

## ORIGINAL ARTICLE

# On the role of abnormal DL<sub>CO</sub> in ex-smokers without airflow limitation: symptoms, exercise capacity and hyperpolarised helium-3 MRI

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## ABSTRACT

**Background** The functional effects of abnormal diffusing capacity for carbon monoxide (DL<sub>CO</sub>) in ex-smokers without chronic obstructive pulmonary disease (COPD) are not well understood.

**Objective** We aimed to evaluate and compare well established clinical, physiological and emerging imaging measurements in ex-smokers with normal spirometry and abnormal DL<sub>CO</sub> with a group of ex-smokers with normal spirometry and DL<sub>CO</sub> and ex-smokers with Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage I COPD.

**Methods** We enrolled 38 ex-smokers and 15 subjects with stage I COPD who underwent spirometry, plethysmography, St George's Respiratory Questionnaire (SGRQ), 6 min Walk Test (6MWT), x-ray CT and hyperpolarised helium-3 (<sup>3</sup>He) MRI. The 6MWT distance (6MWD), SGRQ scores, <sup>3</sup>He MRI apparent diffusion coefficients (ADC) and CT attenuation values below -950 HU (RA<sub>950</sub>) were evaluated.

**Results** Of 38 ex-smokers without COPD, 19 subjects had abnormal DL<sub>CO</sub> with significantly worse ADC (p=0.01), 6MWD (p=0.008) and SGRQ (p=0.01) but not RA<sub>950</sub> (p=0.53) compared with 19 ex-smokers with normal DL<sub>CO</sub>. Stage I COPD subjects showed significantly worse ADC (p=0.02), RA<sub>950</sub> (p=0.0008) and 6MWD (p=0.005), but not SGRQ (p=0.59) compared with subjects with abnormal DL<sub>CO</sub>. There was a significant correlation for <sup>3</sup>He ADC with SGRQ (r=0.34, p=0.02) and 6MWD (r=-0.51, p=0.0002).

**Conclusions** In ex-smokers with normal spirometry and CT but abnormal DL<sub>CO</sub>, there were significantly worse symptoms, 6MWD and <sup>3</sup>He ADC compared with ex-smokers with normal DL<sub>CO</sub>, providing evidence of the impact of mild or early stage emphysema and a better understanding of abnormal DL<sub>CO</sub> and hyperpolarised <sup>3</sup>He MRI in ex-smokers without COPD.

## INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterised by chronic progressive expiratory flow limitation that develops as a result of the lung's inflammatory response to inhaled toxic gases and particles, primarily from tobacco smoke.<sup>1</sup> In COPD, airflow limitation is caused by both small airway disease (obstructive bronchiolitis) and parenchymal destruction (emphysema)<sup>1</sup> but the relative contributions of these pathologies vary from person to person.

## Key messages

### What is the key question?

- ▶ The functional effects of abnormal DL<sub>CO</sub> in ex-smokers without airflow limitation are not well understood. To try to better understand the role of abnormal DL<sub>CO</sub> in ex-smokers without COPD, we evaluated and compared clinical, physiological and emerging imaging measurements in ex-smokers with normal spirometry and DL<sub>CO</sub>, ex-smokers with normal spirometry but abnormal DL<sub>CO</sub> and those with GOLD stage I COPD.

### What is the bottom line?

- ▶ We evaluated 53 ex-smokers including 15 subjects with stage I COPD and 38 subjects without airflow limitation. Of the 38 ex-smokers without airflow limitation, 19 had abnormal DL<sub>CO</sub> and significantly worse symptoms, 6MWD and <sup>3</sup>He ADC compared with the 19 ex-smokers with normal DL<sub>CO</sub> although CT derived measurements of emphysema were not significantly different.

### Why read on?

- ▶ Abnormal DL<sub>CO</sub> in ex-smokers without airflow limitation was related to worse symptoms, exercise capacity and <sup>3</sup>He ADC compared with ex-smokers with normal DL<sub>CO</sub>, providing evidence of the impact of DL<sub>CO</sub> abnormalities consistent with early or very mild emphysema and revealed by <sup>3</sup>He MRI but not CT. Abnormal DL<sub>CO</sub> measurements in ex-smokers without COPD should be followed-up to evaluate potential progression of disease.

When COPD is suspected based on symptoms, such as dyspnoea, chronic cough or sputum production, and/or a history of exposure to risk factors,<sup>1</sup> airflow limitation is measured using spirometry and severity is determined according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria.<sup>1</sup> This approach, however, has been acknowledged to potentially result in an over diagnosis of COPD in the elderly,<sup>2</sup> as well as under diagnosis of mild or early stage COPD.<sup>3</sup>

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The COPDGene study recently reported low forced expiratory volume in 1 s (FEV<sub>1</sub>) and normal FEV<sub>1</sub>/forced vital capacity (FVC) in ex-smokers with significant symptoms and decreased 6 min Walk Distance (6MWD), and defined these patients as GOLD unclassified (GOLD-U).<sup>4</sup> Until now, ex-smokers with GOLD-U or those with 'non-obstructive' or 'pure' emphysema without airflow limitation have been systematically excluded from COPD studies. With respect to non-obstructive emphysema, there have been a few case reports<sup>5-7</sup> and pilot studies<sup>8</sup> that described significant smoking history, severe symptoms and abnormal diffusing capacity for carbon monoxide (DL<sub>CO</sub>) in patients concomitant with normal expiratory airflow. A recent study also reported that otherwise normal asymptomatic smokers with abnormal DL<sub>CO</sub> showed evidence of endothelial microparticles in the circulation—a marker of early lung destruction associated with emphysema.<sup>9</sup> Although abnormal DL<sub>CO</sub> in ex-smokers is a valuable marker of lung function impairment, even in the absence of airflow limitation, the relationship between DL<sub>CO</sub> with other functional markers (ie, symptoms and exercise limitation) is not well understood. We hypothesised that subjects with abnormal DL<sub>CO</sub> without airflow limitation would have imaging evidence of early or mild emphysema with measurable functional consequences.

Multidetector CT and hyperpolarised helium-3 (<sup>3</sup>He) MRI have been used independently to measure emphysema and airways disease as distinct phenotypes in COPD.<sup>10-11</sup> In particular, hyperpolarised <sup>3</sup>He MRI apparent diffusion coefficients (ADC)<sup>12-13</sup> provide a way to sensitively measure regional lung tissue destruction—the hallmark of emphysema. Abnormally elevated <sup>3</sup>He ADC have previously been reported in asymptomatic smokers without COPD<sup>14-15</sup> although the relationship between <sup>3</sup>He MRI ADC in early disease with symptoms and other physiological measurements has never been reported and their functional impact is not known. To better understand the consequences of early or mild disease in ex-smokers, we have evaluated well established clinical, physiological as well as emerging imaging measurements in ex-smokers with normal spirometry but abnormal DL<sub>CO</sub> as well as ex-smokers with GOLD stage I COPD and those with normal spirometry and DL<sub>CO</sub>.

## MATERIALS AND METHODS

### Study subjects

All subjects provided written informed consent to the protocol approved by the local research ethics board and Health Canada, and the study was compliant with the Personal Information Protection and Electronic Documents Act (Canada) and the Health Insurance Portability and Accountability Act (USA). Ex-smokers were recruited from a local tertiary care centre and by advertisement. Thirty-eight subjects were enrolled who were ex-smokers without a diagnosis of COPD and 15 ex-smokers were enrolled with a previous diagnosis of GOLD stage I COPD,<sup>1</sup> all of whom were 60–85 years of age, with a smoking history ≥10 pack-years. Subjects without a diagnosis of COPD had no history of previous chronic or current respiratory disease and were classified according to American Thoracic Society/European Respiratory Society recommendations<sup>16</sup> on the approximate lower limits of normal for DL<sub>CO</sub>,<sup>17</sup> such that normal is defined as DL<sub>CO</sub> ≥75%<sub>pred</sub> and abnormal DL<sub>CO</sub> <75%<sub>pred</sub>.

### Spirometry, plethysmography and other tests

Spirometry was performed using an EasyOne spirometer (Medizintechnik AG, Zurich, Switzerland) according to the American Thoracic Society guidelines.<sup>18</sup> Lung volumes were measured using body plethysmography and DL<sub>CO</sub> was assessed

using the attached gas analyser (MedGraphics Corporation, St Paul, Minnesota, USA). The St Georges Respiratory Questionnaire (SGRQ) was administered<sup>19-20</sup> and a standard 6 min Walk Test (6MWT)<sup>21</sup> was performed.

### Image acquisition

MRI was performed on a whole body 3.0 T Discovery 750MR (General Electric Health Care, Milwaukee, Wisconsin, USA) MRI system.<sup>22</sup> <sup>3</sup>He gas was polarised to 30–40% (HeliSpin) and doses (5 ml/kg body weight) were administered in 1.0 l Tedlar bags diluted with medical grade nitrogen (N<sub>2</sub>) (Linde, Ontario, Canada). <sup>3</sup>He MRI diffusion weighted images were acquired using a fast gradient recalled echo sequence immediately following inhalation of the <sup>3</sup>He/N<sub>2</sub> gas mixture during breath hold conditions.<sup>22</sup> Two interleaved images were acquired (14 s total data acquisition, repetition time (TR)/echo time (TE)/flip angle=7.6 ms/3.7 ms/8°, field of view (FOV)=40×40 cm, matrix 128×128, seven slices, 30 mm slice thickness, 0 gap), with and without additional diffusion sensitisation with b=1.6 s/cm<sup>2</sup> (gradient amplitude (G)=1.94 G/cm, rise and fall time=0.5 ms, gradient duration=0.46 ms, diffusion time=1.46 ms).

CT was performed on a 64 slice Lightspeed VCT scanner (General Electric Health Care) (64×0.625 mm, 120 kVp, 100 effective mA, tube rotation time=500 ms, pitch=1.0). A single spiral acquisition was acquired in breath hold after inhalation of 1.0 l of N<sub>2</sub> from functional residual capacity. Reconstruction was performed (1.25 mm) using a standard convolution kernel.

To minimise the potential for differences in the levels of inspiration between <sup>3</sup>He MRI and CT, extensive coaching was performed prior to the imaging sessions to ensure subjects could completely inspire the contents of the 1.0 l bag. The order of <sup>3</sup>He MRI and CT acquisition was randomised for each subject.

### Image analysis

Regions of signal void were quantified as the <sup>3</sup>He ventilation defect per cent (VDP).<sup>23</sup> <sup>3</sup>He ADC maps were also generated as previously described.<sup>24</sup> Regional differences in ADC were evaluated in the anterior–posterior (AP) direction.<sup>25</sup> The AP gradient (AP<sub>G</sub>) was the slope of the line of best fit that described the change in ADC as a function of distance (in cm). Analysis of CT was performed using the Pulmonary Workstation 2.0 (VIDA Diagnostics Inc, Coralville, Iowa, USA). Wall area per cent (WA%) was measured for the segmental and subsegmental airways<sup>10</sup> and the relative area with attenuation values below –950 HU (RA<sub>950</sub>) was generated.<sup>26</sup>

### Statistical methods

A multivariate analysis of variance was performed using IBM SPSS Statistics V.20.0 (SPSS Inc, Chicago, Illinois, USA). Univariate comparisons were performed using an unpaired two tailed t test, and Welch's correction was used when the F test for equal variances was significant using GraphPad Prism V.4.00 (GraphPad Software Inc, San Diego, California, USA). A Fisher's exact test was performed for categorical variables. Linear regression (r<sup>2</sup>) and Pearson correlation coefficients (r) were used to determine correlations using GraphPad Prism V.4.00. Results were considered significant when the probability of making a type I error was less than 5% (p<0.05).

## RESULTS

We enrolled 53 ex-smokers, 38 subjects without a diagnosis of COPD and 15 subjects diagnosed with stage I COPD. Of the 38 ex-smokers without COPD, half had normal DL<sub>CO</sub> without airflow obstruction (ND, n=19) and the other half had

**Table 1** Clinical, functional and radiographic measurements of asymptomatic ex-smokers with normal and abnormal diffusion capacity of the lung for carbon monoxide, compared with GOLD stage I chronic obstructive pulmonary disease

	ND (n=19)	AD (n=19)	Stage I COPD (n=15)	Significance of difference (p)	
				ND-AD	AD-COPD
Subject demographics					
Age (years)	71 (7)	74 (7)	77 (5)	0.09	0.30
No of women (n)	3	11	2	0.02	0.01
BMI (kg/m <sup>2</sup> )	29.5 (3.4)	28.6 (4.0)	28.4 (4.0)	0.46	0.91
Pack-years	25 (12)	32 (23)	49 (36)	0.25	0.11
Time since quitting (years)	26 (9)	24 (14)	21 (14)	0.63	0.63
Pulmonary function tests					
FEV <sub>1</sub> % <sub>pred</sub>	107 (13)	99 (12)	95 (13)	0.07	0.34
FVC % <sub>pred</sub>	98 (12)	93 (12)	108 (14)	0.16	0.001
FEV <sub>1</sub> /FVC	80 (6)	80 (7)	63 (5)	0.73	<0.0001
IC % <sub>pred</sub>	112 (17)	103 (22)	103 (17)	0.17	0.99
RV % <sub>pred</sub>	103 (17)	107 (25)	114 (29)	0.58	0.45
TLC % <sub>pred</sub>	101 (10)	101 (15)	109 (13)	0.96	0.12
RV/TLC % <sub>pred</sub>	101 (13)	104 (16)	103 (18)	0.49	0.77
DL <sub>CO</sub> % <sub>pred</sub>	89 (9)	59 (13)	68 (19)	<0.0001	0.12
6MWT					
Pre 6MWT SpO <sub>2</sub> %	97 (2)	95 (2)	95 (2)	0.06	0.57
Δ 6MWT SpO <sub>2</sub> %	0 (2)*	0 (2)†	-1 (3)	0.55	0.25
Distance (m)	430 (99)	341 (95)	417 (41)	0.008	0.005
SGRQ					
Symptoms	18 (17)*	36 (30)*	36 (22)	0.04	0.99
Activity score	19 (21)†	41 (24)	36 (25)‡	0.006	0.54
Impact score	6 (11)†	17 (18)	15 (13)‡	0.04	0.68
Total score	12 (14)§	29 (21)*	25 (17)‡	0.01	0.59
CT measurements					
RA <sub>950</sub>	1.36 (1.25)	1.60 (1.06)	5.50 (3.16)	0.53	0.0008
WA%	57 (4)	59 (2)	58 (2)	0.17	0.23
<sup>3</sup> He MRI measurements					
ADC (cm <sup>2</sup> /s)	0.27 (0.03)*	0.30 (0.03)§	0.36 (0.08)	0.01	0.02
VDP (%)	6 (3)*	7 (4)§	9 (5)‡	0.40	0.07

Values are mean (SD).

Missing values: SpO<sub>2</sub> not recorded post-6MWT (n=1, normal DL<sub>CO</sub>; n=2, abnormal DL<sub>CO</sub>); incomplete SGRQ questionnaire (n=3, normal DL<sub>CO</sub>; n=1, abnormal DL<sub>CO</sub>; n=1, COPD); and image acquisition failures (n=1, normal DL<sub>CO</sub>; n=2, abnormal DL<sub>CO</sub>; n=1, COPD).

\*n=18, †n=17, ‡n=14, §n=16.

AD, abnormal DL<sub>CO</sub>; ADC, apparent diffusion coefficient; BMI, body mass index; COPD, chronic obstructive pulmonary disease; DL<sub>CO</sub>, diffusion capacity of the lung for carbon monoxide; FEV<sub>1</sub>, forced expiratory volume in 1 s; FVC, force vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease; IC, inspiratory capacity; 6MWT, 6 min Walk Test; ND, normal DL<sub>CO</sub>; RA<sub>950</sub>, relative area with attenuation values below -950 HU; RV, residual volume; SGRQ, St George's Respiratory Questionnaire; SpO<sub>2</sub>, peripheral oxygen saturation; TLC, total lung capacity; VDP, ventilation defect per cent; WA%, wall area per cent.

abnormal DL<sub>CO</sub> without airflow obstruction (AD, n=19). Table 1 shows the subject demographics as well as pulmonary function, SGRQ, 6MWD, CT and <sup>3</sup>He MRI measurements for all subjects categorised according to their spirometry and DL<sub>CO</sub> results.

Subjects with abnormal DL<sub>CO</sub> without airflow obstruction (AD) were not significantly different from ex-smokers with normal DL<sub>CO</sub> (ND) and stage I COPD subjects with respect to age, BMI, pack-years, years since smoking cessation, change in SpO<sub>2</sub> after the 6MWT, CT WA% and <sup>3</sup>He VDP. However, there were significantly more female AD subjects than ND (p=0.02) and stage I COPD (p=0.01) subjects.

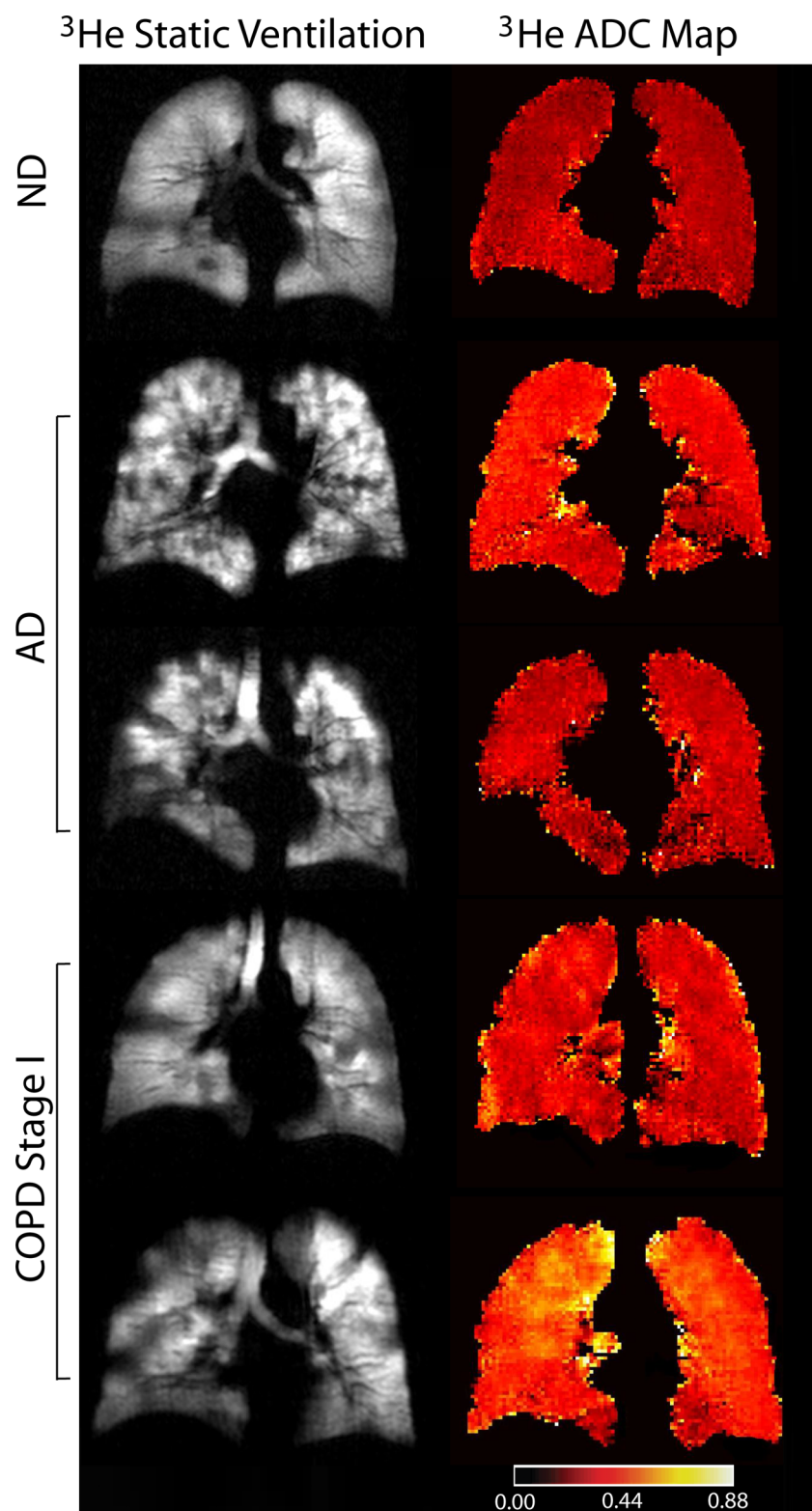
Figure 1 shows the central coronal <sup>3</sup>He MRI static ventilation image and <sup>3</sup>He MRI ADC map for subjects with ND, AD and stage I COPD. As shown in table 1, AD subjects had a significantly worse <sup>3</sup>He ADC (0.30±0.03 cm<sup>2</sup>/s; p=0.01), 6MWD (341±95 m; p=0.008) and SGRQ total score (29±21; p=0.01) compared with ND subjects, but there was no significant difference for RA<sub>950</sub> (p=0.53). In comparison with stage I COPD, AD subjects had a significantly reduced 6MWD (341±95 m;

p=0.005), FVC (93±12%<sub>pred</sub>; p=0.001), RA<sub>950</sub> (1.6±1.1; p=0.0008) and ADC (0.30±0.03 cm<sup>2</sup>/s; p=0.02), and a significantly greater FEV<sub>1</sub>/FVC (80±7%; p<0.0001) and no significant difference for SGRQ total score (p=0.59).

Figure 2A shows the mean ADC on a slice by slice basis in the anterior to posterior direction for ND, AD and stage I COPD subjects. For AD ex-smokers, the ADC gradient in the anterior-posterior direction (ADC AP<sub>G</sub>) was significantly lower than for ND (p=0.02) and not significantly different from COPD subjects (p=0.20). Figure 2B shows the significant correlation between ADC AP<sub>G</sub> and the 6MWD (r=-0.51, p=0.0002).

Figure 3 shows the correlations between <sup>3</sup>He ADC and CT RA<sub>950</sub> with DL<sub>CO</sub>, SGRQ and 6MWD. There was a significant correlation between <sup>3</sup>He ADC and DL<sub>CO</sub> (r=-0.55, p<0.0001) and SGRQ (r=0.34, p=0.02) but not 6MWD (r=-0.17, p=0.24), and as shown in figure 2B, ADC AP<sub>G</sub> was significantly correlated with 6MWD. RA<sub>950</sub> was significantly correlated with DL<sub>CO</sub> (r=-0.31, p=0.03) but not SGRQ (r=0.24, p=0.10) or 6MWD (r=0.0013, p=0.99).

**Figure 1** Helium-3 ( $^3\text{He}$ ) MRI static ventilation images and  $^3\text{He}$  apparent diffusion coefficient (ADC) maps for a representative ND and two representative AD and COPD stage I ex-smokers. ND subject is a 70-year-old man with  $\text{FEV}_1=101\%_{\text{pred}}$ ,  $\text{FEV}_1/\text{FVC}=0.75$ ,  $\text{DL}_{\text{CO}}=113\%_{\text{pred}}$ ,  $^3\text{He}$   $\text{ADC}=0.26\text{ cm}^2/\text{s}$  and  $\text{CT RA}_{950}=1.25$ . AD subject No 1 is a 74-year-old man with  $\text{FEV}_1=89\%_{\text{pred}}$ ,  $\text{FEV}_1/\text{FVC}=0.77$ ,  $\text{DL}_{\text{CO}}=41\%_{\text{pred}}$ ,  $^3\text{He}$   $\text{ADC}=0.31\text{ cm}^2/\text{s}$  and  $\text{CT RA}_{950}=1.52$ . AD subject No 2 is a 74-year-old man with  $\text{FEV}_1=95\%_{\text{pred}}$ ,  $\text{FEV}_1/\text{FVC}=0.85$ ,  $\text{DL}_{\text{CO}}=63\%_{\text{pred}}$ ,  $^3\text{He}$   $\text{ADC}=0.29\text{ cm}^2/\text{s}$  and  $\text{CT RA}_{950}=0.52$ . GOLD stage I COPD subject No 1 is a 74-year-old man with  $\text{FEV}_1=86\%_{\text{pred}}$ ,  $\text{FEV}_1/\text{FVC}=0.59$ ,  $\text{DL}_{\text{CO}}=45\%_{\text{pred}}$ ,  $^3\text{He}$   $\text{ADC}=0.37\text{ cm}^2/\text{s}$  and  $\text{CT RA}_{950}=6.14$ . GOLD stage I COPD subject No 2 is a 78-year-old man with  $\text{FEV}_1=118\%_{\text{pred}}$ ,  $\text{FEV}_1/\text{FVC}=0.62$ ,  $\text{DL}_{\text{CO}}=71\%_{\text{pred}}$ ,  $^3\text{He}$   $\text{ADC}=0.38\text{ cm}^2/\text{s}$  and  $\text{CT RA}_{950}=5.52$ . AD, abnormal  $\text{DL}_{\text{CO}}$ ; COPD, chronic obstructive pulmonary disease;  $\text{DL}_{\text{CO}}$ , diffusion capacity of the lung for carbon monoxide;  $\text{FEV}_1$ , forced expiratory volume in 1 s;  $\text{FVC}$ , force vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease; ND, normal  $\text{DL}_{\text{CO}}$ ;  $\text{RA}_{950}$ , relative area with attenuation values below  $-950$  HU.

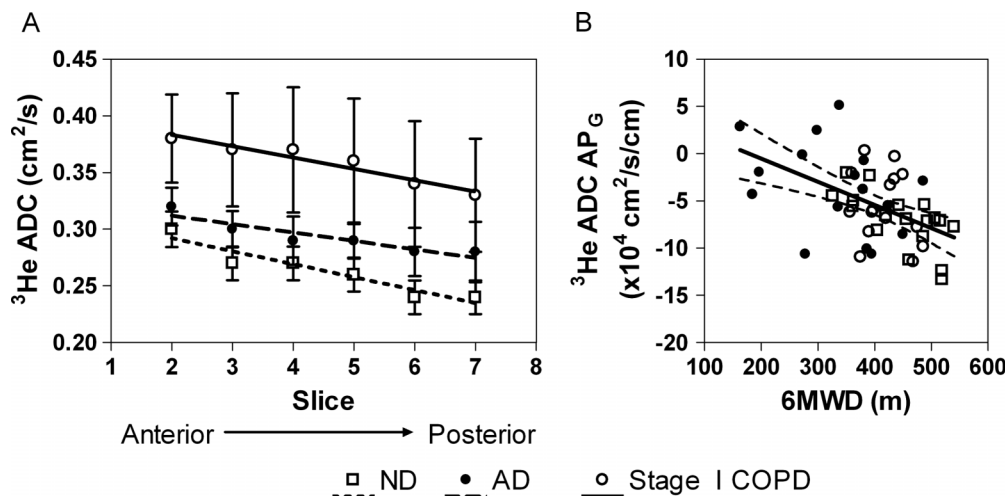


## DISCUSSION

To better understand the relationship between lung structural markers, symptoms and physiological measurements in ex-smokers, we evaluated 53 ex-smokers, including 38 subjects who did not have a diagnosis of COPD and 15 subjects with stage I COPD, and observed the following. (1) Nineteen of 38 ex-smokers showed normal spirometry and CT but abnormal  $\text{DL}_{\text{CO}}$  and 19/38 ex-smokers showed normal spirometry,

CT and  $\text{DL}_{\text{CO}}$ . (2) Subjects with abnormal  $\text{DL}_{\text{CO}}$  had significantly worse 6MWD compared with stage I COPD ex-smokers and significantly worse  $^3\text{He}$  ADC, SGRQ and 6MWD compared with subjects with normal  $\text{DL}_{\text{CO}}$ . (3) Subjects with abnormal  $\text{DL}_{\text{CO}}$  had significantly smaller  $^3\text{He}$  MRI ADC AP gradients compared with subjects with normal  $\text{DL}_{\text{CO}}$ .

We were surprised that half of the ex-smokers without COPD showed abnormal  $\text{DL}_{\text{CO}}$  and significantly worse  $^3\text{He}$  ADC, but



**Figure 2** Regional helium-3 ( $^3\text{He}$ ) MRI ADC anterior-posterior gradients ( $\text{AP}_G$ ) for ND, AD and stage I COPD subjects, and correlation between  $^3\text{He}$  ADC  $\text{AP}_G$  with 6MWD. (A) Mean  $\text{AP}_G$  was statistically significantly different for AD and ND subjects (AD:  $\text{AP}_G = -3.55 \times 10^{-4} \pm 4.85 \times 10^{-4} \text{ cm}^2/\text{s}/\text{cm}$ ; ND:  $\text{AP}_G = -7.03 \times 10^{-4} \pm 3.03 \times 10^{-4} \text{ cm}^2/\text{s}/\text{cm}$ ;  $p=0.02$ ) but not between the AD and stage I COPD subjects (COPD:  $\text{AP}_G = -5.58 \times 10^{-4} \pm 3.73 \times 10^{-4} \text{ cm}^2/\text{s}/\text{cm}$ ;  $p=0.20$ ). Error bars represent the ADC SD for each image slice. (B)  $^3\text{He}$  ADC  $\text{AP}_G$  was significantly correlated with 6MWD ( $r=-0.51$ ,  $p=0.0002$ ,  $r^2=0.26$ ,  $p=0.0002$ ,  $y=-0.02x+4.4$ ). Dotted lines represent the 95% CIs of the regression. AD, abnormal  $\text{DL}_{\text{CO}}$ ; ADC, apparent diffusion coefficient; COPD, chronic obstructive pulmonary disease;  $\text{DL}_{\text{CO}}$ , diffusion capacity of the lung for carbon monoxide; 6MWT, 6 min Walk Test; ND, normal  $\text{DL}_{\text{CO}}$ .

with normal CT, which, based on previous studies,<sup>14 15</sup> was an unexpected result. Although we were not able to confirm significant disease other than emphysema that could account for these findings, we note that a previous evaluation<sup>14</sup> of 10 younger asymptomatic smokers (mean age=47 years, range=23–73) showed that three of five subjects aged 60 years or older also reported  $\text{DL}_{\text{CO}} < 75\%_{\text{pred}}$ . In ex-smokers, abnormal  $\text{DL}_{\text{CO}}$  is thought to reflect diminished lung surface area available for gas exchange although  $\text{DL}_{\text{CO}}$  also reflects the volume of blood in the pulmonary capillaries and thickness of the alveolar capillary membrane,<sup>27</sup> related to bronchiectasis and interstitial lung disease.<sup>28</sup> Abnormally low  $\text{DL}_{\text{CO}}$  is also consistent with pulmonary vascular disease,<sup>29</sup> and such patients exhibit normal spirometry, dyspnoea on exertion<sup>30</sup> and a decline in oxygen saturation with exertion.<sup>31</sup> In the current study, AD subjects did not show reduced oxygen saturation during the 6MWT nor did they report a history of pulmonary vascular disease, so there was no evidence to support the notion that pulmonary vascular disease was responsible for the abnormal exercise performance and dyspnoea observed here. Although  $\text{DL}_{\text{CO}}$  is a very sensitive marker of emphysema in smokers,<sup>8</sup> reproducibility can be low, and in some cases, low to moderate correlations have been reported between  $\text{DL}_{\text{CO}}$  and pathological assessments of emphysema.<sup>32 33</sup>

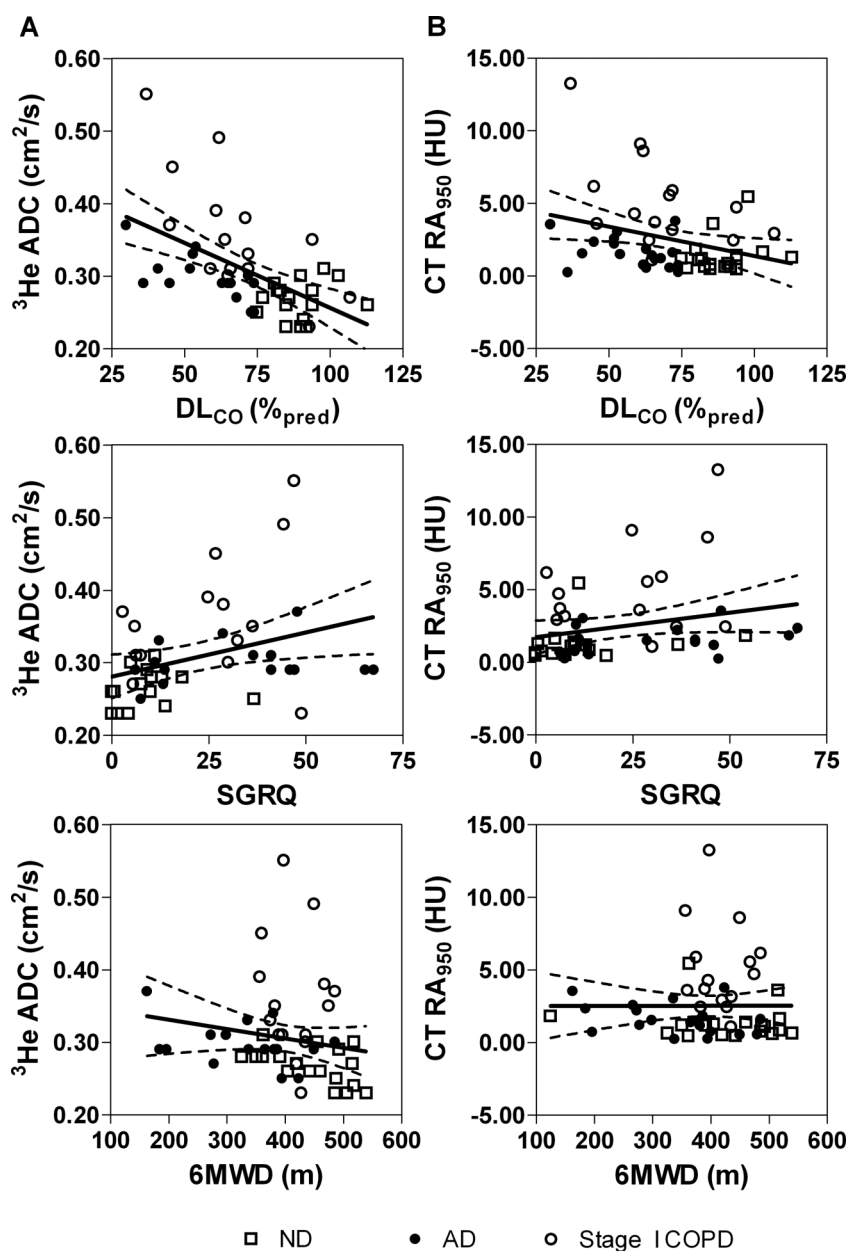
Previous work by Woods and Hogg<sup>34</sup> compared  $^3\text{He}$  ADC with histology measurements of emphysema in explanted lungs and showed that ADC values could be used to distinguish normal from emphysematous lung tissue with greater precision than the mean linear intercept measurement from histology samples. Another previous study in COPD showed that while  $^3\text{He}$  ADC correlated significantly with CT measurements (ie,  $\text{RA}_{950}$ ), stronger correlations were observed for  $^3\text{He}$  ADC and  $\text{DL}_{\text{CO}}$  than for  $\text{RA}_{950}$  and  $\text{DL}_{\text{CO}}$ .<sup>35</sup> In asymptomatic smokers,  $^3\text{He}$  ADC was shown to correlate with  $\text{DL}_{\text{CO}}$ , but there was no significant correlation between  $\text{DL}_{\text{CO}}$  and CT  $\text{RA}_{950}$ .<sup>14</sup> Finally, abnormally elevated  $^3\text{He}$  ADC values were previously observed in never smokers exposed to significant second hand-smoke<sup>36</sup> compared with never smokers with no such exposure. Taken together, these previous findings support the observation here

that elevated  $^3\text{He}$  ADC in ex-smokers with abnormal  $\text{DL}_{\text{CO}}$  may reflect mild emphysema not detected by CT. Our observations are also consistent with previous reports<sup>5–8 37</sup> and the identification of mild emphysema using histology that was not predicted using preoperative CT.<sup>38 39</sup> While we cannot rule out the presence of small airways disease in subjects with AD, there was no significant difference between the AD and ND subjects for  $^3\text{He}$  VDP and CT WA%, both of which provide estimates of airways disease. Taken together, these results suggest that  $^3\text{He}$  ADC is sensitive to very mild emphysema in subjects with abnormal  $\text{DL}_{\text{CO}}$  who have no CT evidence of airways disease or emphysema.

Concomitant with significantly elevated  $^3\text{He}$  ADC, we observed significantly worse 6MWD in AD compared with COPD and ND ex-smokers. This is an important finding and the first to provide evidence of a relationship between  $^3\text{He}$  MRI ADC reflective of early or mild emphysema and exercise capacity. It is also important to note that the ratio of female/male ex-smokers with AD was 11/8 (1.4), and for ND this ratio was 3/16 (0.2). Although the current study was not powered to evaluate sex differences, previous evidence suggests that female sex is significantly associated with early onset COPD.<sup>40 41</sup> However, previous studies have also shown that emphysema dominates in men compared with women,<sup>42</sup> whereas here the sex ratio was reversed. We note that imaging was performed at a fixed volume and because there were more women in the AD group (who potentially had smaller lungs), we investigated the relationship between lung size and  $^3\text{He}$  ADC and observed no correlation for  $^3\text{He}$  ADC with height ( $r=-0.36$ ,  $p=0.18$ ), total lung capacity ( $r=0.33$ ,  $p=0.21$ ) or thoracic cavity volume ( $r=-0.20$ ,  $p=0.45$ ). Therefore, the elevated ADC in the AD subjects observed here was not related to lung size and cannot explain the preponderance of female subjects in the AD subgroup. Consistent with our findings, the 6MWD in COPD was also previously shown to be lower for  $\text{FEV}_1$  matched women versus men.<sup>43</sup>

We took advantage of the fact that  $^3\text{He}$  MRI diffusion weighted images were acquired in the supine position and

**Figure 3** Correlation between helium-3 ( $^3\text{He}$ ) ADC and CT RA<sub>950</sub> with DL<sub>CO</sub>, SGRQ and 6MWD for ND, AD and stage I COPD subjects. (A)  $^3\text{He}$  ADC was significantly correlated with DL<sub>CO</sub> ( $r=-0.55$ ,  $p<0.0001$ ,  $r^2=0.31$ ,  $p<0.0001$ ,  $y=-0.0018x+0.44$ ) and SGRQ ( $r=0.34$ ,  $p=0.02$ ,  $r^2=0.12$ ,  $p=0.02$ ,  $y=0.0012x+0.28$ ) but not with 6MWD ( $r=-0.17$ ,  $p=0.24$ ,  $r^2=0.03$ ,  $p=0.24$ ,  $y=-0.00013x+0.36$ ). (B) CT RA<sub>950</sub> was significantly correlated with DL<sub>CO</sub> ( $r=-0.31$ ,  $p=0.03$ ,  $r^2=0.09$ ,  $p=0.02$ ,  $y=-0.040x+5.42$ ) but not with SGRQ ( $r=0.24$ ,  $p=0.10$ ,  $r^2=0.06$ ,  $p=0.10$ ,  $y=-0.034x+1.71$ ) or 6MWD ( $r=0.0013$ ,  $p=0.99$ ,  $r^2<0.0001$ ,  $p=0.99$ ,  $y=0.00003x+2.5$ ). Dotted lines represent the 95% CIs of the regression. AD, abnormal DL<sub>CO</sub>; ADC, apparent diffusion coefficient; COPD, chronic obstructive pulmonary disease; DL<sub>CO</sub>, diffusion capacity of the lung for carbon monoxide; 6MWT, 6 min Walk Test; ND, normal DL<sub>CO</sub>; RA<sub>950</sub>, relative area with attenuation values below -950 HU; SGRQ, St George's Respiratory Questionnaire.



measured compression of the dependent lung due to gravity. Several sites have reported smaller  $^3\text{He}$  ADC in the dependent lung (or posterior slices) relative to the non-dependent lung,<sup>25 44 45</sup> likely due to gravitational compression of the parenchyma. In COPD subjects,<sup>25 44</sup> this anterior to posterior difference is significantly smaller and this is thought to be due to regional gas trapping that counteracts gravitational compression of the dependent regions. Here we observed that these gradients were significantly smaller in AD subjects compared with ND subjects, suggesting that regional gas trapping was greater in the AD subgroup.

Finally, we showed that  $^3\text{He}$  ADC was significantly correlated with SGRQ and that  $^3\text{He}$  ADC AP<sub>C</sub>s were significantly correlated with the 6MWD. The significant relationships between  $^3\text{He}$  ADC with respiratory symptoms and exercise capacity suggest that in early emphysema, symptomatic changes can go unnoticed in older patients even when standardised tests report significant changes in health related quality of life and exercise capacity. While elevated  $^3\text{He}$  ADC in asymptomatic ex-smokers

was previously described,<sup>14 15</sup> the imaging to exercise capacity and imaging to symptoms correlations observed here in very early emphysema are novel findings. The unexpected finding of  $^3\text{He}$  ADC AP gradient correlations with 6MWD also provides more evidence about the role of mild emphysema and regional gas trapping that may together lead to exercise limitation even in early disease. AD ex-smokers also reported a SGRQ that was not significantly different from the stage I COPD ex-smokers, and worse than ND subjects, which supports previous reports of compromised health related quality of life<sup>46</sup> and reduced work capacity in very early disease.<sup>47</sup>

This study was limited by the relatively small number of subjects evaluated, although we note that this is the single largest prospective study that directly compared CT, symptoms, exercise capacity and  $^3\text{He}$  MRI in ex-smokers with and without airflow obstruction. We admit that we were surprised to find such a large proportion of asymptomatic ex-smokers without airflow limitation and abnormal DL<sub>CO</sub> in this study. This finding raises the important question of whether this subgroup is

atypical or perhaps this is a unique finding because 'asymptomatic' ex-smokers are rarely administered the SGRQ or the 6MWT. Importantly, the selection criteria, manner and location for subject recruitment are those we have previously used for the recruitment of older ex-smokers, and typical of other studies. It is possible that in this unique subgroup, patients were less likely to recognise and report symptoms. Our results certainly raise many intriguing questions regarding whether these subjects are unusual or whether we have simply uncovered a group of older ex-smokers with both unrecognised mild emphysema and functional limitations.

In summary, we evaluated 38 ex-smokers without airflow limitation and 15 ex-smokers with COPD. In the absence of spirometry or CT abnormalities, half of the ex-smokers without COPD showed abnormal DL<sub>CO</sub> and abnormally elevated <sup>3</sup>He ADC, consistent with early or mild emphysema. These subjects had significantly and markedly worse 6MWD and SGRQ compared with ex-smokers with normal ADC and DL<sub>CO</sub>, and worse 6MWD than subjects with COPD. These findings provide a better understanding of abnormal DL<sub>CO</sub> in ex-smokers without COPD.

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