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A new approach to detect early lung functional impairment in very light smokers

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ABSTRACT

Purpose: The aim of our study is to investigate if lung carbon monoxide diffusing capacity (DLCO) measured during effort is able to detect early respiratory functional impairment.
Methods: We enrolled 25 very light smokers and 20 healthy non smokers. Subjects underwent plethysmography, DLCO (single breath technique) and calculated effective pulmonary blood flow (Qc) by rebreathing method. During exercise by cycle ergometer (duration 10 ± 2 min; recovery 11 ± 3 min) DLCO and Qc were calculated at 25% and 50% of theoretical maximum workload.
Results: At baseline lung function and Qc did not differ between groups. DLCO and DLCO/Qc measured during exercise were significantly greater in non smokers ($p < 0.001$); Qc was not statistically different. In very light smokers, DLCO, DLCO/Qc measured during exercise significantly correlated with the number of pack years ($r = -0.60$ $p < 0.001$; $r = -0.58$ $p < 0.05$; $r = -0.55$ $p < 0.05$, respectively).
Conclusions: In very light smokers there is lung function impairment and our data show that DLCO during exercise may reveal this underlying early damage.

1. Introduction

Cigarette smoking is the principal risk factor for the development of chronic obstructive pulmonary disease (COPD) (Hanzahan et al., 1996; Higgins, 1991; Clark et al., 1998). COPD is an irreversible disease and according to the GOLD statement 2012 it is defined as "a disease state characterized by airflow limitation that is not fully reversible; the airflow limitation is usually both progressive and associated with an abnormal inflammation response of the lung to noxious particles or gases" (Fletcher and Peto, 1977).

COPD diagnosis requires lung function tests including diffusing lung capacity measurement which can be performed in nearly every hospital.

Smoking cessation is the only effective treatment in slowing down the decline in FEV_1 (Kanner et al., 1999; Anthonisen, 1997). Many studies have aimed at determining the impact of smoking on symptoms of asthma and other respiratory disorders, but these studies enrolled only elder subjects with a long history of smoking and did not completely assess lung functional damage (Lindstrom et al., 2001). In addiction to bronchial obstruction, smoking significantly decreases lung diffusing capacity for carbon monoxide (DLCO), which is inversely related to smoking history (Fletcher and Peto, 1977).

Cigarette smoking is responsible for lung damage in all subjects, but the severity of this injury is different between individuals, probably due to personal susceptibility.

The major mechanisms thought to be responsible for the development and progression of COPD include the protease-antiprotease hypothesis, inflammation, oxidative stress, alveolar cell apoptosis, and matrix remodelling. (Yoshida and Tuder, 2007; Barnes, 2000; Suki et al., 2003). Probably, these are not distinct processes and each can contribute to disease complexity (Barnes, 2000).

Approximately 30% of smokers do not show chronic symptoms or abnormal lung function. Nevertheless, even these so-called very light smokers show small changes in lung morphology, inflamma-

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tion and function (Rijcken and Britton, 1998; Samet and Lange, 1996; Lange et al., 1990; Rizzi et al., 2004).

Very light smokers are defined fairly consistently as daily smoking of fewer than 6 cigarettes per day, while the definition of light smokers varies from 10 to 20 cigarettes per day, according to different studies (Husten, 2009); the world health organization defines heavy smoking as 20 or more cigarettes per day (WHO, 1998).

Many young people start smoking believing that low numbers of cigarettes per day will not harm their health, and that only a high number of daily cigarettes will lead to serious consequences for their lung function (Towns et al., 2015).

The early detection of COPD using respiratory function tests is a real challenge for the pulmonologists; but even more difficult is to demonstrate the early lung damage caused by cigarette smoking and other common risk factors.

The most important parameter used to assess respiratory impairment is the FEV_1, but its reduction is not an early indicator for lung damage. Light and recent smokers don't usually show alterations of lung function including DLCO at rest.

It is known that during exercise COPD patients experience a worsening of respiratory symptoms due to worsening bronchial obstruction and lung hyperinflation. It is not known whether the early lung damage in smokers is worsened during exercise as a stress test for the respiratory system.

The aim of this study was to evaluate whether DLCO variations, measured at increasing effort levels, could be an indicator of early lung damage caused by cigarette smoking in very light smokers with normal pulmonary function tests and DLCO at rest.; these results could be useful to encourage smoking cessation on a large scale to prevent future COPD.

2. Methods

2.1. Subjects

Between November 14th 2013 and March the 4th 2014, twenty five very light smoking subjects, defined for a daily smoking of fewer than 6 cigarettes per day (Husten, 2009) were enrolled at the respiratory function laboratory of "Ospedale L. Sacco" in Milan, Italy, for an assessment of respiratory function; another twenty healthy non smoking subjects were enrolled as a control group, age and gender matched. Inclusion criteria were: normal resting lung function, normal ECG findings, normal blood pressure values, absence of cardiovascular and pulmonary signs and symptoms. Exclusion criteria included obesity (Body Mass Index; BMI $\geq 30\,kg/m^2$), anaemia or presence of musculoskeletal or rheumatologic disorders which may limit exercise capacity.

Before lung function tests, all subjects were given the COPD Assessment Test (CAT) for an evaluation of respiratory symptoms (Jones et al., 2009).

All subjects of the smokers' group were asked to stop smoking on the day before the access to the laboratory.

Written informed consent was obtained for all subjects participating in this study. Approval of the local Human Ethics Committee was obtained.

The two groups (very light smokers and non smokers) did not differ in terms of age (32 ± 4 vs. 33 ± 3 years), BMI (25 ± 3 vs. $24 \pm 4\,kg/m^2$), sex (60% males vs. 59% females), COHb at baseline (1.1 ± 0.5 vs. 1.0 ± 0.4% Hb tot), CAT score (1.1 ± 0.5 vs. 0.9 ± 0.5 points), theoretical maximum workload (180 ± 5 vs. 178 ± 6 W) or socioeconomic status. In the smokers group the average smoking habit was 2.8 ± 1.6 pack years [PKY = (number of cigarettes smoked per day x years smoked)/20] and 4 ± 2 cigarettes per day.

2.2. Pulmonary function tests

Lung volumes and dynamic parameters were assessed and analyzed by computed spirometry and plethysmography (VMAX227 Autobox V6200; Sensor Medics; Yorba Linda, CA, USA), in accordance with the European Respiratory Society criteria (Quanjer et al., 1993). DLCO was measured with the single breath technique, using a mixture of carbon monoxide and methane (O_2 20%, CO 0.3%, CH_4 0.3%) according to the recommendations from the European Respiratory Society (Cotes et al., 1993): the subject performs a maximal expiration and then a maximal inspiration; breath holding time was at least 10 s, followed by an expiration to residual volume; washout volume was 0.75 L.

DLCO was automatically adjusted for the level of haemoglobin and carboxyhemoglobin (COHb) measured at rest with a blood gas analyzer (Critical Care Laboratory Synthesis 35; Instrumentation Laboratory; Paderno Dugnano, Italy). Blood samples were inserted into a cuvette which is enlightened with different wavelengths; optical absorbance was then registered within ten seconds and deoxy-, *meta*- and carboxy-haemoglobin concentrations were measured and shown on display. The following equation was used (Mohsnifar and Tashkin, 1979):

$$\text{Adjusted DLCO} = \text{COHb} - \text{measured DLCO}[1 + (\%\text{COHb}/100)]$$

The carbon monoxide transfer factor coefficient (Kco) was derived from the following equation:

$$\text{Kco} = \text{DLCO}/\text{alveolar volume}.$$

The mass-flow sensor was calibrated before each test using a three-litre syringe connected to the mass-flow sensor and stroked five times to measure the volume inspired and expired by the syringe. Correction factors were then calculated to fine tune the volumes measurement.

2.3. Exercise testing

Exercise testing was performed according to a standardized procedure for the cardiopulmonary exercise test (ATS/ACCP, 2003). After calibration of the oxygen and carbon dioxide sensors, the study subjects were asked to sit on an electromagnetically braked cycle ergometer (Ergometric 800 Sensor Medics, Yorba Linda, CA, USA) and the seat was adjusted properly to avoid maximal extension of the knee. After a 3-min rest period sitting on the ergometer, exercise began with a 3-min warm-up period at 0 W, followed by a progressively increasing ramp protocol of 10–25 W/min, according to anthropometric data of the subjects (age, sex, weight, height), in order to perform an exercise time lasting 8–12 min (depending on the theoretical maximum workload of the subject, and on the ramp protocol used in each test). All subjects had to maintain a cycling frequency of 60 rpm indicated by a digital display placed on the monitor of the ergometer. At this time we measured heart rate (Hr), breath rate (Br), oxygen consumption (VO_2) and tidal volume (Vt), before starting the test and at 25% and 50% of theoretical maximum workload measured in Watts (25%tmw; 50%tmw).

In all subjects sitting on the ergometer, DLCO and effective pulmonary blood flow (Qc) were calculated with CO_2-rebreathing method (R-CO_2) before starting the test and 25%tmw; 50%tmw. (Jones, 1988; Collier, 1985).

Qc was evaluated using Fick's formula during R-CO_2

$$Qc = VCO_2/CvCO_2 - CaCO_2$$

VCO_2 is CO_2 production; $CvCO_2$ is the CO_2 content in venous mixed blood; $CaCO_2$ is arterial CO_2.

$CvCO_2$ was obtained after 10–15 s. of breathing a mixture of 7% CO_2 in O_2; expired CO_2 is measured with a breath to breath system;