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Prevalence of reduced lung diffusing capacity and CT scan findings in smokers without airflow limitation: a population-based study

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ABSTRACT

Background Population distribution of reduced diffusing capacity of the lungs for carbon monoxide (DLCO) in smokers and main consequences are not properly recognised. The objectives of this study were to describe the prevalence of reduced DLCO in a population-based sample of current and former smoker subjects without airflow limitation and to describe its morphological, functional and clinical implications.

Methods A sample of 405 subjects aged 40 years or older with postbronchodilator forced expiratory volume in 1 s/forced vital capacity (FVC) >0.70 was obtained from a random population-based sample of 9092 subjects evaluated in the EPISCAN II study. Baseline evaluation included clinical questionnaires, exhaled carbon monoxide (CO) measurement, spirometry, DLCO determination, 6 min walk test, routine blood analysis and low-dose CT scan with evaluation of lung density and airway wall thickness.

Results In never, former and current smokers, prevalence of reduced DLCO was 6.7%, 14.4% and 26.7%, respectively. Current and former smokers with reduced DLCO without airflow limitation were younger than the subjects with normal DLCO, and they had greater levels of dyspnoea and exhaled CO, greater pulmonary artery diameter and lower spirometric parameters, 6 min walk distance, daily physical activity and plasma albumin levels (all $p<0.05$), with no significant differences in other chronic respiratory symptoms or CT findings. FVC and exhaled CO were identified as independent risk factors for low DLCO.

Conclusion Reduced DLCO is a frequent disorder among smokers without airflow limitation, associated with decreased exercise capacity and with CT findings suggesting that it may be a marker of smoking-induced early vascular damage.

Trial registration number NCT03028207.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Diffusing capacity of the lungs for carbon monoxide (DLCO) provides information on the effective alveolar-capillary surface area available for gas transfer in the lungs.

WHAT THIS STUDY ADDS

⇒ Reduced DLCO is a frequent finding among smokers without airflow limitation.

⇒ This functional disorder is associated with a limited exercise tolerance and reduced daily physical activity, as well as a larger pulmonary artery diameter.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Absence of differences in the lung parenchyma attenuation suggests that DLCO may be a surrogate marker of smoking-induced early vascular lung damage.

INTRODUCTION

Cigarette smoke has been identified as a major risk factor for chronic obstructive pulmonary disease (COPD), a progressive destructive lung disease with persistent chronic inflammation characterised by airflow limitation,[1 2] that is, usually identified by spirometry, the most common lung function test.

Diffusing capacity of the lungs for carbon monoxide (DLCO), a complex test that provides a quantitative measure of the effective alveolar-capillary surface area available for gas transfer in the lungs, is also currently used in the clinical setting for the characterisation of subjects with COPD.[3] DLCO is useful in distinguishing COPD phenotypes[4 5] and for predicting increased symptoms, poor quality of life, decreased exercise tolerance and severe exacerbations in patients with COPD.[3] Moreover, among patients with airflow limitation, DLCO negatively correlates with the extend of emphysema on lung CT scans.[6]

Moreover, DLCO seems to have a role in the evaluation of at-risk subjects, in early stages of lung disease or when they have not yet developed it.[7] Thus, some evidence suggests that a

reduced DLCO predicts a future decline in forced expiratory volume in 1 s (FEV_1),[8 9] and it has been reported that, in smokers with normal spirometry, a low DLCO increases the risk of developing COPD.[10] Even a DLCO below 85% predicted has been found to be a significant predictor of all-cause mortality.[11]

This is particularly important, since DLCO could provide more integrated information about the lung abnormalities induced by cigarette smoking. Although most interest has focused on the contributions of cigarette smoke towards injuring the extracellular matrix and pulmonary epithelial cells, critical interdependence has been recognised between alveolar epithelial and microvascular endothelial cells to maintain the airspace structure, and loss of endothelial cells within the lung directly contributes to emphysematous remodelling.[12] In fact, along with persistent inflammation, protease-antiprotease imbalance and oxidative stress, smoking-induced endothelial damage has been linked to pulmonary lesions and systemic comorbidities, including pulmonary hypertension and atherosclerosis.[13]

Although DLCO provides insight into respiratory physiology beyond that obtained with spirometry, including indirect measurement of pulmonary vascular abnormalities, no previous information is available about the population prevalence of low DLCO in subjects with a history of smoking without airflow limitation as well as its clinical and physiological consequences.[14] The second Epidemiology of COPD in Spain (EPISCAN II) study, a population-based epidemiological study designed with the main objective of determining the prevalence of COPD in Spain through the analysis of a representative sample of adults from all Spanish regions,[15] provides an ideal opportunity to analyse these aspects. This manuscript presents the results of a secondary objective which was to assess the prevalence of DLCO alterations in a population-based sample of current or former smoker subjects without airflow limitation, to identify its determinants and to evaluate the physiological and clinical parameters related to DLCO in these subjects.

METHODS

EPISCAN II is a national, multicentre, cross-sectional, population-based epidemiological study, whose protocol, fieldwork and methods have been previously described.[15 16] Briefly, the study was conducted at 20 teaching hospitals throughout Spain from April 2017 to February 2019.

Patient and public involvement

Patients and public were not involved in the development of the research question, design or recruitment; no patient advisors were required, and data were analysed anonymously. The results will be disseminated to the scientific community in academic writing.

Study subjects

Subjects from the general population, who resided in the postal code areas nearest to the participating hospitals, were selected. A list of random telephone numbers was obtained, stratified according to these postal codes and quotas for sex and age groups. We selected men or women aged 40 years or more, with no physical or cognitive difficulties that would prevent them from completing spirometry or any of the study procedures. To evaluate the secondary outcomes, the first 35 subjects with airflow limitation and the first 35 subjects without airflow limitation from 12 preselected sites were consecutively invited to undergo a DLCO test and thoracic CT scan, with the aim to recruit a total of approximately 400 individuals for each group.

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

For the purposes of this prespecified secondary objective of EPISCAN II, a current smoker was defined as an individual who smoked at least one cigarette, cigar or pipe a day and a former smoker was defined as an individual who had discontinued using any form of tobacco at least 6 months before the study visit. Current or former smoker subjects were included in the analysis if they had a postbronchodilator FEV_1/forced vital capacity (FVC) >0.70 and a cumulative tobacco use of at least 10 pack-years. As a control group, never-smoker subjects with a postbronchodilator FEV_1/FVC >0.7 were included.

Procedures and measurements

Demographic information on anthropometric characteristics, level of education, family conditions, smoking history and comorbidities were collected. Comorbidities were assessed using the Charlson and COPD-specific co-morbidity test (COTE) indices.[17 18] Health status was assessed by the COPD Assessment Test (CAT) questionnaire,[19] and the respective questions of the European Community for Coal and Steel Questionnaire (ECSC)[20] were used to identify respiratory symptoms (chronic cough, chronic bronchitis, chronic expectoration, dyspnoea or wheezing). The degree of dyspnoea was evaluated by the modified Medical Research Council dyspnoea scale.[21] Physical activity was measured by the Yale Physical Activity Survey questionnaire validated for the Spanish population and the elderly population, providing a summary of the physical activity level, as well as the time and energy cost of weekly physical activity.[22]

Prebronchodilator and postbronchodilator spirometry was performed using a pneumotachograph (Vyntus Spiro, Carefusion, Germany), according to American Thoracic Society (ATS)/European Respiratory Society (ERS) standardisation[23] and using the Global Lung Initiative equations as reference values.[24] DLCO was measured by the single-breath method with the same equipment in all study centres (MasterScreen diffusion, Carefusion, Germany) in accordance with ATS/ERS

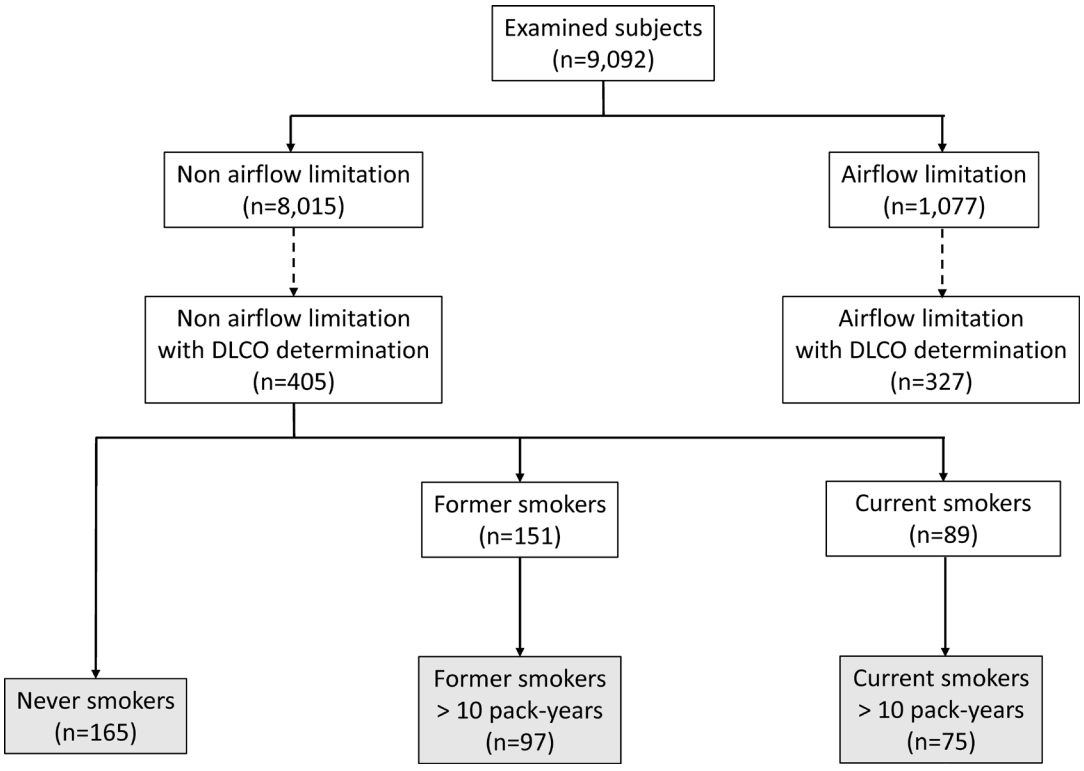

Figure 1 Flow chart of study participants. DLCO, diffusing capacity of the lungs for carbon monoxide.

recommendations.[5] The DLCO was corrected to body temperature, pressure, water vapour saturated conditions and a minimum of two acceptable manoeuvres that matched within 10% or less of the alveolar volume (VA) and DLCO was required. We excluded tests with a breath-holding time <9 or >11 s, an inspiratory capacity less than 85% of the largest previously measured vital capacity, expiration in >4 s, or with evidence of leaks or Valsalva or Müller manoeuvres. Adjustments were made for atmospheric pressure, haemoglobin levels and CO back pressure using non-invasive estimation of carboxyhaemoglobin by a CO-oximeter according to equations recommended in current standardisation.[25] Cotes equations were used as reference values,[26] and values below the lower limit of normal were considered reduced.

The 6 min walk test was performed in accordance with ATS guidelines,[27] and the Body Obstructive Dyspnoea Exercise (BODE) index[28] was calculated accordingly. From each participant, 20 mL of venous blood were collected for routine blood analysis, C reactive protein, fibrinogen and albumin.

CT images were acquired during maximal inspiration, without contrast and with low-dose radiation (120 kVp as acquisition voltage). The images obtained underwent semiautomatic postprocessing for determination of the percentage of emphysema, areas of low attenuation and bronchiolar airway wall thickness, as previously described.[16 29] The pulmonary artery (PA) diameter was measured at the level of the PA bifurcation, and the average of two perpendicular measurements of the ascending aorta diameter were taken on the same CT image using mediastinal windows.[30] PA enlargement was defined as a PA diameter ≥29 mm in men and ≥27 mm in women.[31]

Statistical analysis

Categorical variables were presented as numbers with percentages, and continuous variables as mean with SD or median (IQR), according to their distribution. Comparisons between subgroups have been performed using the Student's t-test, analysis of variance, Mann-Whitney U test or χ^2 test. The relationship between variables has been assessed with the Pearson correlation analysis. Multiple logistic regression analysis has been used to investigate factors associated with a reduced DLCO. Only variables with a level of significance <0.01 in the bivariate analysis were included in the multiple regression model. The ORs and the coefficient of determination (r^2) were calculated for the model. Data were analysed with the SPSS V.25.0 software, considering a p value of 0.05 statistically significant for all tests.

RESULTS

The EPISCAN II population included 9092 subjects who were able to perform a valid spirometry. Among those, 8015 had no airflow limitation, and 405 of them were invited to a longer office visit with DLCO determination and a CT scan. This subgroup was similar to the remaining patients without airflow limitation in terms of demographic characteristics, smoking intensity, comorbidities and spirometric parameters (online

Table 1 Demographic and clinical characteristics of the study subjects*

	Current smokers (n=75)	Former smokers (n=97)	Never-smokers (n=165)	P value
Females, n (%)	46 (61.3)	43 (44.3)	115 (69.7)	<0.001
Age, years	56±8	62±10	61±12	<0.001
BMI, kg/m^2	26.1±4.6	28.1±3.9	27.4±5.2	0.023
Level of studies, n (%)				0.051
No studies	0	1 (1.0)	2 (1.2)	
Primary education	7 (9.3)	24 (24.7)	33 (20.0)	
Secondary education	23 (30.7)	14 (14.4)	32 (19.4)	
University	44 (58.7)	58 (59.8)	98 (59.4)	
Lives alone, n (%)	14 (18.7)	22 (22.7)	22 (13.3)	0.143
Pack-years	32±14	32±23	–	0.839
Charlson Comorbidity Index	0.3±0.7	0.5±1.1	0.3±0.8	0.294
COTE index	1.4±2.6	0.8±1.8	1.05±2.4	0.212
CAT score	9.2±6.6	6.8±5.4	6.2±5.6	0.001
Chronic cough, n (%)	27 (36.5)	12 (12.5)	18 (11.2)	<0.001
Chronic bronchitis, n (%)	10 (15.2)	3 (3.2)	4 (2.5)	<0.001
Chronic expectoration, n (%)	21 (28.0)	14 (14.7)	14 (8.5)	<0.001
Dyspnoea, n (%)	6 (8.2)	16 (16.7)	26 (16.5)	0.209
Wheezing, n (%)	42 (56.8)	27 (27.8)	41 (24.8)	<0.001
Baseline SaO_2, %	96±1	96±1	96±2	0.638
Exhaled CO, ppm	25±16	3±4	4±5	<0.001
Prebronchodilator FVC, z-score	0.07±0.96	0.21±1.00	0.26±0.93	0.365
Prebronchodilator FEV_1, z-score	−0.17±1.04	0.05±1.09	0.21±1.01	0.031
Prebronchodilator FEV_1/FVC, z-score	−0.46±0.77	−0.31±0.85	−0.13±0.83	0.014
Postbronchodilator FVC, z-score	0.10+0.85	0.24+0.96	0.32+0.99	0.164
Postbronchodilator FEV_1, z-score	−0.15+0.98	0.06+1.11	0.19+1.12	0.011
Postbronchodilator FEV_1/FVC, z-score	−0.45+0.80	−0.32−0.91	−0.15+1.07	0.002
6MWD, m	508±102	523±94	510±101	0.539
DLCO, z-score	−0.80±0.60	−0.30±0.71	−0.26±0.68	<0.001
DLCO/VA, z-score	−0.10±0.12	−0.02±0.12	−0.01±0.15	0.005
VA, z-score	−0.46±0.66	−0.31±0.68	−0.25±0.66	0.086
Haemoglobin, g/L	148±14	146±14	140±14	<0.001
Eosinophils, %	2.1±1.5	2.3±1.6	2.0±1.6	0.440
Fibrinogen, g/L	3.9±0.8	3.8±0.9	3.8±1.0	0.910
C reactive protein, mg/L	1.4±2.8	0.9±1.5	1.5±2.8	0.134
Albumin, g/l	12.1±16.2	17.1±18.4	12.5±16.3	0.717
BODE index	1.0±0.5	1.1±0.5	1.1±0.6	0.195
YPAS—physical activity summary	55±22	62±24	57±21	0.236
Total emphysema volume, %	1.9±4.4	2.6±3.7	1.7±3.4	0.276
Low density volume, %	47±20	51±18	41±18	0.005
P15, HU	−910±31	−919±26	−904±26	0.003
Secondary bronchi WA, %	25.4±5.7	25.3±5.7	24.5±5.4	0.392
Pulmonary artery diameter, mm	26±4	27±4	24±4	0.074
Pulmonary artery:aorta ratio	0.78±0.11	0.78±0.11	0.79±0.12	0.811

Comparison between groups using ANOVA or chi-squared test.
*Data are mean±SD or number (percentage).
ANOVA, analysis of variance; BMI, body mass index; BODE, Body Obstructive Dyspnoea Exercise; CAT, chronic obstructive pulmonary disease assessment test; CO, carbon monoxide; COTE, Chronic obstructive pulmonary disease-specific co-morbidity test; DLCO, lung diffusing capacity of carbon monoxide; FEV_1, forced expiratory volume at 1 s; FVC, forced vital capacity; HU, Hounsfield units; 6MWD, 6 min walk distance; P15, 15th percentile; SaO_2, oxygen saturation; VA, alveolar volume; WA, wall area; YPAS, Yale Physical Activity Survey.

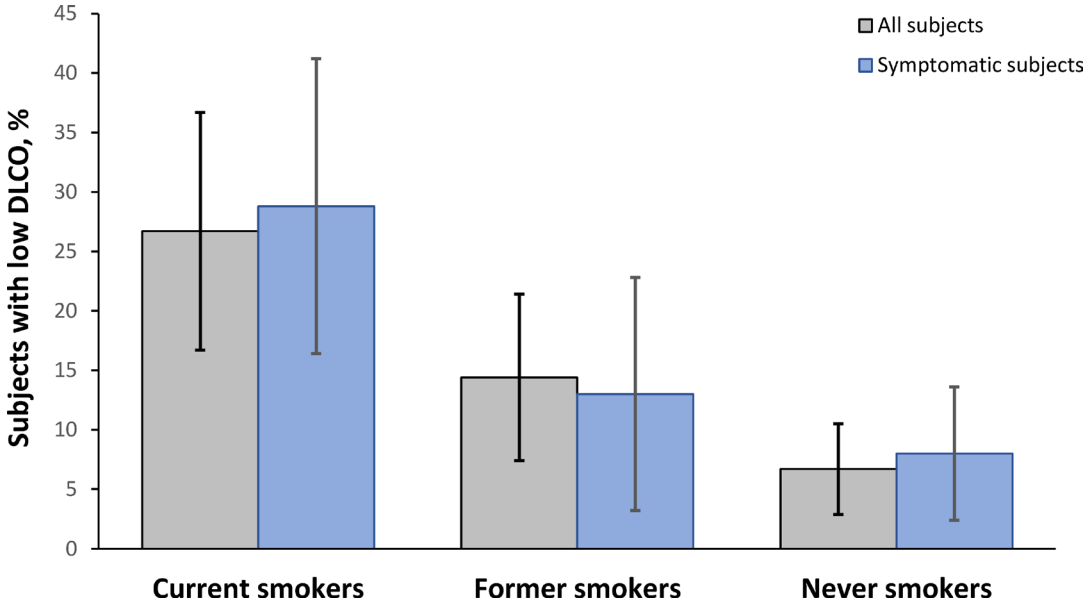

Figure 2 Frequency of a reduced pulmonary diffusion capacity among the different subgroups of the study, considering all the subjects analysed and only those who reported chronic respiratory symptoms. DLCO, diffusing capacity of the lungs for carbon monoxide.

supplemental table 1). According to previous definitions, these subjects were classified as never-smokers (n=165), former smokers (n=151) or current smokers (n=89). Last, in the two subsamples of smokers only those subjects with a cumulative use of tobacco greater than 10 pack-years were selected (figure 1). Table 1 summarises the main characteristics of the three study groups.

Prevalence of lung diffusing capacity reduction

In the subjects selected for the analysis, the frequency of a DLCO below its lower limit of normal was higher in current smokers (26.7%; 95% CI 16.7% to 36.7%) than in former smokers (14.4%; 95% CI 7.4% to 21.4%) or never-smokers (6.7%; 95% CI 2.9% to 10.5%) (p<0.001). When the analysis was limited to subjects with self-reported chronic respiratory symptoms using the ECSC questionnaire, the frequencies obtained in the three subgroups showed a similar pattern (figure 2).

Clinical and functional variables related to DLCO

In current and former smoker subjects, women presented lower z-scores than men, both for DLCO (−0.64±0.57 vs −0.34±0.80, p=0.001) and DLCO/VA (−0.11±0.12 vs 0.01±0.12, p<0.001). Table 2 shows the relationship of both the DLCO and the DLCO/VA ratio with the main clinical, functional and morphological variables in all subjects with previous or current tobacco use. In them, DLCO presented an inversely proportional relationship with nicotine dependence assessed by the Fagerström test, health status and exhaled CO levels. In turn, DLCO was directly proportional to spirometric parameters, exercise tolerance (assessed by the distance walked for 6 min), and the level of daily physical activity. Finally, a negative relationship was identified between both DLCO and the DLCO/VA ratio with the diameter of the PA, although without differences in its ratio with the ascending aorta diameter. No other differences in DLCO or DLCO/VA ratio were detected based on the presence of chronic respiratory symptoms (online supplemental table 2).

The analysis of parameters related to DLCO and DLCO/VA ratio carried out separately in current and former smoker subjects (online supplemental tables 3 and 4) showed a trend similar to those described in the joint analysis of both subgroups.

Differences of smoker subjects with reduced or normal DLCO

The comparison of current or former smokers with a DLCO below their lower limit of normal versus subjects with normal DLCO (table 3) shows that DLCO reduction occurs in younger subjects with a higher level of exhaled CO. In addition, smokers with reduced DLCO have lower spirometric parameters, higher levels of dyspnoea, lower exercise tolerance and less daily physical activity. In fact, subjects with reduced DLCO perform less physical activity (median (IQR)) (35 (24–48) vs 57 (30–82) hour/week, p=0.005) and reach a lower energy cost (117 (82–120) vs 187 (99–281) MET × hour/week, p=0.004). Moreover, smokers with low DLCO also have worse BODE scores and lower plasma albumin levels. In turn, larger PA diameters were found in smoker subjects with reduced DLCO compared with those with normal DLCO. In fact, subjects with reduced DLCO showed a tendency towards PA enlargement, although without reaching

Table 2 Relation between diffusion parameters and clinical, functional and morphological variables in subjects with smoking history of more than 10 pack-years*

	DLCO, z-score		DLCO/VA, z-score	
	R	P value	R	P value
Age, years	0.017	0.829	−0.079	0.303
BMI, kg/m^2	0.127	0.097	**0.295**	**<0.001**
Pack-years	−0.054	0.478	−0.041	0.595
Fagerström test	**−0.291**	**0.010**	**−0.270**	**0.017**
Charlson index	0.020	0.799	0.103	0.179
COTE index	−0.115	0.134	0.010	0.892
CAT total score	**−0.194**	**0.011**	−0.084	0.271
mMRC dyspnoea scale	−0.299	0.565	0.041	0.938
Baseline SaO_2, %	0.093	0.227	0.046	0.549
Exhaled CO, ppm	**−0.370**	**<0.001**	**−0.302**	**<0.001**
Prebronchodilator FVC, z-score	**0.352**	**<0.001**	**−0.136**	**0.074**
Prebronchodilator FEV_1, z-score	**0.393**	**<0.001**	−0.023	0.7666
Prebronchodilator FEV_1/FVC, z-score	0.148	0.053	**0.194**	**0.011**
6MWD, m	**0.206**	**0.007**	0.020	0.793
Haemoglobin, g/L	0.087	0.261	0.117	0.133
Eosinophils, %	0.041	0.599	**0.154**	**0.047**
Fibrinogen, g/L	−0.073	0.390	−0.098	0.248
C reactive protein, mg/L	−0.079	0.326	−0.020	0.800
Albumin, g/L	0.295	0.182	−0.114	0.613
BODE index	−0.020	0.791	**0.174**	**0.023**
YPAS—physical activity summary	**0.218**	**0.043**	0.148	0.175
Total emphysema volume, %	0.020	0.812	−0.064	0.440
Low density volume, %	0.050	0.547	−0.021	0.801
P15, HU	−0.089	0.280	−0.038	0.650
Secondary bronchi WA, %	0.002	0.978	0.008	0.926
Pulmonary artery diameter, mm	**−0.294**	**<0.001**	**−0.381**	**<0.001**
Pulmonary artery:aorta ratio	0.027	0.748	0.082	0.321

Bold values highlight significant relationships.
*Pearson correlation coefficient (r) and p value are shown.
BMI, body mass index; BODE, Body Obstructive Dyspnoea Exercise; CAT, Chronic Obstructive Pulmonary Disease Assessment Test; CO, carbon monoxide; COTE, Chronic obstructive pulmonary disease-specific co-morbidity test; DLCO, lung diffusing capacity of carbon monoxide; FEV_1, forced expiratory volume at 1 s; FVC, forced vital capacity; HU, hounsfield units; mMRC, modified Medical Research Council; 6MWD, 6 min walk distance; P15, 15th percentile; SaO_2, oxygen saturation; VA, alveolar volume; WA, wall area; YPAS, Yale Physical Activity Survey.

the level of statistical significance (26.7 vs 12.6%, p=0.083). Online supplemental tables 5 and 6 present the comparison between subjects with reduced and normal DLCO, evaluating current smokers and former smokers separately.

Finally, from all those variables that reached statistical significance in the comparison between reduced and normal DLCO in current or former smokers, the multiple logistic regression model only retained prebronchodilator FVC and exhaled CO as independent predictors of reduced DLCO (table 4), although the model contribution to explained variance in DLCO was modest (r^2=0.153, p<0.001). The same variables were retained in multiple logistic regression models separately for current and former smokers (online supplemental tables 7 and 8).

DISCUSSION

The main finding of our study is that reduced DLCO is present in more than a quarter of current smokers without airflow limitation, being associated with lower spirometric parameters, as well as reduced exercise tolerance and less daily physical activity. Furthermore, a reduced FVC and increased exhaled CO have been

Table 3 Comparison between current or former smoker subjects according to the lung diffusing capacity*

	Reduced DLCO (n=34)	Normal DLCO (n=138)	P value
Females, n (%)	21 (61.8)	68 (49.3)	0.133
Age, years	**54 (48–64)**	**60 (54–67)**	**0.028**
BMI, kg/m^2	26.7 (21.6–29.1)	27.1 (24.4–30.0)	0.237
Level of studies			0.070
No studies	1 (2.9)	0	
Primary education	7 (20.6)	24 (17.4)	
Secondary education	6 (17.6)	31 (22.5)	
University	19 (55.9)	83 (60.1)	
Lives alone	5 (14.7)	31 (22.5)	0.227
Pack-years	25 (20–39)	30 (20–40)	0.529
Fagerström test	4 (3–6)	4 (2–5)	0.682
Charlson Comorbidity Index	0.4±0.8	0.4±1.0	0.861
COTE index	1.9±2.7	0.9±2.0	0.066
CAT total score	5.0 (4.5–15.5)	4 (2-8)	0.531
mMRC dyspnoea level			**0.037**
0	**23 (67.6)**	**105 (76.1)**	
1	**8 (23.5)**	**30 (21.7)**	
2	**1 (2.9)**	**3 (2.2)**	
3	**2 (5.9)**	**0**	
Chronic cough, n (%)	8 (24.2)	31 (22.6)	0.503
Chronic bronchitis, n (%)	2 (6.9)	11 (8.5)	0.565
Chronic expectoration, n (%)	7 (20.6)	28 (20.6)	0.604
Wheezing, n (%)	15 (44.1)	54 (39.4)	0.378
Baseline oxygen saturation, %	97 (96–97)	96 (96–97)	0.863
Exhaled CO, ppm	**31 (29–32)**	**12 (2–31)**	**0.006**
Prebronchodilator FVC, z-score	**–0.43 (–1.00 to 0.40)**	**0.36 (–0.29 to 0.95)**	**<0.001**
Prebronchodilator FEV_1, z-score	**–0.44 (–1.32 to –0.10)**	**0.03 (–0.54 to 0.91)**	**0.001**
Prebronchodilator FEV_1/FVC, z-score	–0.48 (–0.85 to –0.06)	–0.34 (–0.99 to 0.28)	0.501
Reduced VA, n (%)	3 (8.8)	2 (1.4)	0.053
6MWD, m	**465 (450–480)**	**555 (533–605)**	**0.047**
Haemoglobin, g/L	147 (144–149)	153 (148–170)	0.821
Eosinophils, %	0.1 (0.1–0.1)	0.2 (0.1–1.9)	0.435
Fibrinogen, mg/dL	4.4 (3.9–4.9)	3.5 (2.9–4.1)	0.698
C reactive protein, mg/L	0.7 (0.2–1.3)	0.4 (0.1–3.5)	0.404
Albumin	**4.2 (4.2–4.3)**	**4.7 (4.6–39.6)**	**<0.001**
BODE index			**0.006**
0–2 points (survival: 80%)	**30 (88.2)**	**137 (99.3)**	
3–4 (survival: 67%)	**4 (11.8)**	**1 (0.7)**	
Physical activity summary	**29 (24–62)**	**53 (34–69)**	**0.048**
Total emphysema volume, %	0.69 (0.18–2.80)	0.34 (0.17–2.79)	0.261
Low density volume, %	53 (34–63)	48 (24–54)	0.210
P15, HU	–911 (–917 to –886)	–905 (–930 to –889)	0.127
Secondary bronchi WA, %	25.8 (22.8–27.5)	25.3 (20.2–29.8)	0.928
Pulmonary artery diameter, mm	**25 (24–27)**	**23 (21–24)**	**0.002**

Continued

Table 3 Continued

	Reduced DLCO (n=34)	Normal DLCO (n=138)	P value
Pulmonary artery:aorta ratio	0.77 (0.72–0.79)	0.70 (0.65–0.71)	0.578
Pulmonary artery enlargement	8 (26.7%)	16 (13.4%)	0.095

Comparisons were performed by Student's t-test, Mann-Whitney U or χ^2 tests.
Bold values highlight significant differences.
*Data are mean±SD, median (IQR) or number (frequency) according to their type and distribution.
BMI, body mass index; BODE, body obstructive dyspnoea exercise; CAT, chronic obstructive pulmonary disease assessment test; CO, carbon monoxide; DLCO, lung diffusing capacity for carbon monoxide; FEV_1, forced expiratory volume at 1 s; FVC, forced vital capacity; LLN, lower limit of normal; mMRC, modified Medical Research Council; 6MWD, 6 min walk distance; P15, 15th percentile; VA, alveolar volume; WA, wall airway.

identified as independent predictors of reduced DLCO in smokers without airflow limitation.

As previous methodological comments, it is worth mentioning that the procedure followed for study subject selection generated a sample with a prevalence of current and former smokers that are the same as in the general population of our country (22% and 25%, respectively).[32] In fact, to our knowledge, this is the first article that shows a population approach to the evaluation of DLCO with simultaneous measurements of low dose CT scan in smokers without airflow limitation.

The population prevalence of DLCO below the LLN identified in our study in current and former smokers (19.8%) is consistent with the 17% previously described in active smokers selected from advertisements to assess lung health[10] and 24% identified in a retrospective analysis of a lung function database.[33] In addition, our data confirm the relationship of DLCO with some previously identified variables, such as male gender or spirometric parameters.[34–36] However, the sample analysed has not identified a relationship between DLCO and the presence of chronic respiratory symptoms. This finding is also consistent with a small study of 58 smokers without airflow limitation, in which subjects who reported non-specific respiratory problems, chronic bronchitis or wheezing had lower spirometric parameters, but no differences were found in DLCO or with the single-breath nitrogen method, suggesting that symptoms are more related to physiological changes in the central airways than in the peripheral airways.[37]

Although some previous studies have described a weak relationship between the DLCO of heavy smokers (>20 pack-years) and lung attenuation,[6 38] they included a notable percentage of patients with airflow limitation. Thus, in a subsample of smokers or former smokers from the GenKOLS study, DLCO was related to the percentage of low-attenuation areas and to the standardised airway wall thickness only in patients with COPD, while in subjects without airflow limitation the same relationship was not significant.[39] Similarly, in 38 former smokers without airflow limitation, no differences in low-attenuation areas or bronchial wall thickness were identified between those with normal or low DLCO.[40] This suggests that, in early phases of smoking-induced lung damage, the decrease in DLCO is not attributable to emphysema-like changes. As DLCO decreases in a wide variety of pathologic conditions, including reduction in alveolar surface area, decreased perfusion, even ventilation or inflammation or fibrosis of the alveolar wall impairing alveolar diffusion,[5] impaired gas exchange may not necessarily reflect early emphysema, since it may be due to other smoking-related changes, including altered membrane diffusion or pulmonary vascular changes.[41] The contribution of peripheral airways to DLCO reduction has been suggested in never-smokers from the COPDGene cohort, in which small airway dysfunction correlated significantly with lower DLCO among both non-obstructed and GOLD 1–2 subjects.[42] This might justify that, in our smoker subjects, FVC was retained as an independent predictor of low DLCO, instead of FEV_1, since the former

Table 4 Multiple logistic regression model to detect decreased DLCO in current and former smoker subjects*

Variable	B	SE	P value	OR	95% CI
Prebronchodilator FVC, z-score	−0.815	0.225	<0.001	0.443	0.285 to 0.688
Exhaled CO, ppm	0.030	0.013	0.018	1.030	1.005 to 1.056
Constant	−1.910	0.301	–	–	–

*Exhaled CO level, prebronchodilator FVC, prebronchodilator FEV_1, serum albumin level and pulmonary artery diameter were entered into the model. The BODE index was not entered because it was considered redundant and not strictly applicable to subjects without airflow limitation.
B, regression coefficient; BODE, body obstructive dyspnoea exercise; CI, confidence interval; CO, carbon monoxide; DLCO, diffusing capacity of the lungs for carbon monoxide; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; OR, odds ratio.

better represents the contribution of the whole bronchial tree, including its most distal portions.

In this study, the other risk factor independently associated with the presence of reduced DLCO was increased exhaled CO. The DLCO correction for exhaled CO suggests that its contribution does not seem to be exclusively dependent on CO backpressure, so it is interesting to consider that exhaled CO is a recognised indicator of oxidative stress,[43] one of the main causes of endothelial damage. This possibility is reinforced by the identification in smokers with reduced DLCO of low levels of albumin, a negative acute-phase reactant with anti-inflammatory and antioxidant properties.[44] Indeed, it has been suggested that albumin level may be a marker of susceptibility to the oxidative response resulting from smoking, while albuminuria should be a non-invasive marker of arterial stiffness.[45] Although merely speculative, these findings are in agreement with some current evidence suggesting that peripheral airway destruction might be initiated, in part, by early smoking-induced damage to the pulmonary vascular endothelium mediated by plasma endothelial microparticles with apoptotic features, which are elevated in smokers with normal spirometry and reduced DLCO.[46] It is particularly attractive to consider that, in the absence of lung parenchymal damage, DLCO provides a window into the microvasculature of smoker subjects. Thus, the lower DLCO of smokers probably reflects the presence of greater ventilation-perfusion inequalities, lower carbon dioxide elimination efficiency and, consequently, greater ventilatory stimulation,[47] which, in turn, would justify the higher degree of dyspnoea reported by our smokers with reduced DLCO. This basic physiological proposal is in line with studies about exercise response in smokers at risk of COPD, in which exercise dyspnoea is mainly explained by increased inspiratory neural drive.[47] Furthermore, this could also explain their lower exercise tolerance, consistently with the previous description of a correlation between low DLCO and reduced 6 min walk distance in former smokers with normal chest CT scans and spirometry.[40] However, since the EPISCAN study protocol did not include the determination of functional residual capacity or inspiratory capacity, it is not possible to completely rule out the existence of a certain degree of hyperinflation not detected by lung imaging tests, which is a main determinant of exercise tolerance in patients with airflow limitation.[48]

Finally, one last aspect of our study to highlight is the larger diameter of the PA found in smokers with reduced DLCO compared with those with normal DLCO. Although this difference does not lead to significant differences in the PA enlargement between the two smoker subgroups or in the relationship between the diameter of the PA and the ascending aorta, which is the most consistent indicator of pulmonary hypertension,[49] they might also reflect an early impact of smoking on the pulmonary vascular bed. In this regard, it is interesting to note that cigarette smoking has recently been shown to contribute to pulmonary arterial remodelling through several hypoxia-independent pathways that promote K+channel dysregulation in the absence of clinically established COPD.[50]

Our study has several limitations that are worth discussing. First, the cross-sectional design does not allow for cause-and-effect relationships to be established, although the data reveal that reduced DLCO is associated with differences in dyspnoea level, exercise tolerance and PA diameter. Second, the sample size may be limiting for some subanalyses, despite providing a representative sample of smokers at the population level and achieving statistical significance in most comparisons. Third, other spirometric parameters suggestive of peripheral airway disease have not been evaluated, such as the FEV_3/FEV_6 ratio, whose decrease is highly prevalent in smokers with preserved pulmonary function but impaired indices of physical function and quality of life.[51 52] And fourth, the EPISCAN II study did also not include specific measurements of small airway function or pulmonary microcirculation to allow for the specific assessment of their relationship with a reduced DLCO.

In conclusion, this study shows that reduced DLCO is a frequent finding among smokers without airflow limitation and is associated with a limited exercise tolerance and reduced daily physical activity, as well as a larger PA diameter. These findings, together with increased exhaled CO and reduced plasma albumin, in the absence of differences in the lung parenchyma attenuation, allow us to speculate that, in these subjects, DLCO may be a surrogate marker of smoking-induced early vascular lung damage.

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