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Plasma AGEs and skin autofluorescence are increased in COPD

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Abstract

Chronic obstructive pulmonary disease (COPD) is associated with systemic inflammation and oxidative stress. These conditions may lead to formation of advanced glycation end-products (AGEs). In this study we investigated in 88 COPD patients and 55 control subjects (80% ex-smokers) the association of the plasma protein-bound AGEs N^{ε}- (carboxymethyl)lysine (CML), pentosidine, N^{ε}-(carboxyethyl)lysine (CEL) and AGE accumulation in skin by skin auto fluorescence (AFR) with lung function. Plasma CML (COPD: 61.6 ± 15.6, never smokers: 80.7 ± 19.8 , ex-smokers: 82.9±19.3 nmol/mmol lysine) was decreased and CEL (COPD: 39.1±10.9, never smokers: 30.4±5.0, ex-smokers : 27.7±6.4 nmol/mmol lysine), and AFR (COPD: 3.33±0.67, never smokers: 2.24±0.45, ex-smokers: 2.31±0.47 AU) were increased in COPD compared to the controls. Disease state was inversely associated with CML, and linearly with CEL and AFR. Performing regression analyses in the total group, CEL and AFR were negative and CML was positive associated with lung function, even after correction for potential confounders. In conclusion, CEL and AFR were negatively and CML was positively associated with disease state. Only in the total group, the AGEs showed an association with FEV_1. Our data suggest that AGEs are involved in the pathophysiology of COPD, although their exact role remains to be determined.

Introduction

Chronic obstructive pulmonary disease (COPD) is a major and increasing global problem predominantly associated with lung pathology. It is a chronic inflammatory disease of the lower airways caused by long term inhalation of noxious gasses, especially cigarette smoke. The pathologic characteristics of COPD are destruction of lung parenchyma or emphysema, inflammation of the central airways, and an increase in the number of mucus producing cells. The airflow limitation measured by reduced forced expiratory volume in the first second (FEV_1) is usually progressive. Apart from pulmonary pathology, COPD is characterized by systemic inflammation and oxidative stress [1], of which the origin is not known yet. Chronic inflammation and local/systemic oxidative stress may lead to the increased formation and accumulation of systemic levels of advanced glycation end products (AGEs).

AGEs are a class of compounds formed from non-enzymatic glycation and oxidation of proteins and lipids. Production of these molecules through the classical pathway takes weeks to months and involves many reversible intermediates that finally lead to the formation of irreversible AGEs [2]. An alternative pathway is induced by increased oxidative, inflammatory, and glycemic stress and will result in the formation of AGEs by reactive dicarbonyl compounds within hours [3]. The best characterized AGEs to date are N^{ε}-(carboxymethyl)lysine (CML), pentosidine, and N^{ε}-(carboxyethyl)lysine (CEL). AGEs are ligands of the receptor for advanced glycation end products. Similar to other ligands of this receptor, including high-mobility group box-1 and serum amyloid A, binding leads to the activation of the transcription factor nuclear factor kappa B, resulting in the production of inflammatory cytokines [4, 5].

Some AGEs, such as pentosidine, are characterized by their yellow-brown fluorescent color and their ability to form stable inter and intra molecular cross links [6] on long lived proteins such as skin collagen and lens proteins [7-10]. Because of these special fluorescent characteristics their accumulation on skin collagen can be estimated non-invasively with a skin autofluorescence (AFR) reader also called AGE reader [11]. Recent evidence has shown that

AFR increases with age and is increased in patients with diabetes [12-14]. In COPD, it has been shown that CML is increased in epithelial lining fluid (ELF) from peripheral airways as compared to healthy ex - and current smokers [15]. Because systemic oxidative stress and persistent systemic inflammation are present in COPD, we hypothesize that AGEs, in plasma and skin, are increased in COPD. This study was therefore designed to investigate primarily the plasma concentrations of protein-bound CML, CEL and pentosidine, as well as AFR in COPD patients compared to healthy controls and to examine their relation to disease state, and lung function. Secondly, the relation of plasma AGEs with AFR was examined.

Methods

Study population:

The study population included 114 clinically stable, moderate-to severe COPD patients referred for pulmonary rehabilitation, which were recruited from the Center of expertise for chronic organ failure (Ciro), Horn, the Netherlands and 61 healthy (ex/never) smoking controls. Clinical history of COPD and the degree of disease severity were assessed according to the published American Thoracic Society (ATS), Global Initiative for Chronic Obstructive disease (GOLD) guidelines, which is post-bronchodilator FEV_1<80% predicted and FEV_1/FVC <70% predicted. Exclusion criteria were history of tumor, diabetes, and an exacerbation of the disease for <4 weeks before blood draw and skin reading. Control subjects were judged healthy by a standardized health questionnaire. This study was approved by the local medical ethical committee.

The number of pack years (PY; the number of packs of cigarettes smoked per day divided by 20 multiplied by the number of years smoked) and the smoking status (never/current/ex-smoker) were recorded. People with 0 PY were considered as never smoker, >10 PY were

considered smokers and who stopped smoking at least 1 year prior to recruitment were considered ex-smokers.

Lung function was determined using spirometry and forced expiratory volume in 1 sec (FEV_1) and forced vital capacity (FVC) were calculated from the flow-volume curve. Height and weight were measured in every participant and BMI was calculated (weight divided by height^2 (kg/m^2)). Self-reported medication history and associated co-morbidities of the patients and controls were recorded. 63.7% of patients were on long and /or short acting muscarinic antagonists (LAMA), 52.9% on combination therapy of LABA and inhaled corticosteroids, 39.2% on long-acting beta-2 agonists (LABA), 22.5% on short-acting beta-2 agonists (SABA), 16.7% were on low dose oral steroids, 10.8% on inhaled steroids, 8.8% on N-acetylcysteine and 20.6% were on statins.

Blood collection and determination of plasma markers:

Blood was collected in an evacuated tube containing EDTA (Sherwood Medical, St Louis, Missouri, USA) and immediately centrifuged at 800 rpm for 10min at 4°C. The plasma samples were subsequently stored at -80°C until analysis. Plasma protein-bound CML and CEL were measured by liquid chromatography tandem mass spectrometry [16] and pentosidine was measured by HPLC with fluorescence detection [17] and expressed per lysine concentrations.

Oxidative damage of plasma protein was assessed by measuring plasma protein carbonyl's by a spectrophotometric assay. In brief, plasma protein carbonyls were derivatised with 2,4- dinitro-phenyl hydrazine (DNPH) to form protein hydrazine, which can be measured at 360-385nm [18]

Plasma levels of high density lipoproteins (HDL), triglycerides, glucose, C-reactive proteins (CRP) and creatinine were measured in an auto-analyzer (ABX Pentra 400, HORIBA ABX S.A.S, France). Glomerular filtration rate (GFR) was calculated using the Cockcroft-Gault formula [19].

Skin autofluorescence:

AFR was determined by an AGE Reader (version 2.1, DiagnOptics technologies BV, Groningen, the Netherlands). AFR was measured on the volar side of the lower part of the dominant arm in every participant, at room temperature and in a dark environment. Care was taken to perform the measurement in an area without any scar, visible vessels, lichenifiction, or other skin abnormalities. In brief, the excitation source is a 4W UV-A emitting lamp that emits light with a wavelength of 300-420 nm (peak 360 nm). Light reflected and emitted in the 300-600 nm range from the skin is measured by the inbuilt spectrometer using a UV glass fiber. In addition, dark and white reference readings were performed before every measurement to correct for dark current background light and to calculate reflectance, respectively. To correct for difference in light absorption, AFR was calculated as ratio of excitation light (300-420 nm) to emitted light (420-600 nm), and expressed as arbitrary units (AU) [20, 21]. Only AFR with a reflectance >0.1 was considered for further investigation.

Statistical methods:

SPSS (version 17, Chicago, IL) was used for the data analysis. Variables with skewed distribution like CRP, FEV_1%predicted, pentosidine and triglycerides were log transformed before further analysis. Comparison of characteristics between groups was performed by student's *t* or Chi-square tests, for continuous or categorical data, respectively. Multiple linear regression analysis was used to investigate whether disease state (COPD) and lung function were associated with increased plasma AGEs and AFR. All analyses were first adjusted for age and sex (model 1) and further for smoking (model 2) and GFR, BMI, HDL cholesterol and triglycerides (model 3). In addition, we added CRP, as a marker of low-grade inflammation to the model to investigate the extent to which this variable explained (i.e. attenuated) the associations of plasma AGEs and AFR

with COPD (model 4). We additionally used linear regression analysis to investigate the associations of AFR with plasma AGEs. Again we first adjusted for age and sex (model 1), next smoking (model 2) and then GFR (model 3). All results are expressed as standardized regression coefficients to enable comparison of the strength of the associations between variables.

Results

Out of 61 healthy controls and 114 patients included, 6 controls and 26 patients were excluded from the analyses because of reflectance <0.1. Table 1 describes the baseline characteristics of the final study group. As expected, FEV_1 was significantly lower in the COPD patients compared to the control group ($p<0.001$). Based on the GOLD criteria 34 patients were categorized GOLD II, 39 as GOLD III and 15 as GOLD IV. BMI was not different between controls and patients. COPD patients had smoked more PY compared to healthy controls. Furthermore, no differences were observed in the plasma levels of HDL cholesterol, triglycerides, and glucose. Plasma creatinine and GFR levels were not significantly different. CRP was found to be increased in COPD patients compared to controls ($p<0.001$). Lastly oxidative stress marker plasma protein carbonyl's showed no difference between patients and controls.

Plasma AGEs and AFR in COPD patients and controls

Figure 1A demonstrates that (unadjusted) levels of plasma CML (COPD: 61.6 ± 15.6 and never smoking controls: 80.7 ± 19.8, ex-smoking controls: 82.9±19.3 nmol/mmol lysine), were decreased in the COPD group compared to the never/ex smoking control group. On the other hand the plasma concentrations of CEL (figure 1B) (COPD: 39.1 ± 10.9 and never smoking controls: 30.4±5.0, ex-smoking controls: 27.7±6.4 nmol/mmol lysine) was increased in COPD patients compared to never/ex smoking control group. ~~and~~ Other plasma AGE pentosidine (figure

1C) (COPD: 0.68 ± 0.45 and never smoking controls: 0.56±0.09, ex-smoking controls: 0.61±0.16 nmol/mmol lysine) showed no difference between COPD group and never/ex smoking control group. AFR (figure 1D) was found to be increased in COPD compared to controls (COPD: 3.33 ± 0.67 and never smoking controls: 2.05 ± 0.31, ex-smoking controls: 2.31 ± 0.47 AU). Plasma levels of AGEs and AFR did not differ between never and ex-smoking controls (figure 1). Plasma levels of AGEs and AFR did also not differ between ex and currently smoking COPD patients or patients of different GOLD stages (data not shown).

Associations of COPD and lung function with plasma AGEs and AFR

Table 2 shows that, after adjustment for age, sex and PY (model 2), COPD was associated with a lower plasma level of CML [β = -1.086 (95%CI: -1.396 to -0.777)], higher plasma levels of CEL [β = 1.002 (95%CI: 0.683 to 1.321)] and higher AFR [β = 1.042 (95%CI: 0.776 to 1.308)] (p <0.001). However, this is not the case for with pentosidine [β = 0.046 (95%CI:-0.308to0.399)]. Additional adjustment for possible other confounders (i.e. GFR, BMI, HDL cholesterol, triglycerides) did not significantly change the associations of COPD with plasma CML,CEL and AFR , which remained significant (model 3). Further in model 4 we evaluated the effect of CRP, as a marker of low-grade systemic inflammation on the associations of plasma AGEs and AFR with disease state. We did however not observe any effect on the associations.

Secondly, the associations of lung function (FEV_1%predicted) with AFR and plasma AGE levels were evaluated by regression analysis in total group. Plasma CML [β=0.518 (95%CI: 0.352 to 0.685)] was a positive, CEL [β=-0.427 (95%CI: -0.598 to -0.256)] and AFR [β=-0.402 (95%CI: -0.556 to -0.248)] were found to be a negative determinants of lung function, even after adjusting for age, sex and PY (p<0.001, table 3, model 3). Additional adjustment for other confounders did not affect the association. Further, analysis was restricted to patients alone; the association of lung functions with AGE's and AFR were no longer significant (supplementary table 1). In addition, Correlation analysis were done for AFR and AGEs with other disease maker

like DLCO, BODE and Charlson score, but there were no significant correlations were observed (supplementary table 2).

Associations of plasma AGEs with AFR

Lastly, the association between the three different plasma AGEs and AFR was evaluated. After adjustment for age and sex, PY plasma CML [β=-0.320 SD (95%CI: -0.502 to -0.138), p=0.000] and CEL [β=0.238 SD (95%CI: 0.052 to 0.424), p=0.013], but not pentosidine [β=0.046 (95%CI: -0.140 to 0.233), p=0.624] showed the association. After additional adjustment for GFR plasma CML and CEL remained the same (table 4, model 3).

Additional analyses

Since steroids are known to have side effects on skin [22], we compared AFR in patients taking or not taking oral steroids, which was found not to be significantly different (3.26 ± 0.64 ~~AU~~ and 3.34 ± 0.68 AU, respectively, p=0.650).A Similar comparison was performed for plasma AGE levels, but also here no significant difference due to oral steroid use were found. Surprisingly, patients who were on combination therapy of LABA and inhaled corticosteroids showed higher AFR than those who were not (3.5 ± 0.65 AU and 3.2 ± 0.68 AU, respectively, p=0.042). We did not observe any difference in plasma AGEs or AFR between individuals who were using statins vs those who were not.

Discussion

The present study is the first to analyze different plasma AGEs and AFR in patients with COPD. While unadjusted plasma levels of CML were decreased, CEL and AFR were found to be increased in COPD patients compared to never and ex- smoking controls. Importantly, after adjusting for potential. confounders CML was negative, CEL and AFR were positive

determinants of disease state. Lung function measured by FEV_1% predicted showed an association with CML, CEL and AFR in total group; but not when the analysis restricted to the patient group.

Among many different AGEs, we investigated three protein-bound AGEs in plasma i.e., CML, CEL, pentosidine. It is known that the three AGEs we have measured in our study have distinct functionalities; pentosidine as a major cross linking AGE, CML as a major ligand for RAGE, and importantly, CEL as a putative marker for intracellular glycation. Interestingly the precursor molecule for CEL [23], methylglyoxal is a highly reactive compound causing cell death [24].

CML was decreased in COPD patients compared to the never and ex-smoking controls. However, previously we and others showed that the plasma CML was not different in COPD patients compared to controls. In our former study, as well as in another publication, CML levels were determined by ELISA and uncorrected for lysine content [25, 26] as opposed to the measurement by mass spectrometry employed here. Interestingly, change in the direction of plasma protein bound CML levels are in line with plasma concentration of sRAGE in COPD. In the epithelial lining fluid (ELF) of small airways in COPD a recent pilot study showed on the other hand an increase in the levels of CML, measured by competitive ELISA. Importantly, this study found a positive association of CML with the oxidative stress marker 8-isoprostane as well as IL-8 and a negative association with FEV_1 [15]. However this study did not measure the CML concentrations in circulation. Therefore, it might be that a change of CML in the lung tissue is not reflected by altered plasma levels.

This is the first report on the AGE, CEL in COPD. Elevated circulating levels of protein bound CEL were found in COPD compared to never and ex-smoking controls. Primarily, it would be interesting to test whether increased plasma CEL levels as determined here could arise from elevated pulmonary levels or have a different source of origin. Secondly, extracellular toxicity /intracellular glycation via methylglyoxal/CEL might be a molecular mechanism in COPD

pathogenesis which needs to be further explored. In the present study we were unable to measure methylglyoxal in the circulation. The fluorescent AGE, pentosidine, which requires oxygen for its formation [27], was not found to be altered in the plasma of COPD patients compared to controls. Studies have shown that AGEs are formed from glucose [23, 28, 29] and lipids [30, 31] by the slow classical pathway. It is important to highlight that diabetic patients were excluded from our study population, which makes it likely that the alterations in AGE levels in COPD are derived from other pathways rather than from glucose. Furthermore, inflammation and oxidative stress [32], even in the absence of increased glycemia [33, 34], may lead to the formation of AGEs in a short time course i.e. within a few hours [35]. However, we currently found that the positive association of COPD with plasma CEL and AFR, negative association of CML was independent of CRP. Although to fully rule out a role for inflammation in the formation of AGEs in COPD, a panel of plasma inflammatory markers may need to measured [36]. Moreover, the association of COPD with plasma levels of CEL and CML was also independent of BMI, blood lipids and GFR. Neither inhaled or oral steroid use, nor the smoking status had effect on plasma AGE's. From our study it is clear that increases in CEL and decrease in CML level in the circulation are due to the disease state. But it needs further investigation to elaborate the exact processes underling these difference in COPD.

Regression analysis showed that plasma CML was independently, negatively but CEL was independently, positively associated with disease state. Interestingly, ~~only~~ CML and CEL were found to be associated with lung function only in the total group and there were no observed correlation of plasma AGEs with other parameter of disease such as DLCO, BODE or Charlson comorbidity score, implying that the cumulative factor from the disease state is a driving force for differences in AGEs instead of FEV1 alone. Our results showed that AGEs may not directly related to the degree of lung function impairment in COPD.

Other studies have shown that AGEs are increased in diseases such as atherosclerosis [37], osteoporosis [38] and diabetes [39], which are common co-morbidities in patients with

COPD. The cardiovascular complications in diabetes are postulated to be due to the high levels of AGEs in the circulation [39]. In addition, CML has been found on the inner walls of arteries in patients suffering from an acute myocardial infraction in the absence of diabetes [40]. Importantly, in present study diabetics were excluded and other co-morbidities were not frequent enough to investigate their contribution to the increased AGE levels in COPD. Therefore, prospective large longitudinal studies are needed to fully elucidate the potential mediating factors including co-morbidities involved in COPD-induced changes in plasma and skin AGEs, and vice versa.

From the present data, we cannot elucidate whether the increase in AFR is due to a direct effect of smoking on skin collagens, whether it is a systemic consequence of the lung pathology or whether it is an effect of medication use. There is indeed evidence that smoking increases AFR [12, 41]. Koetsier et al for instance showed that AFR was increased in current smokers compared to non smokers after correcting for age, but their study group was devoid of ex smokers. In the present study, we did not find a difference in the AFR between current smoking patients and ex smoking patients (data not shown). Adjusting for pack years smoked furthermore did not affect the relation between AFR and disease status, With respect to the latter possibility, Noordzij et al. reported that vasodilatation and vasoconstriction caused a respective decrease and increase in AFR [42]. It is known that corticosteroids cause vasoconstriction and most of the COPD patients are taking either a low dosage of oral or a medium to high dosage of inhaled steroids. We did however not find an effect of oral steroid use on AFR. The combination therapy of LABA and inhaled corticosteroids on the other hand was found to be associated with an increase in AFR, whereas no effect on plasma AGEs was observed. The individual medications did not affect AFR and a possible mechanism behind the combined treatment remains to be investigated.

The study which validated the AGE reader showed a correlation between AFR and collagen linked AGEs from skin but they did not investigate the association between plasma AGEs to the AFR [43]. From this it is not clear if the AGEs are formed locally in the skin or they

accumulated from the circulation. In the present study we thus evaluated the association between AFR and AGEs in circulation and we showed that the CEL and CML were associated with AFR. Surprisingly, we did not found an association of florescent AGE pentosidine with the AFR.

In this study population, from the 114 COPD patients and 61 controls we eliminated 22 patients and 6 controls from further analyses because the AFR reflection value <0.1. During the validation of the AGE reader the major reason for a reflection value <0.1 was skin color, with skin prototypes (SPT) V and VI causing reflection <0.1 [20]. In this population however, SPT II-IV showed a lower reflection value. Importantly the age reader which we used was an older version where we cannot consider the reflection bellow 0.1. However the more recent versions recalculate the AFR with reflection <0.1.

In conclusion, it is clear from our study that specific AGEs in the circulation and AFR are increased in COPD patients. Importantly, CEL and AFR were negatively and CML was positively associated with disease state. Plasma CML, CEL and AFR showed an association with FEV_1, but only in the total group. It is important to further investigate the adverse effect of AGEs and in particular of CEL on the system and specific the lung.

Author's contribution

ER designed the study and provided the samples. JS performed the analysis; CGS provided the equipment and reagents for analysis. FF was a study physician. LE, ER and PG contributed for stastical analysis, PG, NR and ER drafted the manuscript, ER, NR, FF, EW, CGS, LE and PG contributed to acquisition and interpretation of data. All authors read and approved the final version of manuscript.

Competing interests

The authors declare that they have no competing interests.

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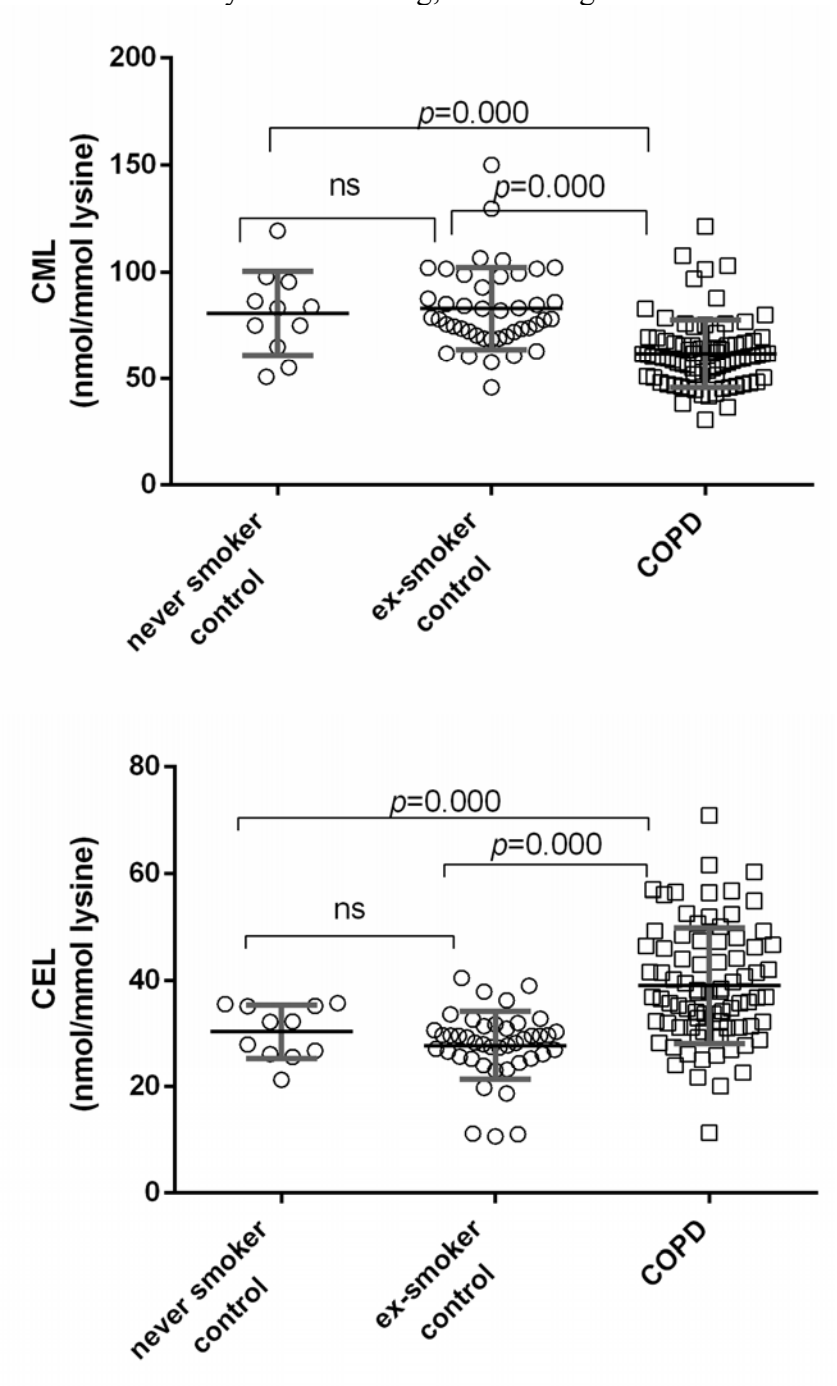
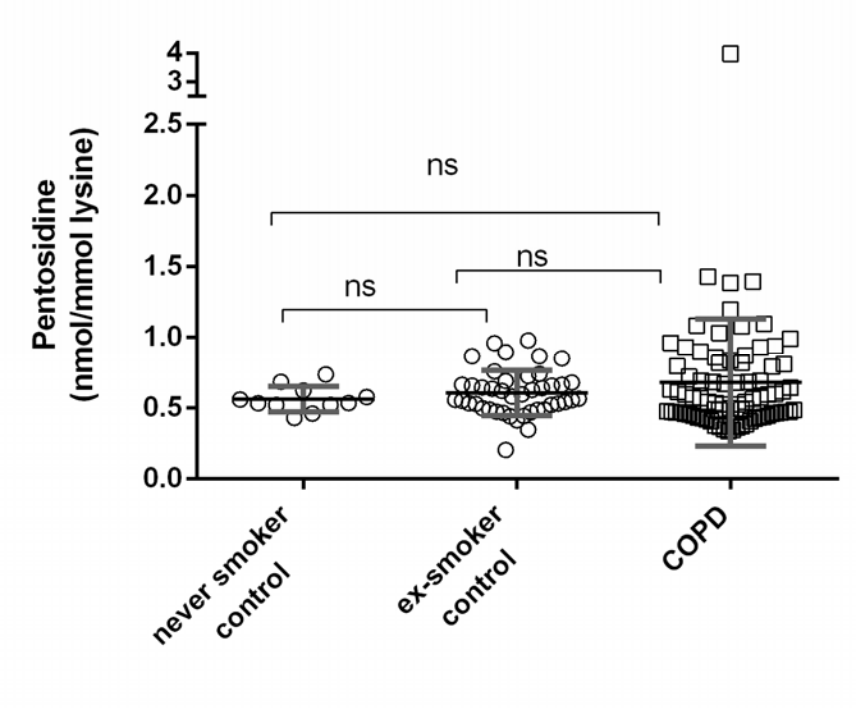
Figure legend:

Figure 1: Plasma levels of CML, CEL and pentosidine, and AFR in COPD patients and control subjects

(A) CML, (B) CEL (C), pentosidine and (D) AFR were measured in COPD patients and healthy never smoking, ex-smoking controls.




Pentosidine
(nmol/mmol lysine)
4
3
2.5
2.0
1.5
1.0
0.5
0.0
ns
ns
ns
never smoker
control
ex-smoker
control
COPD

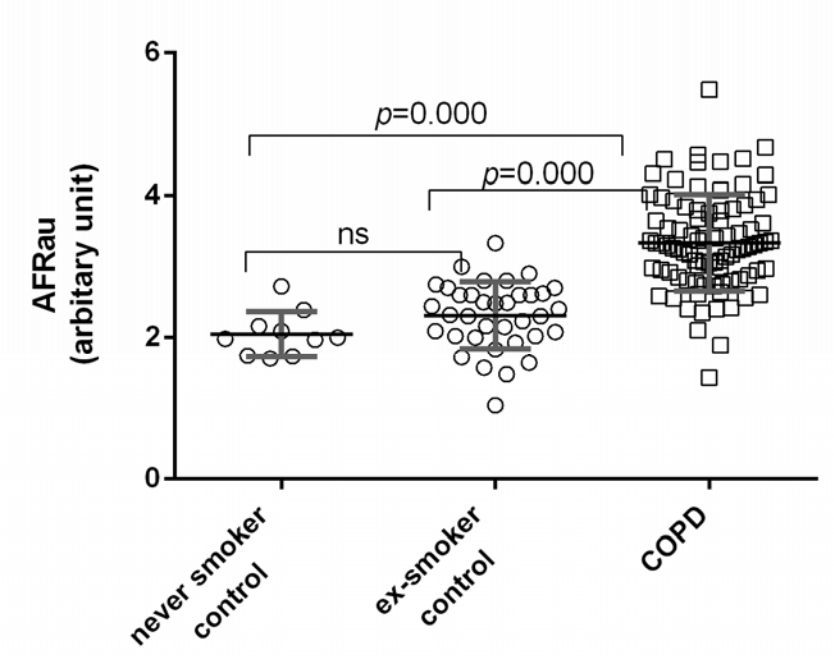

AFRau
(arbitary unit)
6
4
2
0
p=0.000
p=0.000
ns
never smoker
control
ex-smoker
control
COPD

Table 1: General characteristics of COPD patients and controls.

	Controls, n = 55	Stable COPD, n = 88	*p* value
Age	60 ± 6	63 ± 8	=0.016
Male, (n) %	(26) 47.3	(46) 52.3	=0.937
Pack year smoked *	12.2 ± 13.3	35.0 ± 16.1	<0.001
Smoking status, (n) % §	Ex-smoker = (43)78.2 Non smoker = (11)20	Ex-smoker = (64)72.7 Current smoker = (18)20.5	
BMI, Kg/ m^2	27.5 ± 3.9	26.1 ± 5.2	=0.069
FFMI, Kg/ m^2	18.2 ± 2.4	16.94 ± 2.8	=0.004
FEV_1 % predicted	118 (109.0-128.0)	45.50 (32.25-61.0)	<0.001
FEV_1/VC predicted	78.97 (75.2-82.43)	51.88 (38.25-63.25)	<0.000
DLCO % predicted	-	51.46(42.4-73.19)	
BODE score, points	-	3.50(2.0-8.0)	
Charlson comorbidity index, points	-	1.0(0.00-3.0)	
Triglycerides, mg/dl	4.5 (4.3-5.8)	4.69 (4.5-5.7)	=0.748
HDL cholesterol, mg/dl	68.3 ± 19	75 ± 27	=0.096
Glucose, mg/dl	102.5 ± 11.7	102 ± 69	=0.938
Creatinine, µ mol/L	82.3 ± 14.0	90.2 ± 24.8	=0.049
CRP, mg/L	0.64 (0.33-1.59)	3.4 (1.7-8.5)	<0.001
GFR, ml/min	77.1 ± 14.2	73.7 ± 20.8	=0.280
Protein carbonylation, nmol/ml	2.73 (2.7-4.1)	2.8 (2.7-4.0)	=0.254

Unless otherwise stated all data are expressed as mean ± SD or median (IQR).
* indicates = history of pack year smoked is missing for 34 patients but smoking stop date is available for this group.
§ indicates = smoking status of 6 patients and 1 control subject were missing because of missing information on packyear smoked or smoking stopped date.
BMI, body mass index; FFMI, fat free mass index; FEV_1, forced expiratory volume; HDL, high density lipoproteins; CRP, C- reactive proteins.

Table 2 Association of disease state with plasma AGEs and AFR (n=143)

Dependent variable	models	β [a]	95 %CI	*p*
CML	1	-1.054	-1.348 to -0.761	0.000
	2	-1.086	-1.396 to -0.777	0.000
	3	-1.203	-1.507 to -0.899	0.000
	4	-1.223	-1.569 to -0.878	0.000
CEL	1	0.982	0.680 to 1.284	0.001
	2	1.002	0.683 to 1.321	0.001
	3	0.915	0.588 to 1.241	0.001
	4	0.921	0.551 to 1.292	0.001
Ln-Pentosidine	1	0.067	-0.268 to 0.402	0.693
	2	0.046	-0.308 to 0.399	0.799
	3	-0.042	-0.386 to 0.301	0.808
	4	-0.075	-0.465 to 0.315	0.706
AFR	1	1.152	0. 894 to 1.410	<0.001
	2	1.042	0.776 to 1.308	<0.001
	3	1.029	0.751 to 1.306	<0.001
	4	1.080	0.766 to 1.395	<0.001

[a] β, standardized regression coefficient: indicates differences in plasma AGEs and AFR (in SD) in individuals with vs. without COPD.
Model 1, adjusted for age, sex
Model 2, model 1+ pack year
Model 3, model 2 + GFR, BMI, HDL cholesterol, LnTriglycerides
Model 4, model 3 + LnCRP

Table 3. Association of plasma AGEs and AFR with FEV1 %predicted (n=143)

	model	β[a]	95 %CI	*p*
CML	1	0.406	0.256 to 0.556	0.000
	2	0.405	0.249 to 0.562	0.000
	3	0.518	0.352 to 0.685	0.000
	4	0.508	0.316 to 0.701	0.000
CEL	1	-0.462	-0.608 to -0.316	<0.001
	2	-0.464	-0.617 to -0.312	<0.001
	3	-0.427	-0.598 to -0.256	0.000
	4	-0.426	-0.624 to -0.228	0.000
Ln-Pentosidine	1	0.022	-0.139 to 0.183	0.790
	2	0.035	-0.133 to 0.203	0.679
	3	0.087	-0.089 to 0.263	0.331
	4	0.127	-0.076 to 0.330	0.218
AFR	1	-0.443	-0.579 to -0.307	0.000
	2	-0.383	-0.520 to -0.246	0.000
	3	-0.402	-0.556 to -0.248	0.000
	4	-0.400	-0.578 to -0.222	0.000

[a] β, standardized regression coefficient: indicates increase (in SD) in FEV_1% per SD increase in plasma AGEs or AFR.
Model 1, adjusted for age, sex
Model 2, model 1+ pack year
Model 3, model 2 + GFR, BMI, HDL cholesterol, LnTriglycerides
Model 4, model 3 + LnCRP

Table 4 Associations of AFR with plasma AGEs (n=143)

	model	β[a]	95 %CI	*P*
CML	1	-0.324	-0.493 to -0.154	0.000
	2	-0.320	-0.502 to -0.138	0.001
	3	-0.316	-0.499 to -0.134	0.001
CEL	1	0.254	0.080 to 0.428	0.004
	2	0.238	0.052 to 0.424	0.013
	3	0.234	0.048 to 0.421	0.014
Ln-Pentosidine	1	0.056	-0.118 to 0.230	0.527
	2	0.046	-0.140 to 0.233	0.624
	3	0.057	-0.127 to 0.242	0.539

[a] β, standardized regression coefficient: indicates increase (in SD) in AFR per SD increase in plasma AGEs.
Model 1, adjusted for age, sex
Model 2, model 1+ pack year
Model 3, model 2 + GFR

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