, et al. The Rotterdam Study: 2016 objectives and design update. *Eur J Epidemiol*. 2015;**30**(8):661-708.
23. van Waateringe RP, Slagter SN, van der Klauw MM, et al. Lifestyle and clinical determinants of skin autofluorescence in a population-based cohort study. *Eur J Clin Invest*. 2016;**46**(5):481-490.
24. Mesinovic J, Zengin A, De Courten B, Ebeling PR, Scott D. Sarcopenia and type 2 diabetes mellitus: a bidirectional relationship. *Diabetes Metab Syndr Obes*. 2019;**12**:1057-1072.
25. Tabara Y, Ikezoe T, Yamanaka M, et al. Advanced glycation end product accumulation is associated with low skeletal muscle mass, weak muscle strength, and reduced bone density: the Nagahama study. *J Gerontol A Biol Sci Med Sci*. 2019;74(9):1446-1453.
26. Sun K, Semba RD, Fried LP, Schaumberg DA, Ferrucci L, Varadhan R. Elevated serum carboxymethyl-lysine, an advanced glycation end product, predicts severe walking disability in older women: the Women's Health and Aging Study I. *J Aging Res*. 2012;**2012**:586385.
27. Liu P, Hao Q, Hai S, Wang H, Cao L, Dong B. Sarcopenia as a predictor of all-cause mortality among community-dwelling older people: a systematic review and meta-analysis. *Maturitas*. 2017;**103**:16-22.
28. Zhang X, Zhang W, Wang C, Tao W, Dou Q, Yang Y. Sarcopenia as a predictor of hospitalization among older people: a systematic review and meta-analysis. *BMC Geriatr*. 2018;**18**(1):188.
29. Churilov I, Churilov L, Brock K, Murphy D, MacIsaac RJ, Ekinci EI. Sarcopenia is associated with reduced function on admission to rehabilitation in patients with diabetes. *J Clin Endocrinol Metab*. 2021;**106**(2):e687-e695.
30. Mori H, Kuroda A, Ishizu M, et al. Association of accumulated advanced glycation end-products with a high prevalence of sarcopenia and dynapenia in patients with type 2 diabetes. *J Diabetes Investig*. 2019;**10**(5):1332-1340.
31. Alt N, Carson JA, Alderson NL, et al. Chemical modification of muscle protein in diabetes. *Arch Biochem Biophys*. 2004;**425**(2):200-206.
32. Ethgen O, Beaudart C, Buckinx F, Bruyère O, Reginster JY. The future prevalence of sarcopenia in Europe: a claim for public health action. *Calcif Tissue Int*. 2017;**100**(3):229-234.
33. Guillamón-Escudero C, Diago-Galmés A, Tenías-Burillo JM, Soriano JM, Fernández-Garrido JJ. Prevalence of sarcopenia in community-dwelling older adults in Valencia, Spain. *Int J Environ Res Public Health*. 2020;**17**(23).
34. Baig MH, Jan AT, Rabbani G, et al. Methylglyoxal and advanced glycation end products: insight of the regulatory machinery

affecting the myogenic program and of its modulation by natural compounds. *Sci Rep*. 2017;**7**(1):5916.

35. Grillari J, Katinger H, Voglauer R. Aging and the ubiquitinome: traditional and non-traditional functions of ubiquitin in aging cells and tissues. *Exp Gerontol*. 2006;**41**(11):1067-1079.
36. Egawa T, Tsuda S, Goto A, et al. Potential involvement of dietary advanced glycation end products in impairment of skeletal muscle growth and muscle contractile function in mice. *Br J Nutr*. 2017;**117**(1):21-29.
37. Falcon LJ, Harris-Love MO. Sarcopenia and the New ICD-10-CM Code: screening, staging, and diagnosis considerations. *Fed Pract*. 2017;**34**(7):24-32.
38. Verzijl N, DeGroot J, Thorpe SR, et al. Effect of collagen turnover on the accumulation of advanced glycation end products. *J Biol Chem*. 2000;**275**(50):39027-39031.
39. Bender R, Lange S. Adjusting for multiple testing—when and how? *J Clin Epidemiol*. 2001;**54**(4):343-349.
40. Noble WS. How does multiple testing correction work? *Nat Biotechnol*. 2009;**27**(12):1135-1137.