Measures of lung function and their relationship with advanced glycation end-products

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ABSTRACT

Background: Advanced glycation end-products (AGEs) have been implicated in the pathophysiology of chronic obstructive pulmonary disease (COPD). However, the association between AGE accumulation in the skin measured by skin autofluorescence (SAF) and lung function in healthy subjects has not been explored in detail. We use a population-based study of 50–64-year-olds to assess spirometry, diffusing capacity of the lung for carbon monoxide (D_{LCO}) and impulse oscillometry (IOS) in relation to SAF.

Methods: Participants with information on SAF, lung function and potential confounding variables were included from the Swedish Cardiopulmonary Bioimage Study (SCAPIS) cohort (spirometry, n=4111; D_{LCO}, n=3889; IOS, n=3970). Linear regression was used to assess changes in lung function (as measured by spirometry (forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC) and FEV_1/FVC), D_{LCO} and IOS (resistance measured at 5 (R_5) and 20 Hz (R_{20}), R_5-R_{20}, area of reactance, reactance measured at 5 Hz (X_5), and resonant frequency)) by a 1-SD increase in SAF.

Results: FEV_1, FVC and D_{LCO} were significantly and inversely associated with SAF after adjustment for potential confounding factors (per 1-SD increase in SAF: FEV_1 −0.03 L (95% CI −0.04–−0.02 L), p<0.001; FVC −0.03 L (95% CI −0.05–−0.02 L), p<0.001; D_{LCO} −0.07 mmol·min^{-1}·kPa^{-1} (95% CI −0.11–−0.03 mmol·min^{-1}·kPa^{-1}), p<0.001). This association was also found in nonsmokers and in non-COPD subjects. Pulmonary reactance (X_5) but not pulmonary resistance (R_5, R_{20} and R_5-R_{20}) was significantly associated with SAF (per 1-SD increase in SAF: X_5 −0.001 kPa·L^{-1}·s (95% CI −0.003–0.00 kPa·L^{-1}·s), p=0.042), which was mirrored in non-COPD patients but not in current nonsmokers.

Conclusions: AGE accumulation, as measured by SAF, is significantly associated with lung function decrements indicative of changes in the lung parenchyma

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Introduction

Advanced glycation end-products (AGEs) are a group of compounds formed by a nonenzymatic reaction between reducing sugars (such as glucose) and amino acids in proteins, lipids or nucleic acids [1]. This glycation process is thought to be part of normal ageing and is known as the Maillard reaction where intermediate products, such as Schiff bases and Amadori products (*e.g.* glycated haemoglobin (HbA1c)), are formed, subsequently resulting in the formation of AGEs. AGEs are also formed by alternative pathways from dicarbonyl compounds that are a result of auto-oxidation and degradation products of glucose [2]. The presence and accumulation of AGEs can be measured by both circulating plasma AGEs or skin AGEs as measured by skin autofluorescence (SAF). The formation of AGEs is thought to be accelerated in chronic hyperglycaemia and oxidative stress [2, 3] and as such, it is well known that AGE accumulation is associated with diabetes mellitus (DM) and its complications [1, 4, 5], including cardiovascular disease (CVD) [6–8], renal disease [4, 9] and dementia [10, 11].

Plasma AGEs and SAF are thought to be increased in patients with chronic obstructive pulmonary disease (COPD) [12–15]. This relationship has been present independently of smoking, DM, CVD or renal disease [13]. SAF has also been found to be associated with a lower ratio of forced expiratory volume in 1 s (FEV_1) to forced vital capacity (FVC) in patients with COPD [13]. A recent study assessed whether SAF was associated with lung function in those with normal spirometry [16]. It was found that the FEV_1/FVC ratio was negatively associated with SAF only in the elderly age group with normal spirometry. Studies assessing diffusing capacity of the lung for carbon monoxide (D_{LCO}) and SAF have found conflicting results. A significant correlation has been found between D_{LCO} and SAF in a population consisting of COPD patients and healthy controls [17]. However, another study found no significant correlation between plasma AGEs or SAF and D_{LCO} in patients with COPD [12].

Furthermore, the receptor for advanced glycation end-products (RAGE), which is highly expressed in the lung under normal physiological conditions [18], has been implicated in COPD development [14], COPD severity [19] and its progression [20], along with impaired spirometry and D_{LCO} measurements [21]. The ligation and activation of this receptor is thought to increase an inflammatory response [22] *via* various pathways, including NF-κB, which is responsible for chronic inflammation in many conditions. Furthermore, blockage of RAGE has also been implicated as having a protective function in COPD/ emphysema [23]. *AGER* is the gene that encodes RAGE, and has additionally been implicated in the susceptibility to lung function decline [24] and susceptibility to developing COPD [25]. The soluble receptor for advanced glycation end-products (sRAGE) is thought to act as a "decoy" receptor with a potential protective mechanism in which it prevents the ligation of RAGE with AGEs by clearance of circulating AGEs. The levels of sRAGE are reduced in COPD patients compared to heathy controls [26], which further reinforces a pathophysiological link between AGEs and COPD.

Although there has been much evidence to suggest a pathophysiological link between AGEs or SAF and COPD, there has been sparse literature on how changes in SAF can potentially affect lung function. To our best knowledge, there have not been any studies assessing the associations between SAF and impulse oscillometry (IOS) measurements. Measurements such as D_{LCO} and IOS can give additional important information on the nature of respiratory pathology that may be present, and can potentially provide an indication of early disease that is not yet measurable by simple spirometry. The aim of the present study was to assess the association between lung function (as measured by spirometry, D_{LCO} and IOS) and SAF using middle-aged subjects enrolled in a large population-based study in Sweden, the Swedish Cardiopulmonary Bioimage Study (SCAPIS).

Methods

Study population

SCAPIS is a national, multicentre, population-based study formed through collaboration between six Swedish universities (Gothenburg, Linköping, Malmö/Lund, Stockholm, Umeå and Uppsala). The project was initiated as a joint national effort to prevent CVD and COPD by improving the risk prediction of cardiopulmonary conditions using updated knowledge relevant to today's pattern of risk factors [27]. Nationwide, 30 154 men and women participated. From the population of Malmö, 6251 individuals took part in the study and the participation rate in Malmö was 53%. Study participants took part in a comprehensive questionnaire, biochemistry, extensive physical examinations, lung function tests and imaging as previously described [27]. The study was approved by the Regional Ethical Review Board in Umeå. The study participants gave their written and informed consent.

Baseline examinations

Height was measured without footwear to the nearest centimetre. Weight was measured in light indoor clothing and without footwear on a digital scale. Estimated glomerular filtration rate (eGFR) was calculated

according to the Lund–Malmö equation [28]. Venous blood samples for immediate laboratory analysis of C-reactive protein (CRP), plasma glucose and HbA1c were taken after an overnight fast ($\geqslant 8$ h). Smoking status (current, ex- or never-smoker) and pack-year history (estimated from the tobacco consumption of cigarettes, cigars and pipe tobacco) were ascertained from questionnaire responses. Prevalent DM was defined as either self-reported physician-diagnosed DM or a new diagnosis of DM at baseline. Prevalent COPD was defined as self-reported physician-diagnosed COPD, chronic bronchitis or emphysema at baseline.

Lung function measurement

Dynamic spirometry (Jaeger MasterScreen PFT; Carefusion, Hoechberg, Germany) was performed 15 min after bronchodilation using 400 µg salbutamol with subjects in the sitting position and wearing a nose clip. FEV_1 and FVC were obtained according to the American Thoracic Society (ATS) and European Respiratory Society (ERS) standards [29, 30]. Reference values by HEDENSTRÖM and co-workers [31, 32] were used for the calculation of FEV_1 % predicted and FVC % predicted.

D_{LCO} was measured using a single-breath carbon monoxide diffusion test (Jaeger MasterScreen PFT) according to ATS/ERS standards, and was performed in a sitting position and wearing a nose clip. D_{LCO} and alveolar volume (V_A) were calculated using the single-breath D_{LCO} test with helium as the tracer gas. The two measures used for further statistical analysis were D_{LCO} and the carbon monoxide transfer coefficient (D_{LCO}/V_A). European reference values were used for the calculation of D_{LCO} % predicted and D_{LCO}/V_A % predicted [33].

IOS was used for measurements for the Malmö study participants in the SCAPIS cohort (Jaeger MasterScreen PFT). Reactance and resistance were expressed in kPa·L^{-1}·s (area of reactance (A_X) in kPa·L^{-1}) and resonant frequency (f_{res}) in Hz. Examination with IOS gives a measure of respiratory impedance that is the sum of the forces that oppose pressure impulses [34] (respiratory resistance and respiratory reactance), providing information on the mechanical properties of both the central and peripheral airways [35]. It has advantages over spirometry in that no complex respiratory manoeuvre is required for measurement and it has greater sensitivity to detect peripheral airway obstruction (small airways) than spirometry [35]. Resistance at 5 Hz (R_5) is considered to be a measure of total respiratory resistance, while resistance at 20 Hz (R_{20}) reflects mainly the resistance of large airways. Therefore, the difference (R_5-R_{20}) is considered to reflect more resistance in peripheral airway or ventilatory heterogeneity due to peripheral airways obstruction. Respiratory reactance is the phase shift of the input pressure signal, and consists of elastance and inertance [34]; it gives an overall measure of the respiratory system "stiffness". In COPD, there is a shift to more negative values for reactance. Reference equations by SCHULZ et al. [36] were used to calculate % predicted values for IOS measurements in this cohort.

AGE measurement

SAF (the assessment of AGE products in the skin using ultraviolet (UV) light) was performed for SCAPIS study participants in Malmö only (n=4644) using an AGE reader (AGE Reader-mu Connect; DiagnOptics, Groningen, the Netherlands). A skin area of 2 cm^2 on the forearm (free from scarring, tattoos and birthmarks) was illuminated with UV light (300–420 nm) and the light emissions from the subject's skin were registered by a spectrometer [37]. The mean of three acceptable values was taken as the final recorded result reported in arbitrary units (AU). The procedure was noninvasive and took $\leqslant 12$ s to complete.

Analysis of data

All analyses were carried out using SPSS version 24 (IBM Corp., Armonk, NY, USA). Subjects were included in the analysis if information on all key variables was complete: age, sex, height, weight, smoking status, pack-years, eGFR, prevalent DM, HbA1c, plasma glucose and CRP. Any potential data errors of SAF or lung function measures were then excluded (SAF, n=2; D_{LCO}/V_A, n=7; IOS measurements (R_5, n=1; reactance at 5 Hz (X_5), n=1; f_{res}, n=2; A_X values <0 excluded, n=1; R_5-R_{20} values < -0.02 excluded, n=64). Potential confounding variables with skewed distributions were log-transformed (HbA1c, plasma glucose and CRP). Three final study populations were used: subjects with information on relevant baseline examinations and either spirometry (n=4111), D_{LCO} (n=3889) or IOS measures (n=3970).

Linear regression was used to establish the change in the lung function measures per 1-SD increase in SAF (unstandardised β-coefficient with 95% CI) with two models for adjustment for potential confounding variables. For the baseline table analyses, lung function variables with skewed distributions are presented as median values (% predicted values for R_5, R_5-R_{20}, X_5, A_X and f_{res}). For lung function variables with skewed distributions that could not be corrected with log-transformation (IOS measurements: R_5-R_{20}, A_X and X_5), the bootstrapping method in linear regression was used to obtain unstandardised β-coefficients with 95% confidence intervals. The models were adjusted for confounding variables known from the literature. A p-value <0.05 was regarded as statistically significant.

A sensitivity analysis was carried out in subjects who were current nonsmokers (former and never-smokers) and in subjects who had not been diagnosed with COPD.

Results

Baseline characteristics

Baseline characteristics according to tertiles of SAF are shown in table 1. Tertile (T)3 (SAF 2.2–4.6 AU) consisted of an older age group, a larger proportion of current smokers, a higher proportion of subjects with diabetes and COPD, and higher mean values of HbA1c, plasma glucose and CRP, compared to T1–T2 (SAF 1.0–2.1 AU). There was no significant association between eGFR and tertiles of SAF. Mean values of spirometry and diffusing capacity were all lower in T3 *versus* T1–T2 (p<0.001 for all measures). For IOS, higher % predicted values of X_5 and f_{res} were associated with higher SAF values (p=0.001 and p<0.001 respectively). Higher % predicted values of IOS measures reflect an impairment in lung function.

Relationship between AGEs and spirometry

Results from linear regression models for 4111 subjects are shown in table 2. There was a significant reduction in FEV_1 and FVC (in litres) per 1-SD increase in SAF even after full adjustments for potential confounders (per 1-SD increase in SAF: 30-mL reduction in FEV_1, p<0.001; 30-mL reduction in FVC, p<0.001).

TABLE 1 Baseline characteristics by tertiles (T) of skin autofluorescence (n=4111)			
	T1, 1.0–1.8 AU	**T2, 1.9–2.1 AU**	**T3, 2.2–4.6 AU**	**p-value**
Subjects	1480	1345	1286	
Age years	56.8±4.3	57.5±4.1	58.4±4.3	<0.001
Females	55.5%	53.6%	50.7%	0.011
Height cm	171.8±9.9	171.7±9.6	170.9±9.7	0.020
Weight kg	79.9±15.7	80.1±15.4	80.9±16.9	0.087
Smoking				
Current	9.7%	15.9%	26.1%	<0.001
Former	38.5%	41.0%	41.0%	
Never	51.8%	43.0%	32.9%	
eGFR mL·min^{-1}·1.73 m^{-2}	82.9±15.3	82.7±14.3	82.7±16.6	0.643
Diabetes	5.4%	6.5%	13.3%	<0.001
COPD	1.5%	1.6%	3.3%	0.009
HbA1c mmol·mol^{-1} geometric mean	35.9	36.0	37.5	<0.001
Plasma glucose mmol·L^{-1} geometric mean	5.38	5.43	5.58	<0.001
CRP mmol·L^{-1} geometric mean	1.3	1.4	1.6	<0.001
FEV_1 L	3.25±0.76	3.21±0.80	3.06±0.76	<0.001
FEV_1 % predicted	94.9±11.6	94.2±12.4	92.2±12.9	<0.001
FVC L	4.13±0.99	4.09±1.0	3.94±0.94	<0.001
FVC % predicted	97.7±12.7	96.6±14.1	93.6±14.7	<0.001
FEV_1/FVC	0.790±0.06	0.785±0.06	0.779±0.07	<0.001
D_{LCO} % predicted	90.9±12.6	89.8±13.6	86.9±14.6	<0.001
D_{LCO}/V_A % predicted	97.0±13.5	96.1±14.1	94.6±15.8	<0.001
R_5 % predicted	106.9 (93.3–125.6)	107.7 (92.1–127.8)	108.6 (93.3–127.3)	0.312
R_{20} % predicted	128.6±31.3	128.5±31.1	128.2±31.2	0.766
R_5−R_{20} % predicted	54.9 (24.0–91.9)	55.9 (23.1–93.4)	63.3 (29.4–99.9)	0.667
X_5 % predicted	87.2 (68.1–108.9)	88.1 (68.5–110.0)	89.6 (71.1–114.6)	0.001
A_X % predicted	60.6 (38.3–97.0)	61.8 (39.5–101.6)	65.2 (39.7–112.6)	0.348
f_{res} % predicted	79.2 (68.8–95.1)	80.4 (69.3–95.9)	82.3 (68.9–98.6)	<0.001

Data are presented as mean±SD or median (interquartile range), unless otherwise stated. Diffusing capacity parameters: % predicted values calculated for 3889 subjects (T1, n=1408; T2, n=1273; T3, n=1208). Impulse oscillometry parameters: % predicted values presented for 3970 subjects (T1, n=1433; T2, n=1298; T3, n=1239). p-value: ANOVA or Chi-squared for test of linearity for continuous and categorical variables, respectively. Mean values of X_5 in T1–T3 of SAF are: T1, −0.089 kPa·L^{-1}·s; T2, −0.092 kPa·L^{-1}·s; T3, −0.097 kPa·L^{-1}·s. Higher % predicted values of impulse oscillometry measures reflect an impairment in lung function. eGFR: estimated glomerular filtration rate; HbA1c: glycated haemoglobin; CRP: C-reactive protein; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; D_{LCO}: diffusing capacity of the lung for carbon monoxide; V_A: alveolar volume; R_5: resistance measured at 5 Hz; R_{20}: resistance measured at 20 Hz; X_5 reactance measured at 5 Hz; A_X: area of reactance; f_{res}: resonant frequency.

TABLE 2 Change in spirometry measures per 1-SD increase in skin autofluorescence (SAF) (n=4111)			
		Per 1-SD increase in SAF (95% CI)	**p-value**
FEV$_1$ L	Model 1	−0.06 (−0.07–−0.04)	<0.001
	Model 2	−0.03 (−0.04–−0.02)	<0.001
FVC L	Model 1	−0.05 (−0.07–−0.03)	<0.001
	Model 2	−0.03 (−0.05–−0.02)	<0.001
FEV$_1$/FVC	Model 1	−0.005 (−0.007–−0.003)	<0.001
	Model 2	−0.002 (−0.004–−0.000)	0.120

Model 1 adjusted for age, height and sex. Model 2 adjusted for age, height, sex, weight, smoking status (never-, ex- and current smokers), pack-years, estimated glomerular filtration rate, diabetes, log(glycated haemoglobin), log(plasma glucose) and log(C-reactive protein). FEV$_1$: forced expiratory volume in 1 s; FVC: forced vital capacity.

Relationship between AGEs and diffusing capacity

Results from linear regression models for 3889 subjects are shown in table 3. There was a significant reduction in diffusing capacity measures per 1-SD increase in SAF after full adjustments for potential confounders (per 1-SD increase in SAF: 0.07-mmol·min^{-1}·kPa^{-1} reduction in D_{LCO}, p<0.001; 0.01-mmol·min^{-1}·kPa^{-1}·L^{-1} reduction in D_{LCO}/V_A, p=0.002).

Relationship between AGEs and IOS measures

Results from linear regression for 3970 subjects are shown in table 4. Per each 1-SD increase in SAF, there was a 0.001-kPa·L^{-1}·s reduction in X_5 (p=0.042) even after full adjustment for confounding variables. Per each 1-SD increase in SAF, there was a borderline significant increase in f_{res} after adjusting for all potential confounding factors (0.1-Hz increase in f_{res} per 1-SD increase in SAF, p=0.054). The associations between other IOS measures and SAF did not remain significant after full adjustment for potential confounders.

Sensitivity analyses

A sensitivity analysis was performed in current nonsmokers (supplementary table 1). After adjustment for potential confounding factors there was a significant reduction in FEV$_1$, FVC, D_{LCO} and D_{LCO}/V_A per 1-SD increase in SAF. There was no significant association between either FEV$_1$/FVC or IOS measures with 1-SD increase in SAF after adjustment for potential confounding variables in nonsmokers.

Furthermore, a sensitivity analysis was performed in subjects with no known COPD diagnosis (supplementary table 2). Those with either confirmed COPD or no response/missing information for the question about prevalent COPD were excluded. A significant reduction in FEV$_1$, FVC, D_{LCO}, D_{LCO}/V_A and X_5 per each 1-SD increase in SAF remained after adjustment for confounding variables.

Discussion

In ~4000 subjects aged 50–64 years old, SAF was found to have a significant inverse association with measures of spirometry, diffusing capacity and IOS measures such as X_5 even after adjustments for potential confounding variables. This inverse association between SAF and FEV$_1$, FVC or D_{LCO} was consistent in separate analyses in nonsmokers or subjects without a physician-diagnosis of COPD. The ratio of FEV$_1$/FVC was not associated with SAF. The relationship between X_5 and SAF remained significant in subjects with no COPD diagnosis but did not remain significant in current nonsmokers.

TABLE 3 Change in diffusion capacity measures per 1-SD increase in skin autofluorescence (SAF) (n=3889)			
		Change in diffusing capacity per 1-SD increase in SAF (95% CI)	**p-value**
D_{LCO} mmol·min^{-1}·kPa^{-1}	Model 1	−0.16 (−0.20–−0.12)	<0.001
	Model 2	−0.07 (−0.11–−0.03)	<0.001
D_{LCO}/V_A mmol·min^{-1}·kPa^{-1}·L^{-1}	Model 1	−0.02 (−0.03–−0.02)	<0.001
	Model 2	−0.01 (−0.02–0.00)	0.002

Model 1 adjusted for age, height and sex. Model 2 adjusted for age, height, sex, weight, smoking status (never-, ex- and current smokers), pack-years, estimated glomerular filtration rate, diabetes, log(glycated haemoglobin), log(plasma glucose) and log(C-reactive protein). D_{LCO}: diffusing capacity of the lung for carbon monoxide; V_A: alveolar volume.

TABLE 4 Change in impulse oscillometry (IOS) measures per 1-SD increase in skin autofluorescence (SAF) (n=3970)			
		Change in IOS measure per 1-SD increase in SAF (95% CI)	p-value
R_5 kPa·L^{-1}·s	Model 1	0.003 (0.00–0.006)	0.044
	Model 2	−0.001 (−0.003–0.002)	0.628
R_{20} kPa·L^{-1}·s	Model 1	0.00 (−0.002–0.003)	0.732
	Model 2	−0.001 (−0.003–0.001)	0.337
$R_5−R_{20}$ kPa·L^{-1}·s	Model 1	0.002 (0.001–0.004)	0.002
	Model 2	0.00 (−0.001–0.002)	0.509
A_X kPa·L^{-1}	Model 1	0.020 (0.009–0.031)	0.001
	Model 2	0.006 (−0.003–0.015)	0.229
f_{res} Hz	Model 1	0.253 (0.150–0.357)	<0.001
	Model 2	0.100 (−0.002–0.199)	0.054
X_5 kPa·L^{-1}·s	Model 1	−0.003 (−0.004–−0.002)	0.001
	Model 2	−0.001 (−0.003–−0.00006)	0.042

Model 1 adjusted for age, height and sex. Model 2 adjusted for age, height, sex, weight, smoking status (never-, ex- and current smokers), pack-years, estimated glomerular filtration rate, diabetes, log(glycated haemoglobin), log(plasma glucose) and log(C-reactive protein). R_5: resistance measured at 5 Hz; R_{20}: resistance measured at 20 Hz; A_X: area of reactance; f_{res}: resonant frequency; X_5: reactance measured at 5 Hz.

There is a known decline in FEV_1 and FVC with normal ageing, and AGEs accumulate with normal ageing. Similarly, smoking is associated with both a decline in FEV_1 and accumulation of AGEs. However, a significant inverse association remained even after taking into account these two major factors common to the worsening of both lung function and increase in SAF. D_{LCO} assesses the gas exchanging characteristics of the lung parenchyma, and is used mainly in the diagnosis and management of diseases of the lung parenchyma such as emphysema [33]. The association between D_{LCO} and SAF was significant in our study, even after adjustments, and could be found for both D_{LCO} and D_{LCO}/V_A, suggesting that there is a relationship between SAF and impaired alveolocapillary membranes. Previous studies assessing AGEs and D_{LCO} have been carried out with conflicting results. One study found negative correlation between D_{LCO} % predicted and SAF (r^2=0.276, p<0.0001) [17] in a cohort of COPD patients and healthy controls (n=288), whereas another found no correlation between D_{LCO} and SAF (r=0.113, p=0.33) in 88 COPD patients [12]. We found a significant inverse association between D_{LCO} and SAF in our overall cohort, and in the sensitivity analysis where known COPD subjects were excluded. With regards to IOS, respiratory reactance, but not resistance, was associated with SAF as indicated by a significant inverse association between SAF and X_5, and a lack of association between R_5, R_{20} or $R_5−R_{20}$ and SAF. In COPD patients, measures of reactance such as X_5 have been found to be indicative of changes in the elastance of the respiratory system that can be caused by increased heterogeneity, as occurs in airways obstruction [38]. Moreover, bronchodilators improve X_5 in COPD, likely reflecting the improvement of airways obstruction and, subsequently, ventilation heterogeneity [39]. Although the ratio of FEV_1/FVC was not associated with SAF, we found that X_5 was associated with SAF even after adjustment for confounders (and even in non-COPD subjects). This finding can also, to some extent, indicate an association between SAF and airways obstruction.

Both circulating plasma AGEs and SAF accumulation have been found to be associated with conditions related to chronic hyperglycaemia or oxidative stress, including DM, CVD, renal failure and dementia. However, the association with SAF and lung function has not previously been assessed in detail, and many of the existing studies compare AGEs in subjects with COPD *versus* subjects without COPD. A recent study assessed the association between SAF and spirometry [16], and found a correlation between FEV_1/FVC and SAF in elderly subjects (age 69±7 years; r=−0.446, p=0.002) but not in younger subjects (aged 51±8 years). The study cohort consisted of those with normal spirometry at baseline. Therefore, these findings can be compared to our results from a subcohort of subjects not known to be diagnosed with COPD. The age range in the SCAPIS cohort was comparable to this younger cohort; as such, we mirror the findings that at this "younger" cohort age range, SAF was not associated with FEV_1/FVC. Future research should examine whether raised SAF levels in apparently healthy subjects are associated with increased risk of developing COPD.

Our results indicate an association between SAF and changes in the lung parenchyma, as indicated by the significant inverse associations between D_{LCO} and SAF, even in sensitivity analyses. It remains unclear whether these changes would be emphysematous or fibrotic in nature. However, the lack of association between the FEV_1/FVC ratio and SAF, and the significant relationships between lung function measures and SAF in a subcohort of non-COPD subjects would indicate that in this cohort, it is unlikely that

emphysematous changes in the parenchyma would fully explain the associations between SAF and D_{LCO}. Our results potentially indicate an association between a restrictive lung defect and SAF; however, we had no information on total lung capacity (TLC) to confirm this.

The association between restrictive lung diseases such as idiopathic pulmonary fibrosis (IPF) and serum AGEs has also been explored, where it has been found that those with IPF have increased levels of serum AGEs [40, 41]. It has been found that in IPF patients, a change in serum AGEs over time corresponds to a decline in lung function over time; for example, a reduction in D_{LCO} % predicted, FVC and TLC was associated with an increase in serum AGEs [41]. Although it remains unclear if SAF is only a result of locally formed skin AGEs or whether increased SAF can also occur from accumulation from AGEs in the circulation, there seems to be some emerging evidence supporting the latter. A study in 2014 assessed the role of both plasma AGEs and SAF in COPD patients, and found that in addition to both plasma AGE levels and SAF being associated with COPD, certain plasma protein-bound AGEs were also associated with SAF [12]. As the evidence is still very limited, the precise mechanisms by which skin AGE accumulation is associated with lung function can only be speculated. It has been hypothesised that continuous exposure to oxidative stress, such as in conditions like COPD, may lead to the accumulation of AGEs both inside and outside the lung [17]. The level of the protective decoy receptor sRAGE has been found to be reduced in both COPD patients [42] and patients with IPF [41]. Furthermore, it has been thought that an increase in the AGE/sRAGE ratio could also indicate the degree of remodelling due to pulmonary fibrosis [41]. Interestingly, it has also been found that lower levels of sRAGE are associated with higher SAF in COPD subjects, the lack of decoy receptor potentially then resulting in accumulation of AGEs in the skin. This hypothesis could also be extended to the association between sRAGE and SAF in IPF patients as a potential mechanism for the findings linking SAF to parenchymal lung diseases.

Study limitations

Although a significant association was found between certain lung measures and SAF, we are aware that the effect sizes per 1-SD increase in SAF were not large. We appreciate that as such, the clinical relevance may be uncertain. Nevertheless, the highly significant p-values for associations (especially FEV_1, FVC, D_{LCO} and D_{LCO}/V_A with SAF) indicate that although small, a negative association between lung function and SAF was found in this cohort. The presence of an association in a "healthy" cohort such as SCAPIS can potentially provide some insight into how this association may potentially be more pronounced in a more "unhealthy" cohort and highlight the need for longitudinal studies to assess the direction of this small but significant association. The study population consisted of 50–64-year-old subjects; therefore, the findings may not be readily generalisable to younger age groups. However, as the accumulation of AGEs and decline in lung function are generally processes that occur at an older age, these findings may be of greater significance in the 50–64-year-old age group. Study participants were mainly of European origin living in Malmö, Sweden, which again, would limit the generalisability of the results to other populations. In observational studies of this nature, there is also a degree of selection bias to be considered. Those who took part in the study may differ in lifestyle habits from those that declined the invitation to be part of the study. There may therefore be an element of a healthier population agreeing to be part of such a study. However, ~17% of the participants were current smokers, which is comparable to the prevalence of smoking in Sweden (14.7% of men and women aged 55–64 years in 2018) [43]. Due to the nature of this observational cross-sectional study, there may also be the potential for residual confounding in the relationship between SAF and lung function. To take into account the role of inflammation in the association between SAF and low levels of lung function, we adjusted for baseline levels of CRP in our analysis, and found an association to exist between SAF and spirometry, D_{LCO} and X_5 despite this adjustment. However, the role of inflammation could not be fully excluded, as RAGE activation has been implicated with various inflammatory mediators (*e.g.* NF-κB); therefore, inflammation could still, in part, explain the associations observed. Chronic oxidative stress is an important accelerator of AGE formation and is associated with lung diseases such as COPD [17]. Even though many of the adjustment variables in this study are related to oxidative stress to some extent, we had no possibility to specifically adjust the results for oxidative stress. Hence, the role of oxidative stress cannot be excluded. Future studies should directly consider the role of parameters of oxidative stress on the association between SAF and lung function. Although the results indicate a potential relationship between SAF and restrictive lung defect, we did not have information on TLC; hence, we cannot conclusively state an association does indeed exist. Future studies are needed in which total lung volume on computerised tomography (as a proxy for TLC) or from body plethysmography can be used to confirm these findings.

We adjusted for plasma glucose, HbA1c and a diagnosis of DM. Glucose is part of the causal chain leading to the formation of AGEs and, as such, this could be considered as over-adjustment of the relationship between SAF and lung function. However, it is noteworthy that the relationships between SAF and lung function, in most cases, persisted after extensive adjustment for glucose.

Study strengths

This study does, however, have many strengths. Post-bronchodilator measurements were used ,which are advocated for the diagnosis of COPD [44], to ensure an element of reversibility (*e.g.* due to asthma) can be ruled out. The large sample size of the SCAPIS cohort is also an additional strength, adding power to our significant findings. To our knowledge, this is the first population-based study to assess the relationship between AGE accumulation in the skin with extensive lung function assessed according to standardised protocols including spirometry, D_{LCO} and IOS measures in a healthy population of 50–64-year-olds.

Conclusions

An increase in AGE accumulation, as measured by SAF, is significantly associated with lung function decrements indicative of changes in the lung parenchyma.

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