Chapter 4

Advanced glycation end products in the skin are enhanced in COPD

Susan Hoonhorst, Adèle Lo Tam Loi, Jorine Hartman, Eef Telenga, Maarten van den Berge, Leo Koenderman, Jan-Willem Lammers, Marike Boezen, Dirkje Postma, Nick ten Hacken

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ABSTRACT

Rationale: Cigarette smoking is the main cause of chronic obstructive pulmonary disease (COPD) inducing oxidative stress and local tissue injury, resulting in pulmonary inflammation. Advanced glycation end products (AGEs) are produced by glycation and oxidation of proteins and lipids and their formation is accelerated in inflammatory conditions associated with oxidative stress. AGEs are harmful by affecting protein function and triggering secondary messenger pathways after binding to their receptor.

Objectives: To assess whether AGEs in the skin are associated with COPD.

Methods: Mild-to-very-severe COPD patients and old (40-75 years) and young (18-40 years) healthy smokers and never-smokers were included. AGEs were measured by skin autofluorescence (SAF) using the AGE-Reader™. Demographic variables, smoking habits, co-morbidities and lung function values, were obtained in all subjects.
Measurements and main results: 202 COPD patients (FEV_1,%predicted=55) had significantly higher SAF values than 83 old and 110 young healthy controls: 2.5 vs. 1.8 and 1.2 (arbitrary units, $p<0.05$). No differences in SAF values were found between the four GOLD stages. Both SAF and packyears contributed independently to lower lung function (FEV_1/FVC(%), MEF_{50}/FVC, and RV/TLC(%)).

Conclusions: Skin autofluorescence of AGEs is equally increased in COPD patients of different GOLD stages (I-IV), and is significantly enhanced compared to healthy controls, independent of age, smoking and disease severity. We hypothesize that systemic AGEs may play a role in the induction phase of COPD in susceptible smokers. Future studies should further investigate mechanisms underlying AGEs formation and accumulation in COPD, by investigating other tissues than the skin and performing genetic analyses.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterized by progressive airway obstruction, chronic pulmonary inflammation and airway remodeling (1). The main cause of COPD in the western world is cigarette smoking, which induces oxidative stress and tissue injury in the lungs, finally resulting in chronic pulmonary inflammation (2). However, the burden of COPD is not restricted to the lung as COPD patients may show an ongoing systemic inflammation which induces systemic oxidative stress, and this may affect other organ systems and tissues outside the lung (4, 5).

Advanced glycation end products (AGEs) are products of glycation and oxidation of proteins and lipids. Under normal circumstances, AGEs are formed at a slow rate and accumulate in the body with aging. However, their formation and accumulation is accelerated in inflammatory conditions associated with oxidative stress. Importantly once formed, AGEs may cause local tissue injury by cross-linking of proteins thereby affecting their structure and function. In addition, AGEs may also have important consequences via binding the AGE receptors (RAGEs), triggering secondary messenger pathways inducing an increase of oxidative stress and inflammatory cytokine release (6, 7). AGEs are highly associated with glycemic and oxidative stress conditions, leading to a higher accumulation of AGEs in patients with diabetes, renal failure, and cardiovascular risk (6, 8, 9).

There are indications that AGEs might play a role in the pathogenesis of COPD. Recently, a study demonstrated upregulated expression of AGEs and RAGEs in the airways of COPD patients compared with healthy controls (10). In line with this, another study showed over-expression of RAGEs in airway epithelial and smooth muscle cells of COPD patients (11). In addition, soluble RAGEs in serum (sRAGE), which generally act as decoy receptors for RAGE ligands, have been found to be lower in COPD patients than in healthy controls (12-14).

AGE accumulation in the skin can be measured non-invasively by an AGE Reader™ (15). This device measures intrinsic autofluorescence of some AGEs expressed as skin autofluorescence (SAF). SAF has been extensively examined and proven to be a marker for both cumulative glycaemic and oxidative stress since higher SAF values are strongly associated with diabetes, cardiovascular risk, and renal failure (16-19). No studies are available investigating SAF in COPD.

As older age, oxidative stress and systemic inflammation are important characteristics and predictors of COPD, conditions that are also involved in accelerated AGE formation and accumulation, we hypothesize that AGE accumulation in the skin is increased in COPD. In the current study we aimed to assess if SAF is elevated in COPD patients and investigated SAF in mild to very severe COPD patients and in old (40-75 years) and young (18-40 years) healthy smokers and never-smokers using the AGE Reader™.

METHODS

We collected data from three observational studies performed in Groningen and Utrecht, The Netherlands (ClinicalTrials.gov identifiers: NCT00807469, NCT00850863, NCT00848406, and trialregister.nl identifier: NTR1497). These studies with overlapping baseline investigations

performed SAF measurements following the same protocol. The studies were approved by the medical ethic committee of the University Medical Centers Utrecht (UMCU) and Groningen (UMCG), and all subjects provided signed informed consent.

Study population

COPD patients (age 40-75 years) were recruited from outpatient clinics of UMCG and UMCU. Patients with a smoking history of >10 packyears and a post-bronchodilator $FEV_1/FVC<0.7$ were included and classified by Global initiative for chronic Obstructive Lung Disease (GOLD) stages I to IV (1). Old (40-75 years) and young (18-40 years) healthy smokers and never-smokers without airway obstruction ($FEV_1/FVC>0.7$) were recruited by advertisements. Old current smokers had to have a smoking history of >10 packyears, and young current smokers a smoking history of >0.5 packyears. Non-smoking subjects with a smoking history <0.5 packyears were included as never-smokers in both young and old groups. Exclusion criteria for all groups were alpha-1 antitrypsin deficiency and a doctors' diagnosis of asthma; co-morbidities were not excluded.

Study design

Clinical characteristics

All subjects performed post-bronchodilator spirometry and body plethysmography according to the European Respiratory Society guidelines (20). Furthermore, demographic variables, smoking habits and co-morbidities (a.o. diabetes mellitus, cardiovascular diseases, renal failure) were recorded.

Skin autofluorescence (SAF)

SAF was assessed non-invasively by the AGE-Reader™ (DiagnOptics B.V., Groningen, The Netherlands) (15). Technical details of this device have been extensively described elsewhere (18). In short, the AGE reader illuminates approximately 1 cm^2 of the skin, guarded against surrounding light, with an excitation light source between 300 and 420 nm (peak excitation flow ~350 nm). Only light from the skin is measured between 300 and 600 nm with a spectrometer using a 200-µm glass fiber. SAF was calculated by dividing the average light intensity emitted per nm over the 420- to 600-µm range by the average light intensity emitted per nm over the 300- to 420-µm range, using the AGE Reader software version 2.2. The volar surface of subject's forearm was positioned on top of the device, taking care to perform the measurement at normal skin site, i.e. without visible vessels, scars, or other skin abnormalities. SAF was averaged from three consecutive measurements for each subject, measured within a time period of approximately 2 minutes. In all analyses, SAF was expressed as the mean of these three measurements in arbitrary units (AU).

Statistical methods

Mann-Whitney U tests or Chi-square tests were used to compare baseline characteristics and SAF between groups. Linear regression analyses were performed to investigate associations between SAF and relevant clinical characteristics, with SAF as dependent variable and vital signs (blood pressure, BMI), lung function, smoking characteristics (packyears, current

smoking) as independent variables. Each model was adjusted for age, gender and performing center (UMCU/UMCG). Multiple linear regression analyses were performed to investigate the independent effect of SAF on lung function (FEV_1/FVC, MEF_{50}/FVC, RV/TLC), models being adjusted for age, gender, packyears, and performing center.

RESULTS

Subject characteristics

The characteristics of the study groups are presented in Table 1. We included 202 COPD patients, distributed over GOLD stages I-V (n = 60, 54, 54, and 34 respectively) (Table E1 in online data supplement). The old healthy group contained 83 participants, consisting of 28 never-smokers and 55 current smokers. The young healthy group included 110 participants, consisting of 36 never-smokers and 74 current smokers. In the COPD group, GOLD IV patients were significantly younger than GOLD I-III patients. GOLD I patients smoked a significantly higher number of cigarettes per day than GOLD II-IV patients. In the old healthy group, never-smokers had a significantly higher age than smokers. The characteristics of both young groups were similar.

Table 1. Subject characteristics of total groups

	COPD	Old	Young
N	202	83	110
Males,n(%)	135 (67)	60 (72)	55 (50)
Age, years	64 (58-69)	54 (48-61)	22 (20-28)
BMI, kg/m^2	24.9 (22.6-27.2)	24.7 (22.8-27.2)	22.7 (20.8-24.5)
BMI >30 kg/m^2, n(%)	20 (10)	10 (12)	1 (1)
Current smokers, n(%)	79 (39)	55 (66)	74 (67)
Cigarettes per day	0 (0-10)	17 (11-20)	10 (3-15)
Packyears	35 (25-48)	22 (0-31)	1 (0-5)
$FEV_{1,}$ % predicted	55 (37-85)	109 (102-118)	108 (102-116)
FEV_1/FVC, %	47 (34-58)	79 (76-83)	85 (81-90)
MEF_{50}/FVC, /sec	0.19 (0.11-0.33)	0.88 (0.79-0.04)	1.02 (0.89-1.18)
RV/TLC, %	48 (39-56)	31 (28-34)	23 (20-25)
Diastolic blood pressure, mmHg	80 (75-87)	82 (76-90)	78 (70-82)
Diabetes, n (%)	10 (5)	0 (0)	1 (0.9)
Cardiovascular disease, n (%)	50 (25)	3 (4)	1 (0.9)
SAF, AU	2.5 (2.0-2.9)	1.8 (1.6-2.1)	1.2 (1.1-1.5)

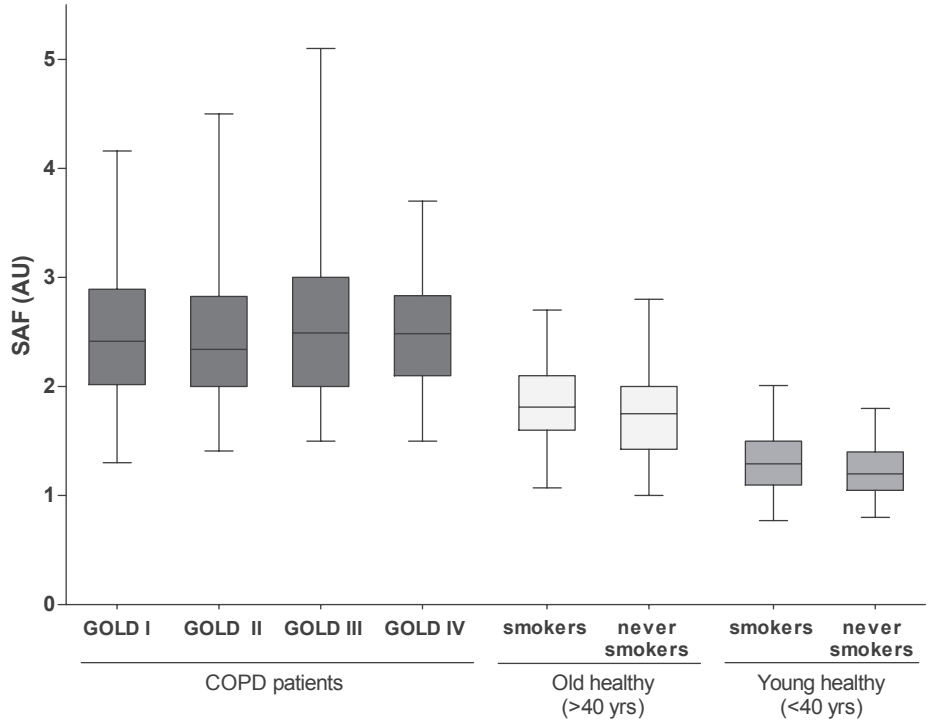
Values are expressed as medians (inter quartile ranges). Values of all baseline characteristics were significantly different between the three groups ($p < 0.05$). n = number, FEV_1 = forced expiratory volume in one second, FVC = forced vital capacity, MEF_{50}= maximal expiratory flow at 50% of vital flow capacity, SAF = skin autofluorescence, AU = arbitrary units.

Skin autofluorescence between groups

When comparing COPD patients, healthy old individuals, and healthy young individuals, SAF was significantly different between all groups (2.5, 1.8, and 1.2 AU respectively, $p<0.001$) (Table 1). SAF was similar between COPD patients GOLD stage I-IV (2.4, 2.3, 2.5, and 2.5 respectively), between healthy old smokers and never-smokers (1.8 and 1.8 respectively), and between young smokers and young never-smokers (1.3 and 1.2 respectively) (Figure 1, Table E1 in online

data supplement).

Figure 1. Skin auto fluorescence (SAF) in COPD patients, old and young healthy smokers and never-smokers



SAF values are significantly different between the total COPD group, the total healthy old group, and total young healthy group, with COPD patients having highest SAF and young groups having lowest SAF ($p < 0.001$). Values are expressed as medians (ranges).

Associations with skin autofluorescence

In the total population, linear regression analyses showed that poorer lung function values and a higher number of packyears were significantly associated with higher SAF values, independently of gender, age, and performing center ($p<0.010$, Table 2 and Figure 2). In all analyses, lung function and packyears were independently associated with SAF since both predictor values remained significant when added together in the models.

In the stratified analyses no significant associations between lung function parameters and SAF were present within any of the groups (Table 2). Only in the young healthy group a lower FEV_1 %predicted was associated with a higher SAF. A higher number of packyears was associated with a higher SAF in both young and old healthy groups ($p\leq0.001$), and this association almost reached significance in COPD patients ($p=0.053$) (Table 2). Within the old healthy group current smoking was associated with higher SAF ($p=0.019$). No associations

Table 2. Linear regression analyses in the total study population and stratified for COPD patients, the old healthy group and the young healthy group

	Total population			COPD patients			Old healthy group			Young healthy group		
Predictors of SAF:	B	S.E.	p	B	S.E.	p	B	S.E.	p	B	S.E.	p
FEV_1, % predicted	**-0.005**	**0.001**	**<0.001**	-0.001	0.002	0.450	-0.003	0.003	0.420	**-0.005**	**0.002**	**0.045**
FEV_1/FVC, %	**-0.008**	**0.002**	**<0.001**	-0.002	0.003	0.518	0.003	0.009	0.783	-0.005	0.005	0.269
MEF_{50}/FVC	**-0.434**	**0.087**	**<0.001**	-0.215	0.299	0.473	0.066	0.187	0.724	-0.100	0.116	0.391
RV/TLC, %	**0.011**	**0.002**	**<0.001**	0.006	0.004	0.127	0.014	0.011	0.211	0.001	0.005	0.790
Packyears	**0.008**	**0.001**	**<0.001**	0.004	0.002	0.053	**0.006**	**0.002**	**0.009**	**0.021**	**0.006**	**0.001**
Current smoking, n/y	0.079	0.055	0.153	0.044	0.091	0.625	**0.223**	**0.094**	**0.019**	0.094	0.049	0.061
BMI, kg/m^2	-0.007	0.007	0.309	-0.015	0.010	0.157	0.016	0.013	0.215	0.016	0.009	0.069
Diastolic blood pressure, mmHg	0.002	0.003	0.411	0.005	0.005	0.326	-0.003	0.004	0.559	0.005	0.003	0.080

Linear regression analyses in the total study population and stratified for COPD patients, old healthy group and young healthy group. Predictor variables were separately added to the regression model with SAF as dependent variable. All models were adjusted for gender, age and center.
Values represented in bold are significant associations ($p<0.05$).
B= unstandardized regression coefficient, S.E. = standard error, (n/y) = no / yes, n = number, FEV_1 = forced expiratory volume in one second, FVC = forced vital capacity, MEF_{50}= maximal expiratory flow at 50% of vital flow capacity, RV = residual volume, TLC = total lung capacity

Table 3. Multiple linear regression analyses in the total study population

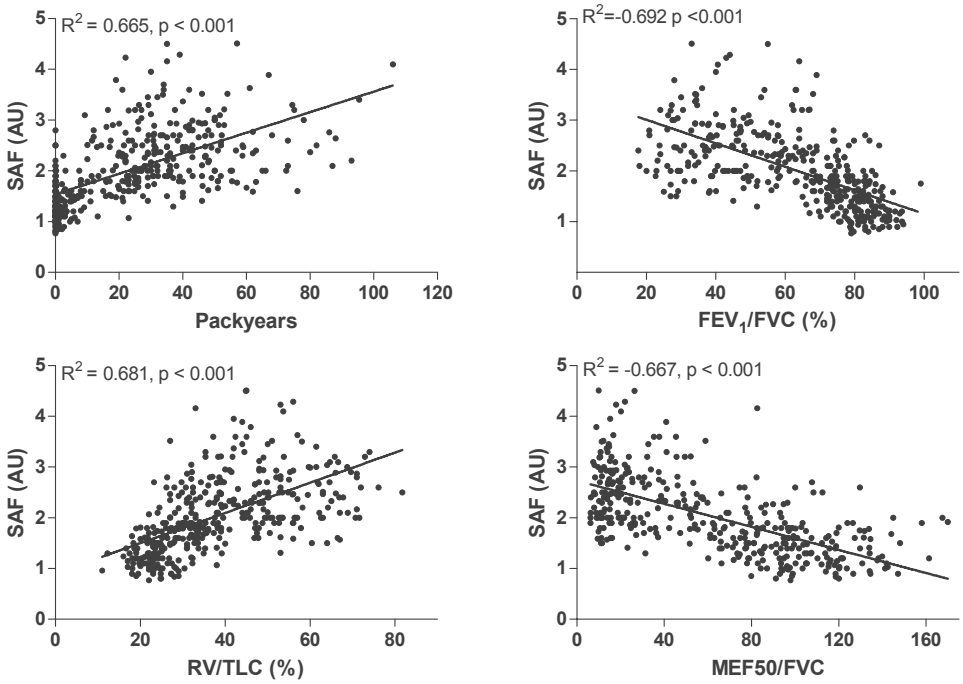
	FEV_1/FVC			MEF_{50}/FVC			RV/TLC		
Predictors of lung function:	β	S.E.	p-value	β	S.E.	p-value	β	S.E.	p-value
Age, years	**-0.375**	**0.059**	**0.000**	**-0.409**	**0.001**	**0.000**	**0.430**	**0.042**	**0.000**
Gender, m/f	**-0.107**	**1,616**	**0.003**	**-0.073**	**0.031**	**0.038**	**0.213**	**1,149**	**0.000**
Packyears	**-0.202**	**0.045**	**0.000**	**-0.205**	**0.001**	**0.000**	**0.140**	**0.032**	**0.004**
SAF, AU	**-0.278**	**1,391**	**0.000**	**-0.263**	**0.020**	**0.000**	**0.250**	**0.995**	**0.000**

Multiple regression analyses in the total study population. Dependent variables were FEV_1/FVC, MEF_{50}/FVC and RV/TLC. Values represented in bold are significant associations. B=standardized regression coefficient, S.E. = standard error, FEV_1 = forced expiratory volume in one second, FVC = forced vital capacity, MEF_{50}= maximal expiratory flow at 50% of vital flow capacity, RV = residual volume, TLC = total lung capacity, SAF = skin autofluorescence, m/f = male / female.

were found between SAF and BMI or blood pressure.

Multiple linear regression analyses in the total study population, including age, gender, packyears and SAF showed that higher SAF was significantly associated with lower lung function values (FEV_1/FVC, MEF_{50}/FVC, RV/TLC) ($p<0.01$, Table 3).

Figure 2. Associations of packyears and lung function variables with skin autofluorescence in the total population



SAF = skin autofluorescence, AU = arbitrary units, R^2 = correlation coefficient, FEV_1 = forced expiratory volume in one second, FVC = forced vital capacity, RV = residual volume, MEF_{50}= maximal expiratory flow at 50% of vital flow capacity, TLC = total lung capacity.

Comorbidities

We investigated the effects of relevant comorbidities, i.e. diabetes and cardiovascular disease on SAF. Cardiovascular disease was most prevalent; in 25% of all COPD patients, 4% of old healthy individuals and 1 % of young healthy individuals (Table 1). Furthermore, prevalence of diabetes was 5%, 0% and 1 % respectively in each group. Median (IQR) SAF values of the 50 COPD patients with cardiovascular disease compared to those without were: 2.6 (2.3-2.9) vs. 2.4 (2.0-2.9)($p<0.05$). Median (IQR) SAF values in COPD patients with diabetes mellitus (n=10) and without (n=192) were 2.9 (2.5-4.2) vs. 2.4 (2.0-2.9) respectively ($p<0.05$).

DISCUSSION

In this study we demonstrated for the first time that AGEs in the skin, measured as skin autofluorescence, are elevated in COPD patients compared with healthy controls, independent of age, current smoking and disease severity. A plausible explanation for this observation is that AGEs reflect higher amounts of cumulative endogenous oxidative stress exposure as a result from chronic systemic inflammation which is generally present in COPD patients (21).

The most important observation of this study is that SAF is elevated in COPD patients compared with healthy controls. Regression models in the total population confirmed that higher SAF associates with lower lung function, independently of age, gender and packyears. These associations were also demonstrated for variables representing small airways dysfunction (MEF_{50}/FVC), which is important because smoking-induced COPD starts in the distal airways. Interestingly, our data showed comparable SAF values between the different stages of disease severity suggesting that disease progression of COPD is not associated with accumulation of AGEs in the skin. Therefore we put forward the notion that AGEs formation may be accelerated particularly during the induction phase of COPD. Apparently, such processes only take place in so called susceptible smokers (22, 23). The old healthy smokers in our study clearly have proven not to be susceptible to develop COPD because they still have normal lung function values after many years of smoking. Even more importantly, their SAF values were completely comparable to those of old healthy never-smokers. We therefore speculate that accelerated AGEs formation in the induction phase of COPD is determined by the genetic make-up of susceptible individuals and that studying the immunological pathways of AGEs formation and accumulation might be very worth wile. Why accelerated AGE formation does not lead to higher SAF values in the higher GOLD stages of COPD is an intriguing finding, however we have to realize that our study is limited by its cross-sectional design.

Skin autofluorescence could be put forward as a useful biomarker of COPD, as it may differentiate COPD patients from healthy controls in an easy and non-invasive way. However, the overlap between groups was large, and it takes many years before SAF is elevated in established COPD. In other words, at an individual level this appears not a useful biomarker to recognize susceptible individuals in a preclinical stage of COPD. As AGEs could play an important pathogenetic role in the pre-clinical stage of COPD it might be attractive to find other surrogate markers for AGE-formation, better reflecting the actual process of AGE-formation than SAF does, and investigate them in the context of susceptible versus non-susceptible smokers with respect to COPD development.

Assuming that AGEs play a role in the development of COPD, a few mechanisms supporting this hypothesis can be considered. Two genome-wide association (GWA) studies have shown that the advanced glycosylation end product-specific receptor (AGER) gene is associated with lower lung function (FEV_1 and FEV_1/FVC) (24, 25). In addition, the minor allele of the AGER SNP was more frequently found in smokers with normal lung function than in smokers with COPD (26). This allele is a missense mutation encoding the sRAGE protein. Thus, one can speculate that in non-susceptible smokers this polymorphism may act as a protective mechanism by increasing the extent of membrane bound RAGE (mRAGE) that is cleaved into sRAGE, resulting in a higher amount of decoy receptors in the circulation protecting

against AGEs driven inflammation and tissue injury in the lungs. As mentioned earlier, other studies showed an upregulation of AGEs and RAGEs in the airways and lower levels of solube RAGE (sRAGE) in the circulation of COPD patients, further indicating the possible important contribution of AGEs in COPD (10-14). Taken together, we speculate that AGE formation is increased in susceptible smokers at a certain time point due to their genetic background, thereby causing harmful effects in cells and tissues, thereby affecting the structure and function of airways and lung tissue.

Earlier studies demonstrated that cigarette smoke is an important exogenous source of reactive glycation products and oxidative stress (27, 28). Therefore cigarette smoking theoretically may induce AGEs directly or indirectly via oxidative stress. Indeed, we demonstrate that a higher number of packyears contributed independently to SAF. This finding is in accordance with a previous study in healthy subjects demonstrating a positive association between the number of packyears and SAF (29). Packyears in our study were more closely related to SAF than current smoking. This is not surprising, as deposition of AGEs in the skin probably needs many years of cumulative oxidative stress, which is more closely related to the number of packyears smoking than to current smoking habits.

Some other conditions affecting the level of SAF have been described in the literature. First of all, older age was found to be strongly related with higher SAF (30). Our study also demonstrated such an association and therefore we adjusted all regression analyses for age. Secondly, it has been shown that individuals with diabetes, cardiovascular diseases, and renal failure have higher SAF values. These comorbidities are very prevalent in COPD, especially cardiovascular disease and diabetes (31, 32). Within our COPD population, SAF was indeed significantly higher in patients with cardiovascular disease and diabetes than those without. This finding is in line with the concept that COPD may be a component of a chronic inflammatory syndrome involving many other organs (33), and that AGEs might act as potential mediators of systemic inflammation. Finally, nutrition habits may have small effects on SAF. One study demonstrated an 8.7 percent increase of SAF in healthy subjects, two hours after a high fat meal containing a sufficient AGEs content (34). In our current study we had no data on recent food intake, but we believe this has not affected our results.

The strength of the current study is that we included a large population of COPD patients with different severity stages (GOLD I-IV). Furthermore, we used large control groups strictly including young and old subjects who were never smokers or current smokers, in this way avoiding contamination with ex-smokers. Of course there are some limitations. The number of non-smokers was smaller than the number of smokers, both in young and old healthy groups. Furthermore, there were no data available on systemic inflammatory markers and on AGEs in other tissues than the skin. In future studies these parameters could help to better interpret SAF data in COPD or COPD development.

In conclusion, we demonstrate that AGEs in the skin, as measured by skin autofluorescence, are higher in COPD patients than healthy controls, independent of age, current smoking and disease severity. We hypothesize that AGEs in the circulation and lung may play a role in the induction phase of COPD in so-called susceptible smokers. Therefore, it is important in future studies to assess mechanisms underlying AGE formation and accumulation

in COPD, by investigating other tissues than the skin and performing genetic analyses and gene expression.

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Chapter 4

Supplement

Table E1.

	COPD				Old		Young	
	GOLD I	GOLD II	GOLD III	GOLD IV	Smokers	Never-smokers	Smokers	Never-smokers
N	60	54	54	34	55	28	74	36
Males, n (%)	40 (67)	42 (78)	39 (72)	14 (41)	40 (73)	20 (71)	42 (57)	13 (36)
Age, years	64 [59-72]	64 [60-69]	65 [60-69]	58 [52-64]‡	51 [46-59]	58 [52-66]*	22 [20-28]	22 [20-29]
BMI, kg/m^2	24.9 [22.8-26.7]	25.7 [23.8-28.3]‡	23.6 [22.4-25.8]	24.2 [20.2-27.6]	24.7 (22.7-27.2)	24.6 (22.8-27.1)	23.1 [21-25]	21.8 [20.4-23.9]
BMI >30 kg/m^2 , n (%)	4 (7)	8 (15)	3 (6)	5 (15)	6 (11)	4 (14)	1 (1)	0 (0)
Current smokers, n	33 (55)	27 (50)	14 (26)	5 (15)	55 (100)	0 (100)	74 (100)	0 (0)
Cigarettes per day	7 [0-16]§	1 [0-12]	0 [0-4]	0 [0-0]	17 [11-20]	---	10 [3-15]	---
Packyears	35 [23-51]	36 [28-47]	40 [26-51]	28 [23-42]	26 [22-39]	---	2.7 [1-6]	---
FEV_1 , % predicted	92 [85-100]‡	65 [55-71]‡	39 [35-45]‡	24 [20-28]‡	107 [101-117]	111 [103-120]	108 [102-116]	108 [103-115]
FEV_1 / FVC, %	62 [57-67]‡	49 [45-55]‡	36 [30-40]‡	27 [23-33]‡	78 [75-83]	79 [77-82]	85 [81-90]	86 [80-91]
MEF_{50}/FVC, /sec	0.39 [0.30-0.49]	0.21 [0.17-0.28]	0.13 [0.10-0.15]	0.10 [0.08-0.11]	0.88 [0.77-1.04]	0.85 [0.79-1.07]	1.03 [0.89-1.19]	1.00 [0.82-1.17]
RV/TLC, %	38 [32-42]	46 [39-49]	56 [48-61]	65 [57-71]	31 [28-34]	32 [27-35]	22 [20-25]	24 [22-27]
Diastolic bloodpressure, mmHg	80 [75-86]	82 [76-88]	80 [76-88]	80 [70-87]	80 [75-88]	83 [77-90]	75 [70-82]	80 [72-84]
Diabetes, n (%)	0 (0)	3 (6)	5 (9)	2 (6)	0 (0)	0 (0)	1 (1)	0 (0)
Cardiovascular disease, n (%)	19 (32)	9 (17)	15 (28)	7 (21)	3 (6)	0 (0)	0 (0)	1 (2,8)
Renal failure, n (%)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
SAF, AU	2.4 [2.0-2.9	2.3 [2.0-2.8]	2.5 [2.0-3.0]	2.5 [2.1-2.8]	1.8 [1.6-2.1]	1.8 [1.4-2.0]	1.3 [1.1-1.5]	1.2 [1.0-1.4]

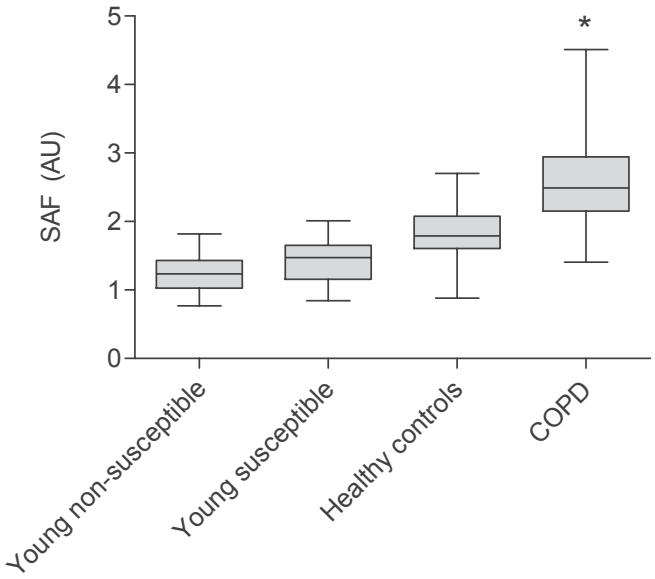
Values are expressed as median (Inter Quartile Range) or as number (percentage).
N = number, m = male, FEV_1 = Forced Expiratory Volume in one second, FVC = Forced Vital Capacity, MEF_{50}= maximal expiratory flow at 50% of vital flow capacity, SAF = skin autofluorescence, AU = arbitrary units.
*$p < 0.05$; †$p < 0.05$ compared with COPD GOLD I, II, III; ‡$p < 0.05$ compared with GOLD III; §$p < 0.05$ compared with GOLD III and IV; ‡$p < 0.01$ between all groups.

Chapter 4

Addendum

No differences were present between young susceptible and non-susceptible individuals. In multiple regression analysis, no association was found between COPD susceptibility and SAF after adjustment for gender, age and packyears.

Figure 1. Skin autofluorescence the susceptibility groups



Skin autofluorescence (SAF) values presented as median (range). * p>0.05 compared with all groups. AU= arbitrary units.

Table 1. Group characteristics

	Young non-susceptible	Young susceptible	Healthy controls	COPD
N, n	33	21	28	94
Male, n (%)	20 (61)	11 (52)	24 (86)	68 (72)
Age, years	21 [20-23]	31 [25-38]	51 [47-62]	63 [58-67]
Packyears, n	1 [0-3]	6 [2-10]	27 [23-39]	35 [26-47]
FEV_1, L	4.6 [3.8-5.4]	4.0 [3.6-4.6]	4.0 [3.5-4.3]	1.8 [1.1-2.8]
FEV_1, % pred	104 [101-114]	110 [104-114]	108 [102-115]	57 [40-85]
FEV_1/FVC *(%)*	86 [84-91]	81 [77-87]	78 [74-84]	48 [37-60]

Data are expressed as median [Inter Quartile Range]. n = number, FEV1 = Forced Expiratoy Volume in 1 second, FVC = Forced Vital Capacity.