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Lung function in smokers

Aspects on COPD diagnosis and associations to
atherosclerosis and alcohol consumption

Sophia Frantz



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DOCTORAL DISSERTATION

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To be defended in room 2007, Inga Marie Nilssons gata 49, SUS Malmö

the 23rd of May 2014 at 9.00 am.

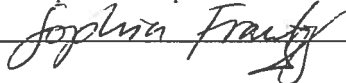
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Title and subtitle Lung function in smokers Aspects on COPD diagnosis and associations to atherosclerosis and alcohol consumption		
Abstract Smoking is the most common and important risk factor for reduced lung function. Chronic Obstructive Pulmonary Disease (COPD) affects mainly smokers and is characterized by airflow obstruction, assessed using one of two major diagnostic spirometric criteria. Aspects of lung function besides spirometry in smokers fulfilling none, either or both of the spirometric criteria for COPD are not well known. Early COPD is hard to reveal, as spirometry has a poor relationship to symptoms. A promising method for early diagnosis of COPD is Impulse Oscillometry System (IOS). Reduced lung function (measured with spirometry) and COPD is associated with cardiovascular disease in a not completely understood way. Smoking is associated with elevated alcohol consumption, but the potential association between lung function and alcohol consumption is unclear. The aim of this thesis was to analyse extensive lung function tests in a population with many smokers with light or no decrease in lung function with special reference to COPD diagnosis and association to atherosclerosis and alcohol consumption. From a previous population-based respiratory questionnaire survey 450 subjects were recruited (never-smokers and smokers, with/without self-reported COPD) and examined with spirometry, body plethysmography, diffusing capacity for CO (D_{LCO}), IOS and ultrasonography of the internal carotid artery. They also answered questionnaires and blood samples were collected. The results show differences in D_{LCO} , residual volume and respiratory symptoms between subjects fulfilling none, either or both of the diagnostic spirometric criteria for COPD in use (paper I). Pulmonary resistance is higher in symptomatic subjects, irrespective of a spirometric COPD diagnosis or not (paper II). Subjects with atherosclerotic plaques in the ICA have lower D_{LCO} and higher residual volume than subjects without plaques (paper III). Among smokers, heavy drinking is associated with lower D_{LCO} and forced expiratory volume in one second (FEV_1) (paper IV). In conclusion, this thesis shows that other aspects of lung function in smokers than FEV_1 are of value in both COPD diagnosis and in understanding associations to atherosclerosis and alcohol consumption.		
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Lung function in smokers

Aspects on COPD diagnosis and associations to
atherosclerosis and alcohol consumption

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List of papers

This thesis is based on the following papers, which will be referred to by their Roman numerals in the text.

- I. Wollmer P, **Frantz S**, Engström G, Dencker M, Löfdahl CG, Nihlén U
Fixed ratio or lower limit of normal for the FEV₁/VC ratio: relation to symptoms and extended lung function tests
Submitted.
- II. **Frantz S**, Nihlén U, Dencker M, Engström G, Löfdahl CG, Wollmer P
Impulse oscillometry may be of value in detecting early manifestations of COPD
Respiratory Medicine; 106 (8): 1116-23, 2012
- III. **Frantz S**, Nihlén U, Dencker M, Engström G, Löfdahl CG, Wollmer P
Atherosclerotic plaques in the internal carotid artery and associations with lung function assessed by different methods
Clinical Physiology and Functional Imaging; 32 (2): 120-5, 2012
- IV. **Frantz S**, Wollmer P, Dencker M, Engström G, Nihlén U
Associations between lung function and alcohol consumption - Assessed by both a questionnaire and a blood marker
Respiratory Medicine; 108 (1): 114-21, 2014

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Abbreviations

ANOVA	Analysis of Variance
ARIC	Atherosclerosis Risk In Communities
ATS	American Thoracic Society
AUDIT	Alcohol Use Disorders Identification Test
AUDIT-C	Alcohol Use Disorders Identification Test, Consumption
AX	An area index of low frequent reactance
BMI	Body Mass Index
CDT	Carbohydrate Deficient Transferrine
CI	Confidence interval
COPD	Chronic Obstructive Pulmonary Disease
CRP	C-reactive protein
CVD	Cardiovascular disease
D _{L,CO}	Diffusing capacity of the Lungs for Carbon monoxide
ECSC	European Coal and Steel Committee
ERS	European Respiratory Society
FEV ₁	Forced Expiratory Volume in one second
FOT	Forced Oscillation Technique
f _{res}	The resonant frequency
FVC	Forced Vital Capacity
GGT	γ -glutamyltranspeptidase
GOLD	Global Initiative for Chronic Obstructive Lung Disease
HbA1c	Glycated haemoglobin
HDL	High-Density Lipoprotein
HPLC	High Performance Liquid Chromatography

ICA	Internal Carotid Artery
IOS	Impulse Oscillometry System
LDL	Low-Density Lipoprotein
LLN	Lower Limit of Normal
OR	Odds Ratio
PN	Predicted Normal
R	Resistance, pulmonary
ROLLS	Role of Low Lung function Study
RV	Residual Volume
SD	Standard Deviation
TLC	Total Lung Capacity
VC	Vital Capacity
X	Reactance, pulmonary

Introduction

Smoking and lung function

The most common and important risk factor for reduced lung function is smoking. The negative effect on lung function caused by tobacco smoke is supposed to be the result of an inflammation as a response to the noxious particles inhaled (Hogg 2004). Another hallmark of the negative effect of tobacco smoke on lung function is oxidative stress, which is caused by both tobacco smoke and the inflammation and is probably both enhancing and enhanced by the inflammation (MacNee 2005). Smokers experience a faster decline of lung function with age compared to never-smokers (Kohansal et al. 2009). Respiratory symptoms associated with smoking are cough and sputum production. Such symptoms are sometimes, but not always, associated with a measurable decrease in lung function (Vestbo and Lange 2002; Vestbo et al. 2013). The lung function variable most often studied in relation to smoking is forced expiratory volume in one second, FEV₁ (Hogg 2004; Kohansal et al. 2009; Jordan et al. 2012), which has a poor correlation to respiratory symptoms (Rabe et al. 2007; Vestbo et al. 2013). Not all smokers develop faster decline of lung function than expected with ageing, and there is still no general agreement on the possibility to tell the difference between a susceptible and non-susceptible smoker before the accelerated lung function decline starts. The presence of respiratory symptoms could be one such marker (de Marco et al. 2007; Kohansal et al. 2009; Vestbo et al. 2013). Smoking cessation is known to have a beneficial effect on lung function decline (Kohansal et al. 2009).

One of the most common respiratory diseases that affects smokers is chronic obstructive pulmonary disease (COPD).

COPD

Chronic Obstructive Pulmonary Disease (COPD) is characterized by chronic, progressive airflow obstruction and can be diagnosed by spirometry. It is a worldwide leading cause of morbidity and mortality and results in an economic and social

burden that is both substantial and increasing. COPD is predominantly caused by smoking, but other factors as occupational exposure and different kinds of indoor pollution are probably also among the risk factors for COPD (Vestbo et al. 2013). COPD is also characterised by exacerbations (sustained worsenings of the patient's symptoms, beyond normal day-to-day variations which are acute in onset and often caused by viral infections) and the presence of coexisting diseases, often called comorbidities.

Pathology, pathogenesis and pathophysiology

In short, in subjects developing COPD, inhaled particles (often from cigarette smoke) cause a modified chronic inflammation, which may induce parenchymal destruction (emphysema) and disruption of small airways (bronchiolitis).

Pathology

In COPD, chronic inflammatory changes are found in the airways, lung parenchyma and pulmonary vasculature. Both increased numbers of inflammatory cell types and structural changes resulting from repeated injury and repair are seen (Hogg 2004). These changes persist despite smoking cessation and increase with increasing disease severity.

Pathogenesis

The pathological changes in COPD are interpreted as an enhanced inflammatory response, compared to normal response to inhaled irritants, most often cigarette smoke. Not all smokers develop COPD, and the reason for this is not known, but at least contribution of genetical determinants is suspected. Never-smokers can also develop COPD, the nature of the inflammatory response in those cases is not understood.

Pathophysiology

The pathophysiological outcome of the inflammatory response in COPD is narrowing of peripheral airways (bronchiolitis) and destruction of lung parenchyma (emphysema) (Hogg et al. 2004), which leads to airflow limitation with a decrease of FEV₁ and gas exchange disturbances. The relative contribution of bronchiolitis and emphysema to airflow limitation is unique in every subject with COPD. The airflow limitation causes air trapping and hyperinflation, which in turn explains symptoms like dyspnea and thereby limitation of exercise capacity. The gas exchange disturbances in COPD are mainly caused by ventilation-perfusion mismatch and in more severe cases, hypoventilation. The gas exchange disturbances also contribute to the symptom of dyspnea in COPD.

Other pathophysiological features of COPD are mucus hypersecretion, present in many but not all COPD patients and pulmonary hypertension, which appears relatively late in disease.

Diagnosis

A diagnosis of COPD should be suspected in any subject with respiratory symptoms as dyspnea, cough and sputum production and/or a history of relevant exposure. There is no general agreement on the spirometric definition of COPD. Several guidelines exist with slightly different approach. In general, FEV₁/vital capacity (VC) is used for diagnosis and FEV₁ as percent of predicted is used for staging.

GOLD

One of the most common guidelines for diagnosing COPD used worldwide is Global initiative for Obstructive Lung Disease (GOLD). This initiative was implemented in 1998 as a cooperation between World Health Organisation (WHO) and National Institutes of Health (NIH) among others. The GOLD Science Committee has released annual reports since 2001 containing state-of-the-art information on COPD, including diagnostic guidelines (www.goldcopd.org, version 2010). According to GOLD, spirometry is required for the diagnosis of COPD. A post-bronchodilator value of FEV₁/forced vital capacity (FVC) <0.7 is the GOLD spirometric criterion for COPD. The known problems with this definition is underdiagnosis of young subjects, mostly women and overdiagnosis of elderly subjects, mostly men (Pellegrino et al. 2005; Vestbo et al. 2013), but as the most widespread problem with COPD diagnostics still is underdiagnosis the haunt for simplicity has been the priority of the GOLD Science Committee. Screening for COPD is controversial and not supported by GOLD. Measuring the degree of the reversibility of airflow limitation is not recommended in COPD patients.

Staging of the disease is based on a combination of the patient's symptoms, risk for exacerbations and severity of airflow limitation, measured as FEV₁ as percent of predicted normal (PN) – I FEV₁ ≥ 80% of PN, II 50% of PN ≤ FEV₁ <80% of PN, III 30% of PN ≤ FEV₁ <50% of PN, IV <30% of PN (Vestbo 2013).

ATS/ERS

The American Thoracic Society (ATS) and European Respiratory Society (ERS) have made a joint statement on diagnostic criteria for COPD (Celli and MacNee 2004; Pellegrino et al. 2005) and advocate the use of the 5th percentile of the age-related FEV₁/FVC ratio (the lower limit of normal, LLN). With the use of LLN both age and gender are taken into account, and the number of probably misdiagnosed subjects is lower. One of the major challenges with the use of LLN for reference value is the choice of reference population, as it has a great impact on interpretation. For

severity classification the same principle is used as with GOLD, but with different limits and five groups, ranging from mild to very severe.

Diagnostic problems

Applying both of the two major guidelines (GOLD and ATS/ERS (LLN)) creates two discordant groups of subjects. One group consists of young subjects with FEV₁/FVC ratio above 0.7 but below LLN. This group may be subjects with pathological airflow limitation, which are not diagnosed with COPD using GOLD (Cerveri et al. 2008). The other group, clinically probably more numerous, consists of elderly subjects with FEV₁/FVC ratio below 0.7 but above LLN. This group may be subjects with lung function decline that is within the range for normal ageing of the lungs, being falsely diagnosed with COPD using GOLD (Hardie et al. 2002). There is still no general agreement on how to treat these two groups, as the longitudinal information on the outcome for subjects in each group is sparse.

Other guidelines

There are several other national guidelines all over the world. Most of them are based on the GOLD criteria, with no or slight changes. Some examples are the guidelines issued by the British National Institute for Health and Clinical Excellence (<http://guidance.nice.org.uk/CG101>), and COPD-X by The Thoracic foundation of Australia and New Zealand (<http://www.copdx.org.au/the-copd-guidelines>). In Sweden the Swedish Respiratory Society has proposed the modification of FEV₁/VC <0.65 as a spirometric criterion for subjects more than 65 years old, to decrease the number of misdiagnosed elderly subjects (<http://slmf.se/kol/huvudpunkter>).

Symptoms

The characteristic symptoms of COPD are chronic and progressive dyspnea, cough, and sputum production. The predictive importance of respiratory symptoms in early stages of COPD and before the development of COPD according to spirometric definitions has been extensively examined, but no consensus has been reached. Studies have shown that chronic cough and sputum production may precede the development of airflow limitation by many years (Lindberg et al. 2005; de Marco et al. 2007). It has also been shown that unhealthy smokers (defined as smokers reporting respiratory symptoms) experience a faster FEV₁ decline than healthy smokers (defined as smokers not reporting respiratory symptoms) (Kohansal et al. 2009). However, longitudinal data from the Copenhagen Heart Study did not show that productive cough was a predictor of COPD development (Vestbo and Lange 2002).

Chronic bronchitis is an independent disease entity, defined as chronic cough or mucus production for at least three months in two successive years. This

phenomenon is not necessarily present among subjects with COPD and not all subjects with chronic bronchitis have COPD (Vestbo et al. 2013).

A general problem is that respiratory symptoms may not always be adequately reported and there is also a poor relationship between respiratory symptoms and spirometric variables (Smith and Woodcock 2006; Vestbo et al. 2013).

Comorbidities

In general

COPD often coexists with other diseases (comorbidities) that may have a significant impact on prognosis (Soriano et al. 2005; Barnes and Celli 2009). The reasons for this vary, sometimes risk factors are shared and sometimes one disease increases the risk of another and in some cases the diseases coexist independently. Systemic inflammation, a general feature of COPD that is shared with other diseases may be a link between COPD and some of its comorbidities (Garcia-Rio et al. 2010). Cardiovascular diseases are major comorbidities in COPD and probably both the most frequent and most important diseases coexisting with COPD (Vestbo et al. 2013). Osteoporosis (Soriano et al. 2005; Fabbri et al. 2008) and depression (Hanania et al. 2011) are other major comorbidities, which are often underdiagnosed and are associated with poor health status and prognosis. Lung cancer is frequently seen in patients with COPD and has been found to be the most frequent cause of death in patients with mild COPD (Anthonisen et al. 2002).

Cardiovascular disease

Cardiovascular diseases are the most common and important among all comorbidities affecting patients with COPD (Soriano et al. 2005; Fabbri et al. 2008). So far the most studied entities studied in this field are ischemic heart disease, heart failure, atrial fibrillation and hypertension.

The increased prevalence of ischemic heart disease in COPD is partly explained by the common risk factors (Johnston et al. 2008). Among patients with COPD about 30% have heart failure, and the same proportion of patients with heart failure has COPD (Vestbo et al. 2013). FEV₁ is a strong predictor of mortality in heart failure. Atrial fibrillation is more common among subjects with than without COPD (Buch et al. 2003). This poses a diagnostic problem mainly because the symptom of dyspnea is very important in both diseases. Finally, hypertension is probably the most common comorbidity of COPD, and also hypertension has implications for the prognosis of the patient (Fabbri et al. 2008).

Apart from the entities described above it has been shown that another manifestation of cardiovascular disease, carotid atherosclerosis is associated with both smoking and low FEV₁ (Ebrahim et al. 1999; Engström et al. 2001) and carotid atherosclerosis is

in turn related to increased risk of stroke and myocardial infarction (Hollander et al. 2002). Most previous studies on the association between cardiovascular diseases and COPD have focused on FEV₁, which gives a limited insight into the pathophysiology of the lungs. In order better to understand the associations between decreased lung function and cardiovascular disease other lung physiological variables could be examined.

Alcohol consumption

Association with lung function

Elevated alcohol consumption is closely associated with smoking (Strine et al. 2005). Still, the effects of alcohol consumption on lung function are still not completely clarified, even though several studies have been performed over the last decades (Emirgil et al. 1974; Emirgil and Sobol 1977; Lyons et al. 1986; Lange et al. 1988; Greene et al. 2008). Large, population-based studies have shown no effect at all of alcohol on lung function (Cohen et al. 1980; Sparrow et al. 1983) or COPD mortality (Greene et al. 2008) as well as a negative effect of high alcohol consumption on lung function (Lange et al. 1988; Tabak et al. 2001). Also positive effects on lung function by light to moderate drinking, compared to abstaining from alcohol has been reported (Siu et al. 2010). Some studies showed abnormal lung function among high consumers of alcohol recruited in hospital settings (Emirgil et al. 1974; Emirgil and Sobol 1977; Garshick et al. 1989). Most studies have measured FEV₁ and VC as the only tests of lung function (Cohen et al. 1980; Lange et al. 1988; Tabak et al. 2001). In order better to understand the associations between lung function and alcohol consumption, other lung physiological variables could be examined.

Assessment

Assessing alcohol consumption is known to be difficult. Most studies on the associations between lung function and alcohol consumption have used self-reported information to assess alcohol consumption (Lange et al. 1988; Hoth et al. 2012). A commonly used questionnaire for self-report of alcohol consumption is the Alcohol Use Disorders Identification Test (AUDIT), a validated questionnaire for examining drinking habits (Bush et al. 1998). Although AUDIT is validated, it is known that all self-reported alcohol consumption could be subject to recall bias (Ekholm 2004), which makes it difficult to find out the true alcohol consumption using this method.

Using biomarkers could be one possible way to assess alcohol consumption more correct. A blood marker that has been used for this purpose is γ -glutamyl-

transpeptidase (GGT), which is a biological marker of alcohol consumption. A negative association between FEV₁ and alcohol consumption was found using GGT (Zureik et al. 1996). Carbohydrate deficient transferrin (CDT) is another, and compared with GGT, more sensitive and specific serum marker of elevated alcohol consumption (Hannuksela et al. 2007; Niemela 2007). CDT has been shown to be associated with FEV₁ in a small group of COPD patients (Nihlén et al. 2001).

Objectives

The main objectives for this thesis were:

- to analyse extensive lung function tests in groups of subjects fulfilling none, either or both of the spirometric criteria for COPD (GOLD and ERS/ATS).
- to examine pulmonary resistance and reactance measured by Impulse Oscillometry System (IOS) in subjects with or without self-reported chronic bronchitis or emphysema or COPD and subjects with or without COPD diagnosed according to the GOLD criteria.
- to examine a broad spectrum of lung function variables and their potential associations with CVD expressed as ultrasound diagnosed atherosclerotic plaques in the internal carotid artery.
- to examine potential effects of alcohol consumption on lung function, by using both a validated questionnaire (AUDIT-C) and a clinically used specific alcohol blood marker (CDT).

Materials and Methods

This thesis is based on a cross sectional study including one clinic visit at the Department of Clinical Physiology, Malmö, Skane University Hospital. The study named ROLLS, Role Of Low Lung function Study, was performed between June 2004 and May 2007 and was approved by the Ethics Committee of Lund University and all participants.

If not otherwise stated, the described methods were used for all the papers included in the thesis.

Study population

In 2000, a postal respiratory questionnaire survey ("Questions about the lungs") was performed in a randomly selected adult population in southern Sweden, (n = 11 933, response rate 78.1%) (Nihlén et al. 2004). For the study upon which this thesis is based (ROLLS) 870 subjects from that population residing in the area of Malmö, in southern Sweden were invited. Three different groups of subjects were recruited (Figure 1):

1. Lung healthy never-smokers = subjects who did not report any respiratory symptoms and never had smoked (controls)
2. Lung healthy current smokers = subjects who did not report any respiratory symptoms and were current smokers (smokers)
3. Subjects with COPD = subjects who responded that they had or had had a diagnosis of chronic bronchitis or emphysema or COPD (COPD)

Questionnaires

Questions about the lungs

This questionnaire was used both for study recruitment purposes and at the study visit. “Questions about the lungs” is a questionnaire about respiratory diseases and symptoms that has been used in several previous surveys and has been described by Montnemery (Montnemery et al. 1998) and Nihlén (Nihlén et al. 2004). The answers to this questionnaire at the study visit were used for paper I, II and IV.

Definitions in the study achieved from “Questions about the lungs”

Self-reported chronic bronchitis or emphysema or COPD (Q+) was defined as a positive answer to any of the questions “Do you have or have had chronic bronchitis or emphysema or chronic obstructive pulmonary disease (COPD)?” and “Did you get the diagnosis chronic bronchitis or emphysema or chronic obstructive pulmonary disease (COPD) by a physician?”. *Symptoms of chronic bronchitis (CB)* were defined as a positive answer to “Have you had periods of 3 months with cough with phlegm during 2 years in a row during the last years?”. *Long-standing cough* was defined as a positive answer to “Have you had long-standing cough during the last 12 months?”. *Dyspnoea on exertion* was defined as a positive answer to the question “Do you get breathless, wheeze or cough during exercise?”. *Airway infection* was defined as a positive answer to the question “During the last 12 months, have you had an airway infection?”. *Any use of pulmonary medication* was defined as a positive answer to “Do you regularly take or have taken any pulmonary medication?”.

AUDIT-C

The Alcohol Use Disorders Identification Test (AUDIT) is a validated questionnaire for examining drinking habits (Bush et al. 1998) that was used in paper IV. The three alcohol consumption questions (AUDIT-C) from this test is a brief screening test for problem drinking. The AUDIT-C questions are:

1. How often do you have a drink containing alcohol?
2. How many drinks containing alcohol do you have on a typical day when you are drinking?
3. How often do you have six or more drinks on one occasion?

The total AUDIT-C score is the sum of scores for the 3 questions resulting in possible total AUDIT-C scores from 0 to 12.

The first question in AUDIT-C “How often do you have a drink containing alcohol?” was used to identify non-drinkers. Subjects that had not answered the first question in AUDIT-C were excluded from further analyses. The total score of AUDIT-C (ranging from 0 to 12) was used to discriminate between moderate and hazardous drinking. For women a total score on AUDIT-C ≥ 3 was regarded as hazardous drinking and for men ≥ 4 (Reinert and Allen 2007).

Smoking habits

Subjects who currently smoked or had stopped smoking within the last 12 months prior to the study visit were classified as smokers. Subjects who had stopped smoking more than 12 months prior to the study visit were classified as ex-smokers. For subjects being current smokers or ex-smokers, the term ever-smokers were used. Subjects who had never been smoking daily for more than one month were classified as never-smokers. The total tobacco consumption was calculated in pack years (one pack year = 20 cigarettes smoked per day for one year).

Lung function tests

All lung function measurements were made 15-45 minutes after inhalation of 1.0 mg of terbutaline (Bricanyl® Turbuhaler®, AstraZeneca, Mölndal, Sweden).

Spirometry and body plethysmography

Both spirometry (all papers) and body plethysmography (papers I, III and IV) were performed according to ERS recommendations (Quanjer et al. 1993), and European reference values were used (Quanjer et al. 1993). A spirometer (Master Screen; Viasys GmbH – Erich Jaeger, Hoechberg, Germany) was used to measure FEV₁ and VC, while TLC and RV were measured using a body plethysmograph (Master Screen, Viasys GmbH – Erich Jaeger).

IOS

The resistance and reactance of the respiratory system was measured using Impulse Oscillometry System (MasterScreen IOS, Viasys GmbH, Hoechberg, Germany)

(papers I and II). Principally, the used method, forced oscillation technique (FOT), implies a loudspeaker generating pressure oscillations composed of multiple frequencies, which are superimposed during 30 seconds of normal tidal breathing. This allows the assessment of resistance and reactance at several frequencies simultaneously, ranging from 5 to 35 Hz. Subjects sat upright, had a nose clip and firmly supported their cheeks with their hands. A minimum of three trials were performed. The following variables were evaluated in ROLLS:

- I. Respiratory resistance at 5 Hz (R5), which is assumed to reflect total airways resistance.
- II. Respiratory resistance at 20 Hz (R20), which is assumed to reflect central airways resistance.
- III. The fall in resistance from R5 to R20 (R5-R20) was used as a surrogate for the frequency dependence of respiratory resistance, which increases with increasing inhomogeneity of peripheral airways (Skloot et al. 2004; Oppenheimer et al. 2007).
- IV. Distal capacitive reactance at 5 Hz (X5), which is assumed to reflect peripheral airways function.
- V. The resonant frequency (f_{res}).
- VI. An area index of low frequent reactance (AX). This parameter incorporates the area, which ranges from the negative reactance between 5 Hz and resonant frequency to the zero line.

Single breath test for CO

Diffusing capacity for carbon monoxide ($D_{L,CO}$) was measured using the single-breath technique (Cotes et al. 1993) (Master Screen) and the reference values for $D_{L,CO}$ were corrected for haemoglobin values according to established procedures (Macintyre et al. 2005) (papers I, III and IV).

Classification and staging of COPD

COPD was diagnosed according to the GOLD (Global initiative for chronic Obstructive Lung Disease) criteria ([www. goldcopd.org](http://www.goldcopd.org), version 2010). In brief, these recommendations state that a post-bronchodilator FEV_1/VC value <0.7 is diagnostic for COPD. Also staging of COPD severity was performed according to the GOLD criteria (version 2010) with stage I (mild): $FEV_1 > 80\%$ of predicted normal (PN),

stage II (moderate): $50\% FEV_1 < 80\% PN$, stage III (severe): $30\% FEV_1 < 50\% PN$, and stage IV (very severe COPD): $FEV_1 < 30\% PN$.

In paper I we also used the current recommendations from the American Thoracic Society and the European Respiratory Society (ERS), that favour the use of the 5th percentile of the age-related FEV_1/FVC ratio (the lower limit of normal, LLN; (Pellegrino et al. 2005)) as a comparison to the GOLD criteria.

Blood analyses

hsCRP and HbA1c

High-sensitivity CRP (hsCRP) was measured in serum by an IMMAGE rate nephelometer, Beckman-Coulter, Brea, CA, USA. The detection limit was 0.2 mg l^{-1} with imprecision 9% (CV) at 1.0 mg l^{-1} , 7% at 13.0 mg l^{-1} and 7% at 75.0 mg l^{-1} (papers III and IV). Glycated haemoglobin (HbA1c) was measured by high performance liquid chromatography (HPLC) (paper III).

Blood lipids

Total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides were measured in plasma on Beckman Coulter LX20 (Beckman Coulter), a routine method in Malmö, Skane University Hospital since 1999 (paper III).

Carbohydrate deficient transferrine (CDT)

CDT was analyzed in serum by the high performance liquid chromatography (HPLC) method (Jeppsson et al. 1993) (paper IV). CDT is expressed as the percentage of transferrin being carbohydrate deficient, a fraction that is increased by heavy alcohol consumption. The level of CDT reflects the alcohol consumption of the last 1-3 weeks. CDT was used to identify subjects with heavy alcohol consumption. Similarly to clinical practice a value of $\geq 2.0\%$ was used for identifying subjects with heavy alcohol consumption in the study (Helander et al. 2003).

Ultrasonography of atherosclerotic plaques in the internal carotid artery (ICA)

A 7.5-MHz linear ultrasound probe was used to screen the ICA, the bulb and the distal portion of the common carotid artery bilaterally for plaques (paper III). Plaques were defined as absent or present. There is no uniform worldwide definition of carotid plaque (Wyman et al. 2006). There are no well-known reference values for thickness of the total vessel wall in this region. In this study, a plaque was defined as a focal thickening of the total vessel wall (of 2 mm or more) relative to adjacent segments protruding into the lumen in at least one side, which is estimated to correspond well to the definition of plaque in this region as the presence of focal thickening at least 50% greater than that of the surrounding vessel wall used in several other studies (Salonen and Salonen 1991; Persson et al. 1992; Hollander et al. 2002; North et al. 2002; Prabhakaran et al. 2006; Roman et al. 2006). All examiners were experienced and not aware of any other study data prior to examination.

Blood pressure

Blood pressure was measured in the right upper arm with subjects in supine position, after 5–10 mins of rest (paper III). Pressure was measured to the nearest 5 mmHg. The mean of two measurements was calculated.

Statistical analyses

IBM SPSS Statistics 20 (SPSS Inc. Chicago, Illinois 60606) was used for the statistical analyses.

Continuous variables are presented as means or medians as described in each study. Standard deviations (SD) or ranges have been used to describe measurement distributions. Categorical variables are presented as numbers and/or percentages.

One-way ANOVA was used for comparisons of continuous variables between groups. Continuous variables not normally distributed (IOS variables, CDT, hsCRP, triglycerides and current daily tobacco consumption) were log-transformed before the analyses. When there was a need to adjust for multiple comparisons between groups Tukey's test was used (paper I).

In paper III, a multivariate logistic regression was used to calculate odds ratios (OR) and confidence intervals (CI) for associations between different factors and the

occurrence of atherosclerotic plaques in the ICA. In paper IV, multivariate linear regression analyses were used to analyse the influence of alcohol consumption on lung function variables. The results were presented as unstandardized B coefficients and standard errors. For all regression analyses all possible relevant adjustments were made.

The IOS variables were adjusted and compared in general linear models (papers I and II). The associations between lung function variables and the occurrence of atherosclerotic plaques in the ICA were also adjusted and analysed in a general linear model. Variables with skewed distributions (IOS variables, hsCRP and triglycerides) were log-transformed before the general linear model analysis. In paper I, a Bonferroni correction was used to adjust for multiple comparisons between groups. After the statistical analyses log-transformed values were back transformed and presented as adjusted geometric means.

Categorical variables were compared using Chi-squared analysis.

A p-value of <0.05 was considered statistically significant.

Results and Discussion

Participation

Of the 870 invited subjects, 450 took part in the study (Figure 1).

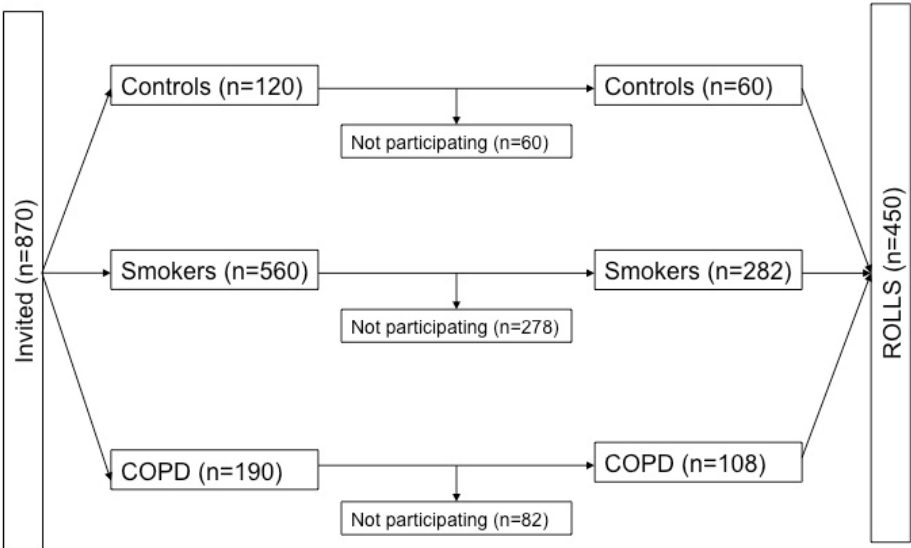


Figure 1. Inclusion in ROLLS. Reasons not to participate included that the subject did not want to participate, had moved away, was not possible to contact, did not call back, did not show up or called back when the inclusion was finished.

The aim with the recruitment of the study population for ROLLS was to generate a population with a large number of ever-smokers, in that way containing several subjects with mild-moderate lung function impairment, which was achieved. In fact, the majority of subjects with COPD according to GOLD at the study visit (n=132) was recruited from the group of smokers not reporting respiratory symptoms in the questionnaire study in 2000 (n=95, not shown). From the invited group reporting a diagnosis of chronic bronchitis or emphysema or COPD (n=108) only 34 had COPD

according to the GOLD spirometry criterion. The remaining subjects in that group consisted of about one third never-smokers (n=27, not shown), finally contributing to the group of never-smokers in ROLLS. When designing the study, we also aimed at including a group of never-smokers reporting no respiratory symptoms, as controls. The 89 never-smokers in ROLLS consisted mainly of subjects from the invited group of never-smokers reporting no respiratory symptoms, but also of never-smokers from the invited group reporting a diagnosis of chronic bronchitis or emphysema or COPD in the questionnaire study in 2000, who had a normal spirometry at the study visit.

The study population is not representative for the general population and we have not analysed the non-participating subjects further.

The 450 subjects participating in ROLLS were classified in different ways for the different studies included in this thesis, based on spirometry results from the study visit and reported respiratory symptoms and drinking habits at the study visit. The different classifications are shown adjacent to the results and discussion of each study.

Characteristics of the study population

Some general characteristics of the study population, divided by smoking habits, are presented in Table 1.

Table 1.

General characteristics of the study population in ROLLS. Continuous variables are presented as mean (SD), categorical variables as numbers (%).

	Never-smokers (n=89)	Ex-smokers (n=141)	Current smokers (n=220)	Total (n=450)
Sex (men/women)	31 (35%)/ 58 (65%)	58 (41%)/ 83 (59%)	96 (44%)/ 124 (56%)	185 (41%)/ 265 (59%)
Age	62.3 (8.2)	62.2 (7.3)	60.7 (7.5)	61.5 (7.6)
Pack years	0	24 (17)	30 (18)	22 (19)
BMI	26.3 (4.9)	28.2 (5.1)	25.5 (4.8)	26.5 (5.0)
COPD I	5 (6%)	35 (25%)	48 (22%)	88 (20%)
COPD II	2 (2%)	11 (8%)	24 (11%)	37 (8%)
COPD III-IV	0	5 (4%)	2 (1%)	7 (2%)
FEV ₁ % of predicted normal	108.9 (17)	97.0 (19)	96.6 (16)	99.2 (18)

The study population has a preponderance of women, most explicit among never-smokers. The age range in the study population is 46-78 years. There is a high preva-

lence of current smokers, 49% (ever-smokers 80%) and the ever-smokers have many pack years. The general prevalence of COPD in the study population according to the GOLD criteria is 29% (132 subjects).

Respiratory symptoms and results of extended lung function tests differ when different diagnostic criteria for COPD are applied (paper I)

For paper I the subjects were classified into two groups with respect to FEV_1/VC : subjects with $FEV_1/VC > 0.7$ and subjects with $FEV_1/VC < 0.7$. The first group was further subdivided into never-smokers (N-NS) and ever-smokers (N-ES). The second group was further subdivided into subjects with $FEV_1/VC > LLN$ (FR+) and subjects with $FEV_1/VC < LLN$ (FR+LLN+) (Figure 2). There was no subject with $FEV_1/VC > 0.7$ but $< LLN$.

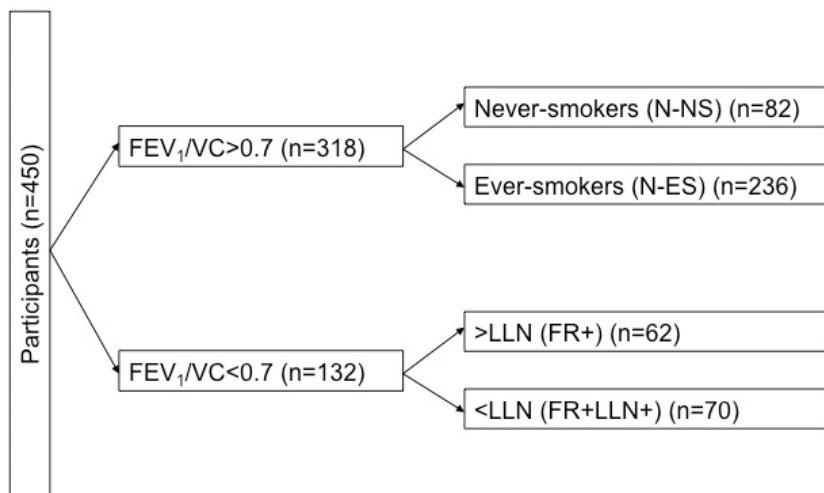


Figure 2. Flow chart demonstrating the classification of participants in paper I depending on their spirometry results.

Subject characteristics are shown in Table 2. There was no statistically significant difference between groups concerning gender. There were small but statistically significant age differences between N-ES and both FR+ and FR+LLN+. Total tobacco consumption was somewhat lower in the N-ES group than in the FR+ ($p=0.066$) and FR+LLN+ ($p=0.04$) groups, but was identical in the two latter groups.

Table 2.

General characteristics of the study groups. LLN = lower limit of normal, a = significantly different from FR+ ($p < 0.001$) and FR+LLN+ ($p < 0.01$), b = significantly different from FR+LLN+ ($p = 0.04$)

	FEV ₁ /VC >0.7 and >LLN		FEV ₁ /VC <0.7 and >LLN	FEV ₁ /VC <0.7 and <LLN
	N-NS	N-ES	FR+	FR+LLN+
N	82	236	62	70
Sex (men/women)	28/54	90/146	37/25	30/40
Age (years)	62 ± 8	60 ± 7 ^a	65 ± 7	64 ± 6.5
Height (m)	1.68 ± 0.09	1.69 ± 0.08	1.71 ± 0.09	1.71 ± 0.09
Weight (kg)	75.1 ± 16.9	76.7 ± 16.9	77.9 ± 15.9	75.1 ± 15.0
Never-smokers/ex-smokers/current smokers	82/0/0	0/90/146	4/21/37	3/30/37
Smoking history (pack years)	0	25 ± 15 ^b	31 ± 22	31 ± 21

Symptoms and results of lung function tests are shown in Table 3. A general pattern was seen for all symptoms with subjects in the FR+LLN+ having the highest prevalence, the FR+ group lower, the N-ES group lower still and the N-NS group had least symptoms.

There were highly significant differences in FEV₁ between all groups. FEV₁ was thus lower in the N-ES group than in the N-NS group despite the very small difference in FEV₁/VC. There was also a considerable difference in FEV₁ between the FR+ and the FR+LLN+ groups.

Only minor differences between groups were seen in VC and TLC. Larger differences were seen for RV and RV/TLC, where the FR+/LLN+ group differed significantly from all other groups. Our finding of significantly higher RV and RV/TLC ratio as well as lower D_{L,CO} in the FR+ than in the N-ES group indicates that the former group comprises a substantial number of subjects with abnormal lung function. These results are in agreement with the study by Güder (Güder et al. 2012), which showed that RV/TLC and D_{L,CO} contributed to the expert's diagnosis of COPD when all available diagnostic information was used. D_{L,CO} correlates well with the macroscopic as well as the microscopic extent of emphysema (Madani et al. 2006), and may occur in subjects with FEV₁/FVC ratio >0.7 (Fain et al. 2006; Kirby et al. 2013). One possible explanation to the differences in D_{L,CO} found in this study may be attributable to development of early emphysema in smokers.

Table 3.

a = p=0.05 for difference between groups, b = p<0.001 for difference between groups, c = p<0.05 for difference between groups, d = significantly different from all other groups (p<0.001), e = significantly different from N-NS (p<0.05), f = significantly different from N-ES (p<0.05), g = significantly different from N-ES (p<0.001), h = significantly different from N-NS (p<0.001), i = significantly different from FR+ (p<0.01), j = significantly different from FR+ (p<0.01), k = significantly different from N-NS (p<0.01), l = significantly different from N-ES (p<0.01).

	N-NS	N-ES	FR+	FR+LLN+
Long-standing cough (%)	10.2 ^a	13.6 ^a	14.5 ^a	26.2 ^a
Dyspnoea on exertion (%)	19.5 ^b	26.0 ^b	38.3 ^b	51.6 ^b
Airway infection (%)	16.7 ^c	29.7 ^c	23.2 ^c	37.3 ^c
Use of pulmonary medication (%)	3.7 ^b	7.4 ^b	4.9 ^b	27.9 ^b
FEV ₁ /VC	0.79 ± 0.05	0.78 ± 0.05	0.68 ± 0.02	0.57 ± 0.08
FEV ₁ (% pred)	110 ± 16 ^d	103 ± 13 ^d	93 ± 15 ^d	78 ± 18 ^d
VC (% pred)	115 ± 15	109 ± 14 ^e	109 ± 18	111 ± 17
TLC (% pred)	104 ± 12	101 ± 11	105 ± 14 ^f	109 ± 14 ^g
RV (% pred)	105 ± 18	101 ± 19	113 ± 25 ^g	125 ± 27 ^{g h i}
RV/TLC	96 ± 11	96 ± 13	101 ± 15 ^f	108 ± 18 ^{g h j}
R5 geometric mean	0.28	0.30	0.33 ^c	0.34 ^h
R5-R20 geometric mean	0.05	0.06	0.07	0.08 ^c
X5 geometric mean	-0.07	-0.09 ^k	-0.10 ^h	-0.11 ^h
AX geometric mean	0.16	0.23 ^k	0.30 ^h	0.36 ^{h i}
f _{res} geometric mean	10.4	11.4	12.1	12.4 ^c
D _{L,CO} (% pred)	89 ± 15	83 ± 15 ^c	79 ± 17 ^{k l}	69 ± 20 ^{g h i}

The prevalence of abnormal values for FEV₁, RV and D_{L,CO} in the different groups was analysed, as these variables showed the greatest differences at the group level (Figure 3). Despite the highly significant differences between all groups in FEV₁ as a percentage of predicted, only the FR+LLN+ group had a sizeable proportion of individually abnormal findings. The greatest proportion of abnormal values was seen in D_{L,CO}, where nearly half of the subjects in the FR+ group and more than half of the subjects in the FR+LLN+ groups showed reduced values.

Regarding measurements of D_{L,CO} the phenomenon of inter-laboratory differences has to be taken into account (Cotes et al. 1993). One possible solution to this problem is to calculate reference values specific for each lung function testing laboratory, using the results for never-smokers. In this study the healthy never-smokers were too few for

such calculations. Instead the reference equations for $D_{L,CO}$ recommended by ERS were used (Cotes et al. 1993). These equations are a summary of the mean from the literature and the process of the derivation is described in detail in the European Coal and Steel Committee (ECSC) recommendations from 1983 (Quanjer 1983). The use of these reference equations is probably the most important explanation to that in this study population the over-all mean $D_{L,CO}$ as % of predicted normal does not reach 100%.

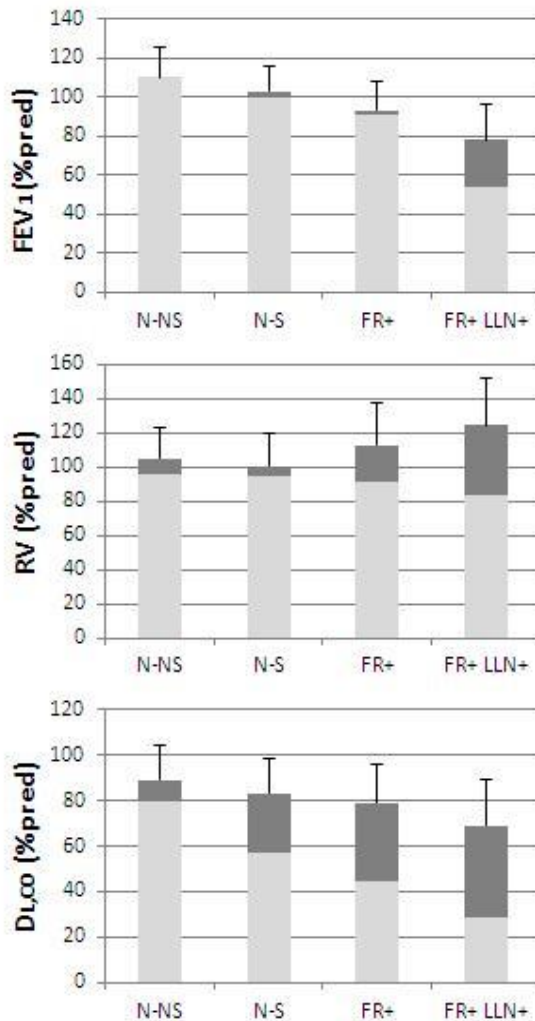


Figure 3. Mean FEV₁, RV and D_{L,CO} as percent of predicted in the different study groups. The darker shaded parts represent the proportion of individually abnormal findings in every study group.

Some guidelines, e.g. those issued by the British National Institute for Health and Clinical Excellence (<http://guidance.nice.org.uk/CG101>), stress that in subjects with $FEV_1 \geq 80$ % of predicted value, a diagnosis of COPD should only be made in the presence of respiratory symptoms, for example breathlessness or cough. We found low prevalence of abnormal FEV_1 as percentage of predicted value in all groups but the FR+LLN+ group, which may indicate that the addition of this variable to the diagnostic algorithm is of limited value.

As for symptoms, IOS variables showed a pattern with increasingly abnormal values going from the N-NS to the N-ES, FR+ and FR+LLN+ groups. The IOS variables showed several significant differences between groups, even if not between the FR+ and FR+LLN+ groups, which suggests that this technique may be of value in early diagnosis of airway disease among smokers.

Our findings that subjects fulfilling both of the two major diagnostic spirometry criteria for COPD have lung function and respiratory symptoms well compatible with a diagnosis is reassuring for clinicians working with possible COPD patients. The finding that subjects fulfilling only the GOLD criterion, in that way belonging to one of the possible discordant groups, still have worse lung function and more respiratory symptoms than ever-smokers with normal spirometry is more complicated. This indicates that it is not possible to easily dismiss these subjects as having ordinary lung function in relation to their age and being falsely diagnosed with COPD according to GOLD. Maybe these subjects are at risk of developing COPD or maybe they have characteristics of very early COPD. Longitudinal studies would be of interest to study this further.

Differences in lung mechanics when comparing subjects with and without reports of respiratory symptoms (paper II)

For paper II the subjects were classified into four different groups based on spirometry results and current answers to the questionnaire (Figure 4). Among subjects with no self-reported respiratory disease ($n = 342$) 252 subjects did not fulfil the spirometric criteria for COPD according to GOLD (Q-/G-) and 90 subjects did (Q-/G+). Among subjects with self-reported respiratory disease ($n = 77$) 43 subjects did not fulfil the spirometric criteria for COPD according to GOLD (Q+/G-) and 34 subjects did (Q+/G+).

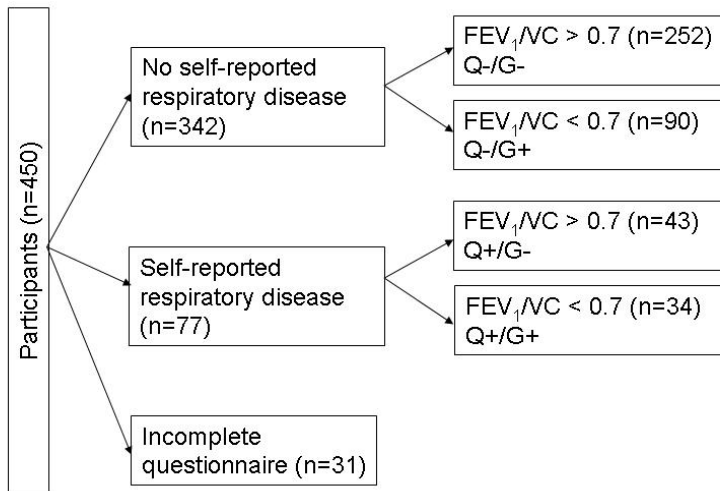


Figure 4.

Flow chart demonstrating the classification of participants in paper II depending on their answers to the questionnaire and their spirometry results.

Subject characteristics are shown in Table 4. Subjects with COPD according to the GOLD criteria (G+) were older than subjects with no COPD according to the GOLD criteria (G-), regardless of concomitant self-reported chronic bronchitis or emphysema or COPD (Q+) or not (Q-) ($p < 0.01$). Q-/G+ subjects had smoked more in terms of pack years compared with Q+/G- ($p < 0.01$).

Table 4.

General characteristics of the study groups in paper II.

	Q-/G- (n=252)	Q-/G+ (n=90)	Q+/G- (n=43)	Q+/G+ (n=34)	TOTAL (n=419)
Sex (men/women)	99/153	49/41	12/31	13/21	173/246
Age, years	60.1 (7.5)	64.1 (6.9)	60.0 (7.5)	65.5 (6.2)	61.4 (7.6)
Never-smokers/ex-smokers/current smokers	65/58/129	3/30/57	10/25/8	1/20/13	79/133/207
Pack years	19 (17)	32 (23)	19 (18)	30 (15)	23 (19)
COPD stage I	0	64 (71.1%)	0	18 (52.9%)	82 (19.6%)
COPD stage II	0	26 (28.9%)	0	10 (29.4%)	36 (8.6%)
COPD stage III-IV	0	0	0	6 (17.6%)	6 (1.4%)

The crude mean levels of resistance and reactance over the full frequency range are plotted in Figure 5 for the four different study groups. Especially for the measurement of resistance, both of the curves representing subjects reporting respiratory symptoms (Q+) are clearly separated from both of the curves representing symptom-free subjects (Q-). These differences between subjects with and without respiratory symptoms and with and without COPD according to GOLD were then further analysed.

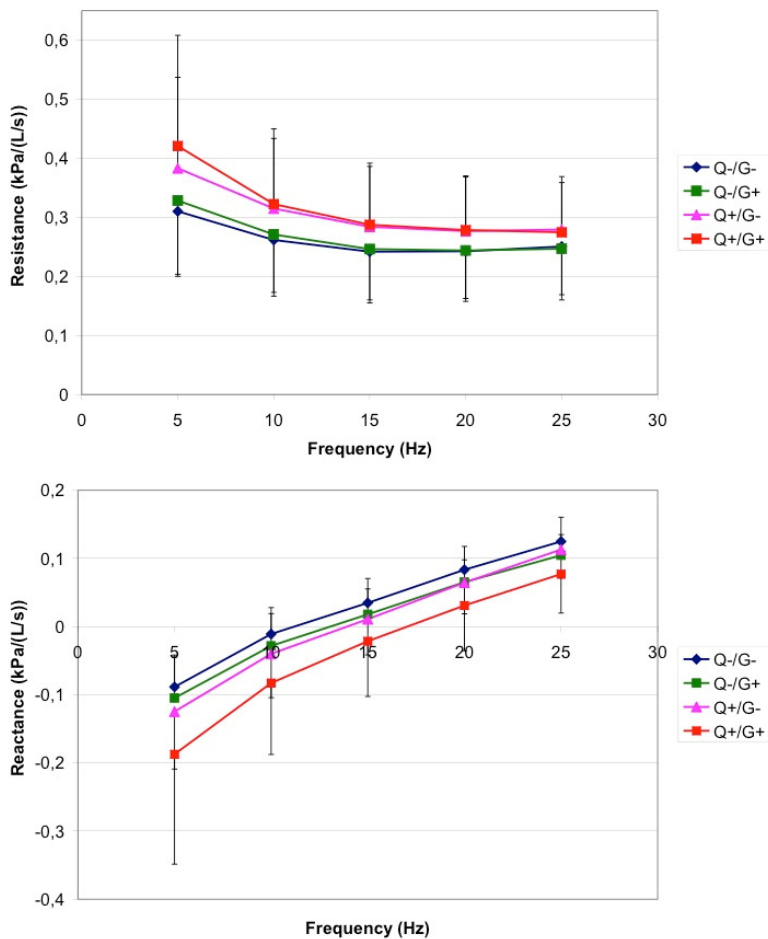


Figure 5. The crude mean values of resistance and reactance at different frequencies in the different study groups. (Q+/Q- = questionnaire positive or negative, G+/G- = fulfilling the spirometric criteria for COPD according to GOLD or not)

The four study groups are compared in Table 5. This shows that among all G+ subjects, lung function variables according to spirometry were lower and pulmonary resistance was higher and pulmonary reactance was lower for Q+ subjects compared to Q- subjects (except for VC% PN, p=0.25 and R20, p=0.11) The same pattern was seen among G- subjects (except for FEV₁/VC% PN, p=0.84).

Table 5.

Spirometry, IOS and questionnaire results at the study visit. For IOS variables geometric means (adjusted for gender and age) are shown. PN = predicted normal value. Letters a, b, c and d show pairwise significant comparisons between indicated groups (p < 0.05). (Q+/- = self-reported chronic bronchitis or emphysema or COPD or not, G+/- = COPD according to GOLD or not).

	Q-/G- (a) (n=252)	Q-/G+ (b) (n=90)	Q+/G- (c) (n=43)	Q+/G+ (d) (n=34)
FEV ₁ % PN	105 ^{b, c, d} (13)	87 ^{a, c, d} (13)	100 ^{a, b, d} (15)	78 ^{a, b, c} (25)
VC % PN	110 ^c (14)	111 ^c (16)	105 ^{a, b} (16)	108 (22)
FEV ₁ /VC	0.78 ^{b, d}	0.64 ^{a, c, d}	0.79 ^{b, d}	0.58 ^{a, b, c}
FEV ₁ /VC % PN	102 ^{b, d} (6)	83 ^{a, c, d} (7)	102 ^{b, d} (7)	76 ^{a, b, c} (16)
R5 (kPa/(L/s))	0.29 ^{b, c, d}	0.32 ^{a, d}	0.35 ^a	0.39 ^{a, b}
R20 (kPa/(L/s))	0.23 ^{c, d}	0.24	0.25	0.27 ^a
R5-R20 (kPa/(L/s))	0.06 ^{c, d}	0.07 ^d	0.08 ^a	0.10 ^{a, b}
X5 (kPa/(L/s))	-0.08 ^{b, c, d}	-0.09 ^{a, d}	-0.10 ^{a, d}	-0.13 ^{a, b, c}
AX	0.19 ^{b, c, d}	0.27 ^{a, d}	0.31 ^{a, d}	0.55 ^{a, b, c}
Symptoms of CB	18 ^{c, d} (7.1%)	12 ^c (13.3%)	15 ^{a, b} (34.9%)	9 ^a (26.5%)
Long-standing cough	36 ^{c, d} (14.3%)	13 ^{c, d} (14.4%)	17 ^{a, b} (39.5%)	13 ^{a, b} (38.2%)
Any use of pulmonary medication	5 ^{b, c, d} (2.0%)	7 ^{a, c, d} (7.8%)	15 ^{a, b} (34.9%)	14 ^{a, b} (41.2%)

The over-all pattern for spirometry variables was, as expected, more abnormal values for the two G+ groups, and healthier values for the two G- groups irrespective of the presence or absence of self-reported disease. The general pattern for the IOS variables was different, with increasingly abnormal values going from the Q-/G- to the Q-/G+, Q+/G- and Q+/G+ groups. This suggests that IOS could be of value for detection of individuals that already have developed typical pathological airway changes (i.e. small airway disease) for COPD, even though not yet meeting the GOLD diagnosis criteria. A previous study on IOS variables performed in subjects with COPD (stage II-IV), smokers and never-smokers without respiratory complaints have shown limited additive value compared to spirometry variables in COPD subjects (Crim et al. 2011). A suggestion from that study though is that complimentary examination with IOS may identify certain subgroups among COPD

patients, for example those with less emphysema and more chronic bronchitis. The contribution from our study in this context is the examination of IOS variables in subjects without COPD according to GOLD, but with respiratory complaints – maybe this group will benefit more than patients with established COPD. This is also mentioned in the study by Crim (Crim et al. 2011), the idea of apparently healthy (normal spirometry) smokers with abnormal IOS variables, which may represent small airway disease, a sign of early COPD. Early detection of COPD is of great value since it increases the possibility to successful treatment, for example smoking cessation (Kohansal et al. 2009). Objective methods that can contribute to early detection are therefore needed. IOS have some attractive features for clinical use, for example it is non-invasive and requires minimal active engagement by the patient. A challenge concerning IOS that has to be addressed in the future is the need for more extensive studies on reference values.

In summary, results from this study suggest that subjects with self-reported chronic bronchitis or emphysema or COPD (Q+) have a higher pulmonary resistance and lower pulmonary reactance than subjects without self-reported chronic bronchitis or emphysema or COPD (Q-), both in subjects with and without spirometry diagnosed COPD according to the GOLD criteria (G+ and G-).

Lower diffusing capacity and higher residual volume in subjects with atherosclerotic plaques in the internal carotid artery (paper III)

For paper III subjects were classified based on the presence or absence of atherosclerotic plaques in the internal carotid artery (Figure 6).

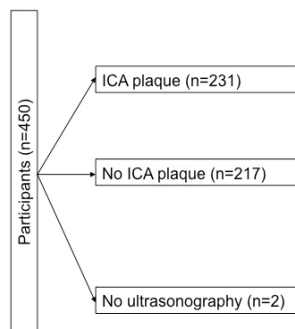


Figure 6.

Flow chart demonstrating the classification of participants in paper III depending on their smoking habits and the presence or absence of atherosclerotic plaques in the internal carotid artery.

Atherosclerotic plaque in at least one of the ICA was detected in 231 (52%) of the subjects. Subject characteristics and pairwise comparisons between subjects with and without atherosclerotic plaques in the internal carotid artery are shown in Table 6. Subjects with plaques were older and had higher systolic blood pressure, heavier smoking history and in addition, higher hsCRP and HbA1c than subjects without plaques in the ICA. Total cholesterol was lower in subjects with plaques than without plaques ($p = 0.051$). The reason is unclear but the finding could perhaps partly be explained by differences regarding statin use. Use of statins was not recorded, and therefore, it is unknown whether statins were more commonly used in subjects with than without ICA plaques.

COPD was more frequently diagnosed in subjects with than without plaques in the ICA. FEV₁, VC and DL_{CO} were statistically significant lower and RV higher in subjects with plaques in the ICA than in subjects without plaques.

Table 6.

Comparisons between subjects with and without ultrasound diagnosed atherosclerotic plaques in the internal carotid artery (ICA) according to ANOVA analysis (continuous variables) and Chi-squared analysis (categorical variables). For continuous variables values are presented as means and standard deviations. For categorical variables values are presented as numbers and percentages. For variables not normally distributed (hsCRP and triglycerides) values are presented as medians and ranges. (PN = predicted normal value)

	Subjects with ICA plaque (n=231)	Subjects without ICA plaque (n=217)	p-value
Sex (Men / Women)	115 (50%)/116 (50%)	68 (31%)/149 (69%)	<0.001
Age (years)	63.6 (7.03)	59.2 (7.56)	<0.001
Body mass index (kg/m ²)	26.4 (4.9)	26.7 (5.2)	0.461
Systolic blood pressure (mmHg)	141 (18)	135 (17)	<0.001
Pack years	28.4 (20.8)	15.9 (15.2)	<0.001
hsCRP (mg/l)	2.8 (0.1-92.1)	2.0 (0.1-25.6)	<0.001
HbA1c (%)	4.81 (0.82)	4.65 (0.41)	0.014
Cholesterol (mmol/l)	5.67 (1.14)	5.87 (1.00)	0.051
LDL (mmol/l)	3.69 (1.05)	3.84 (0.87)	0.097
HDL (mmol/l)	1.27 (0.41)	1.35 (0.39)	0.042
Triglycerides (mmol/l)	1.4 (0.4-4.6)	1.3 (0.4-10.8)	0.283
COPD diagnosis	83 (36%)	47 (22%)	0.001
FEV ₁ (% PN)	95.7 (18)	103.1 (17)	<0.001
VC (% PN)	108.1 (16)	112.4 (15)	0.004
FEV ₁ /VC (% PN)	93.7 (13)	98.0 (11)	<0.001
TLC (% PN)	103.1 (12)	103.7 (13)	0.564
RV (% PN)	110.1 (24)	104.7 (20)	0.012
DL _{CO} (% PN)	78.2 (17)	85.2 (17)	<0.001

Lung function variables were also compared in a general linear model, adjusting for potential confounders (gender, age, systolic blood pressure, pack years, hsCRP, HbA1c, total cholesterol and HDL) (Table 7). The relationships between ICA plaques and RV and $D_{L,CO}$, respectively, remained statistically significant according to general linear analyses with adjustments for potential confounding factors. The general linear analysis did not show any statistically significant differences in FEV₁, VC or TLC between subjects with and without plaques in the ICA.

Table 7.

Comparisons of different lung function measures between subjects with and without ultrasound diagnosed atherosclerotic plaque in the ICA (as a fixed factor) according to general linear analyses with adjustments for gender, age, systolic blood pressure, pack years, hsCRP, HbA1c, total cholesterol and HDL. Adjusted mean values and p-values are given.

	Subjects with ICA plaque (n=226)	Subjects without ICA plaque (n=205)	p-value
FEV ₁ (% PN)	97.9	100.5	0.145
VC (% PN)	109.8	110.7	0.572
TLC (% PN)	104.3	102.6	0.200
RV (% PN)	110.3	104.8	0.020
$D_{L,CO}$ (% PN)	77.4	83.7	0.014

Previous studies on associations between reduced lung function and cardiovascular diseases have mainly been based on FEV₁ and VC (Engström et al. 2001; Schroeder et al. 2005; Mannino et al. 2008) and the results are not completely consistent. The results of this study are in line with those from the Atherosclerosis Risk In Communities (ARIC) study (Schroeder et al. 2005), showing no independent association between lower FEV₁ and the occurrence of atherosclerotic plaques in the ICA. In this study though, results are consistent with the known general association between reduced lung function and cardiovascular disease (Hole et al. 1996; Engström et al. 2000; Sin and Man 2003; Sin et al. 2005; Engström et al. 2006; Maclay et al. 2007; Johnston et al. 2008; Mannino et al. 2008) through other lung function variables than FEV₁ and VC: $D_{L,CO}$ and RV. A proposed link that could contribute to the association between reduced lung function and cardiovascular disease is low-grade systemic inflammation (Engström et al. 2002; Sin and Man 2003; Eickhoff et al. 2008). The results of this study are not in conflict with such an explanation as shown by e.g. a statistically significant higher hsCRP in subjects with ICA plaques than in subjects without plaques. However, similar to the results for e.g. FEV₁, there was no statistically significant association between increase in hsCRP and ICA plaques in the multivariate logistic regression analysis. The study results seem to suggest that also other pathophysiological mechanisms than bronchial disease could be associated with increased risk of atherosclerotic disease. Such mechanism could be degradation of elastic tissue, a central pathological mechanism in the development of

lung emphysema. Previous studies have shown associations between elastin degradation and carotid atherosclerosis (Petersen et al. 2002; Kangavari et al. 2004).

A blood marker of heavy alcohol consumption is associated with lower levels of FEV₁ and D_{L,CO} (paper IV)

For paper IV subjects were classified as non-drinkers (abstainers) or alcohol drinkers depending on their answers to the questionnaire, AUDIT-C (Bush et al. 1998). Fourteen subjects were excluded from further analyses because they had not answered AUDIT-C and then could not be classified as non-drinkers or drinkers. For the analyses drinkers were classified in two different ways: first into moderate or hazardous drinkers using AUDIT-C (Figure 7a) and second into non-heavy or heavy drinkers using CDT (Figure 7b).

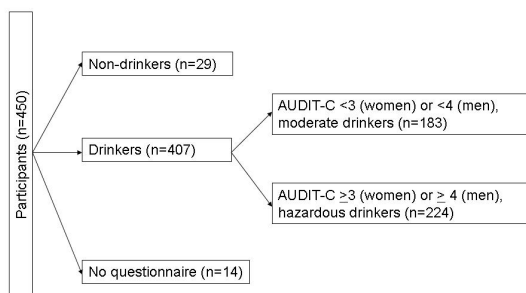


Figure 7a. Flow chart demonstrating the classification of participants in paper IV according to AUDIT-C into moderate or hazardous drinkers.

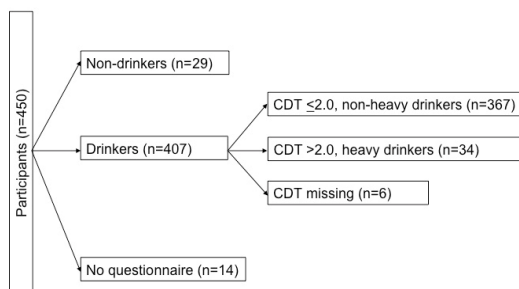


Figure 7b. Flow chart demonstrating the classification of participants in paper IV according to CDT into non-heavy or heavy drinkers.

Pairwise comparisons of general characteristics, lung function and respiratory symptoms within the two different classifications are shown in Table 8.

Table 8.

General characteristics, lung function and respiratory symptoms of the alcohol drinking part of the study population, divided into moderate drinkers and hazardous drinkers based on their total score of AUDIT-C, and into non-heavy drinkers and heavy drinkers based on their CDT levels.

	AUDIT-C classification (n=407)			CDT classification (n=401)		
	Moderate drinkers (n=183)	Hazardous drinkers (n=224)	p-value	Non-heavy drinkers (CDT≤2.0%) (n=367)	Heavy drinkers (CDT>2.0%) (n=34)	p-value
Women	62% (113)	54% (122)	0.168	60% (219)	29% (10)	0.001
Age (years)	62.9 (46-78)	60.1 (46-77)	<0.001	61.2 (46-78)	63.4 (53-77)	0.097
Ever-smokers	73% (134)	87% (194)	0.001	79% (291)	94% (32)	0.062
Current smokers	39% (72)	55% (124)	0.002	45% (165)	85% (29)	<0.001
Pack years	19.6 (0-110)	24.7 (0-135)	0.008	21.3 (0-115)	34.7 (0-135)	<0.001
Current daily tobacco consumption (number of cigarettes)	4.7 (0-54)	8.0 (0-55)	0.029	5.9 (0-55)	14.0 (0-35)	0.057
CDT (%)	1.11 (0.3-3.8)	1.48 (0.6-8.0)	<0.001	1.16 (0.3-1.9)	3.02 (2.0-8.0)	<0.001
FEV ₁ (% of PN)	100 (19)	100 (16)	0.996	100 (17)	96 (20)	0.113
VC (% of PN)	111 (16)	110 (15)	0.562	111 (15)	109 (18)	0.392
FEV ₁ /VC (% of PN)	96 (12)	96 (11)	0.686	96 (11)	92 (13)	0.033
TLC (% of PN)	104 (12)	103 (12)	0.477	104 (12)	103 (13)	0.648
RV (% of PN)	108 (21)	106 (21)	0.391	107 (21)	108 (30)	0.778
D _{LCO} (% of PN)	82 (17)	83 (16)	0.596	83 (16)	74 (20)	0.003
COPD (n)	30% (54)	29% (64)	0.922	27% (99)	50% (17)	0.008
Symptoms of CB	10% (18)	16% (33)	0.152	11% (39)	37% (11)	0.001
Long-standing cough	18% (32)	19% (42)	0.765	17% (61)	40% (12)	0.012
Any use of pulmonary medication	8% (15)	10% (21)	0.782	10% (35)	3% (1)	0.339

Hazardous drinkers were younger, had more pack years, and in addition were more often current smokers, which is in line with previous studies (Strine et al. 2005).

There were no significant differences between hazardous and moderate drinking regarding any of the lung function variables or respiratory symptoms. Levels of CDT were higher among hazardous compared with moderate drinkers. Heavy drinking compared to non-heavy drinking was associated with more pack years and current smoking. Not seen for hazardous drinkers though, heavy drinking was associated with male gender and lower BMI as well as with lower FEV1/VC and DL_{CO}. Heavy drinkers more often had COPD according to the GOLD criteria and more often reported respiratory symptoms.

In table 9, heavy and non-heavy drinkers are compared concerning lung function after adjustments. A significant difference between heavy and non-heavy drinkers was seen for DL_{CO}, both when adjusted for age, sex, height and weight and after additional adjustments for pack years and CRP. Then the continuous associations between CDT and lung function were investigated. After adjusting the crude lung function variables for age, sex, height and weight, a higher CDT was associated with lower FEV1, VC, FEV1/VC and DL_{CO}. The associations between CDT and FEV1 and DL_{CO} remained also after additional adjustments for pack years and CRP.

Table 9.

Relationship between alcohol consumption and lung function variables according to multivariate linear regression analyses. Values are unstandardized B coefficients (standard error, SE). For the continuous relationship between CDT and lung function variables, the B coefficient represents the change after increase of 1 standard deviation of lnCDT. *Adjusted for sex, age, height and weight. **In addition adjusted for pack-years and CRP.

	FEV ₁ (l)	VC (l)	FEV ₁ /VC	TLC (l)	RV (l)	DL _{CO} (mmol/kPa/min)
Heavy vs non-heavy*	-0.170 (0.089) p=0.058	-0.038 (0.094) p=0.686	-2.963 (1.594) p=0.064	-0.011 (0.130) p=0.929	0.045 (0.083) p=0.587	-0.833 (0.251) p=0.001
Heavy vs non-heavy**	-0.065 (0.086) p=0.450	0.036 (0.094) p=0.698	-1.756 (1.567) p=0.263	0.052 (0.130) p=0.693	0.034(0.083) p=0.686	-0.501 (0.239) p=0.037
CDT*	-0.287 (0.071) p<0.001	-0.205 (0.075) p=0.007	-3.231 (1.281) p=0.012	-0.185 (0.104) p=0.077	0.027 (0.067) p=0.684	-0.780 (0.202) p<0.001
CDT**	-0.201 (0.069) p=0.004	-0.145 (0.076) p=0.057	-2.259 (1.267) p=0.075	-0.131 (0.106) p=0.214	0.020 (0.068) p=0.764	-0.489 (0.193) p=0.012

The different results for moderate vs hazardous and non-heavy vs heavy may be an illustration of the effects of different methods for assessing alcohol consumption. In previous studies, most often questionnaires have been used for the assessment, and no

association between alcohol consumption and lung function has been shown (Cohen et al. 1980; Sparrow et al. 1983), which is in line with the findings of the current study. There are, however, also large, community-based studies that with the use of self-reported alcohol consumption indicate an independent, negative effect of alcohol intake on lung function (Lebowitz 1981; Lange et al. 1988). A more recent study (Zureik et al. 1996) has shown a negative association between γ -glutamyltranspeptidase (GGT), another blood marker of alcohol drinking, and FEV₁. In this study we used both a more specific blood marker, CDT (Hannuksela et al. 2007; Niemela 2007) and a validated questionnaire (AUDIT-C) and found different results with the two methods, supporting the idea of the importance of blood markers for assessment of alcohol consumption. Another important probable reason to the different results is that CDT identifies a smaller group of subjects with even heavier alcohol consumption than AUDIT-C does with the current, validated cut-offs (Reinert and Allen 2007). Using higher cut-offs for AUDIT-C would also identify a smaller group of subjects with heavier alcohol consumption, increasing the theoretical possibility to identify associations to lung function, but still the problem with recall bias would remain.

The finding that the difference in DL_{CO} between the groups of heavy and non-heavy drinkers was significant, which was not the case for FEV₁ suggests that DL_{CO} may be an even more sensitive indicator of the effects of alcohol on the lungs.

Results of the multiple linear regression analyses with FEV₁ and DL_{CO} as target variables for drinkers classified as never-smokers or ever-smokers are shown in Table 10.

Table 10.

Relationships between CDT and FEV₁ and DL_{CO} respectively, among never-smoking and ever-smoking alcohol drinkers according to multivariate linear regression analyses. Adjustments have been made for sex, age, height, weight, pack years and CRP. Unstandardized B coefficients and p-values are shown.

Target variable	Alcohol variable	Never-smoking drinkers (n=79)	Ever-smoking drinkers (n=328)
FEV ₁	CDT	0.015 p=0.944	-0.218 p=0.003
DL _{CO}	CDT	0.741 p=0.180	-0.568 p=0.007

CDT was statistically significantly associated with a decrease in FEV₁ and in DL_{CO} in ever-smokers, but not in never-smokers. This implies that the found negative association between CDT and reduced lung function may represent an independent additive negative effect of alcohol among smokers, but not among never-smokers.

Conclusions

- Subjects with FEV1/VC <0.7 and below the LLN frequently have symptoms and findings at extended lung function tests indicative of COPD, supporting the use of this criterion as a cut-off for the diagnosis. Subjects with FEV1/VC <0.7 but above the LLN more frequently than subjects with normal FEV1/VC show findings indicative of COPD at extended lung function tests. Careful evaluation of patient history and extended lung function testing may therefore be warranted in this category of subjects.
- Subjects reporting respiratory symptoms differ in lung mechanics measured by the forced oscillation technique, whether they fulfil the GOLD criteria of COPD or not. The finding indicates that this method better captures small changes in lung function, which may be useful for early detection of COPD.
- Diffusing capacity for carbon monoxide is lower and residual volume is higher in subjects with atherosclerotic plaques in the internal carotid artery, when influence of established risk factors for atherosclerosis is adjusted for. This suggests that associations between reduced lung function and cardiovascular disease may not only be related to bronchial disease, but also to lung parenchymal disease.
- Higher levels of a blood marker for heavy alcohol consumption (CDT) is associated with lower FEV1 and DLCO among smokers. This suggests an additive negative effect of heavy alcohol consumption on lung function among smokers.

Future perspectives

The last decades has taught us a lot about lung function in smokers, both with and without COPD. Still, the nature of early effects of smoking on lung function and the subsequent development of COPD among many smokers is urging to be discovered more thoroughly. With such knowledge the possibilities to influence lung function decline in earlier stages of disease will increase.

Future research on lung function in smokers will have to include more longitudinal studies on outcome. In the search for early predictors that link to future endpoints of disease different modalities will have to be more extensively combined than today. Such modalities include for example morphological methods as computed tomography, biochemical methods using blood markers and physiological methods with a broader span of lung physiology variables, to better reflect different aspects of lung physiology than just airflow limitation.

In the future more sensitive methods to examine lung function like for example IOS may be generally available in primary care all over the world. With such tools and a deeper knowledge of early smoking effects on different aspects of lung function and in turn their effects on future endpoints of disease, appropriate groups among smokers may be selected for screening of lung function. Then smoking-related lung disease as COPD and its concomitant diseases may be earlier detected and more efficiently treated.

But still, the most effective way to improve lung function in smokers - from both a physiological and an economical perspective – is smoking cessation and the major future challenge must be to avoid that people ever become smokers.

Populärvetenskaplig sammanfattning

Rökning är den vanligaste och viktigaste riskfaktorn för att få sänkt lungfunktion. Kroniskt obstruktiv lungsjukdom (KOL) orsakas oftast av rökning, men drabbar inte alla som röker. Det är en sjukdom som ger försämrad lungfunktion och de som drabbas upplever andfåddhet och hosta, ibland med slemproduktion. För att ställa diagnosen KOL krävs att lungfunktionen har undersökts med spirometri. En svårighet är dock att experterna inte är helt överens om var gränsen för sjukligt sänkt lungfunktion ska gå. Idag finns det huvudsakligen två olika riktlinjer i världen att följa. Relativt lite är dock känt om vad som karakteriserar lungfunktionen och vilka symtomen är hos de människor som uppfyller ett eller två av de kriterier som finns eller vad som karakteriserar rökare som ännu inte uppfyller något av kriterierna. Ett annat problem är att KOL ofta upptäcks sent i förloppet, när en stor del av lungfunktionen redan försvunnit och de behandlingar som finns gör sämre nytta. Det vore därför bra om vi blev bättre på att upptäcka KOL tidigare. Just eftersom vi ofta upptäcker KOL relativt sent så vet vi ganska lite om hur lungfunktionen är precis i början av sjukdomen och ännu mindre hur den är tiden innan sjukdomen är ett faktum.

Människor med KOL har ofta många andra samtidiga sjukdomar, av olika skäl – t ex att de delar vissa riskfaktorer som rökning, att de drivs av samma processer i kroppen eller att den ena sjukdomen ökar risken för den andra. De vanligaste samtidiga sjukdomarna vid KOL är hjärt-kärlsjukdomar. För hjärt-kärlsjukdomar är bara delar av sambandet med sänkt lungfunktion och KOL känt.

Det är väl känt att stor alkoholkonsumtion hänger ihop med rökning. Det är däremot inte klarlagt om det finns någon koppling mellan alkoholkonsumtion och lungfunktion. Många studier har gjorts för att försöka klarlägga detta, men det är svårt att få en exakt uppfattning om alkoholkonsumtion genom frågeformulär, vilket gör det något osäkert att använda den metoden i studier av lungfunktion. Det vore bra att veta om det finns någon koppling, för att kunna ge bästa tänkbara råd till rökare angående deras lungfunktion.

Den här avhandlingen syftar till att ta reda på mer om lungfunktionen och vad som hänger ihop med den bland rökare som än så länge mår ganska bra och inte upplever några stora problem med sin lungfunktion. Tidigare har det gjorts en undersökning där tiotusentals frågeformulär skickades ut till slumpmässigt utvalda människor i sydvästra Skåne. Via svaren på frågeformulären bjöds nu människor från tre olika

grupper in att delta i denna studie: människor som aldrig har rökt och som inte har några symtom från luftvägarna, rökare som inte har några symtom från luftvägarna och människor som i frågeformuläret hade svarat att de har kronisk bronkit, emfysem eller KOL. Alla som tackade ja fick komma till sjukhuset i Malmö och genomgå flera undersökningar vid ett och samma tillfälle. Deltagarna gjorde bland annat olika lungfunktionsundersökningar, ultraljud av halskärnen, tog blodprover och besvarade frågeformulär om bland annat lungsjukdomar, symtom från luftvägarna och alkoholkonsumtion.

Vi fann att lungfunktionen mätt på flera olika sätt liksom symtomen från luftvägarna stämde väl överens med en KOL-diagnos hos dem som uppfyllde båda de etablerade kriterierna för KOL, men även att de som bara uppfyllde det ena hade sämre lungfunktion än rökare med normal spirometri (delarbete I).

Vi fann också att de med själv-rapporterad KOL hade högre luftvägsmotstånd än de som inte rapporterade någon KOL, oavsett om de hade KOL enligt spirometri eller inte. Att mäta luftvägsmotstånd skulle således kunna vara av värde för att undersöka rökare som inte har KOL enligt spirometri (delarbete II).

När vi undersökte lungfunktionen hos dem som hade åderförkalkning i halskärnen och jämförde den med dem som inte hade det så såg vi att de som hade åderförkalkning i halskärnen också hade sämre gasutbytesförmåga, som inte bara kunde förklaras med att de också hade rökt mer. Kanske finns det ett samband mellan att ha kärl som blir åderförkalkade och att ha lungvävnad som är mer mottaglig för rök-skador (delarbete III).

Vi kunde också genom att använda ett blodprov som markör för stor alkoholkonsumtion visa att rökare som dricker väldigt mycket alkohol har en sämre lungfunktion än de som dricker mindre mängder, vilket inte bara kunde förklaras med att stor-drickarna också röker mer. Även här visade sig försämringen främst som en sämre gasutbytesförmåga hos de stor-drickande rökarna. Kanske har stora mängder alkohol en oberoende negativ effekt på lungfunktionen hos rökare (delarbete IV).

Sammanfattningsvis har vi visat att det är angeläget att undersöka lungfunktionen på fler sätt än de allra vanligaste för att kunna hitta tidiga förändringar som resultat av rök-skador på lungorna och för att kunna se tidiga kopplingar till hjärt-kärlsjukdom och stor alkoholkonsumtion. I framtiden kan vi förhoppningsvis upptäcka och behandla KOL och dess samtidiga sjukdomar tidigare än idag. Den viktigaste åtgärden mot KOL i framtiden är dock att minska antalet rökare.

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Paper I

Paper II



Impulse oscillometry may be of value in detecting early manifestations of COPD

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Summary

Background: Spirometry is used to diagnose chronic obstructive pulmonary disease (COPD). The Impulse oscillometry system (IOS) allows determination of respiratory impedance indices, which might be of potential value in early COPD, although previous experience is limited. We examined pulmonary resistance and reactance measured by IOS in subjects with or without self-reported chronic bronchitis or emphysema or COPD (Q+ or Q-) and subjects with or without COPD diagnosed according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria (G+ or G-). **Methods:** From a previous population-based study 450 subjects were examined with spirometry and IOS and answered a questionnaire on respiratory symptoms and diseases.

Results: Seventy-seven subjects were Q+, of whom 34 also were G+. Q+/G- subjects (n = 43) reported respiratory symptoms more frequently (35–40% vs 8–14%) but had higher FEV₁ (100% vs 87%) than Q-/G+ subjects (n = 90), p < 0.05 for both comparisons. Q+ subjects had higher pulmonary resistance and lower pulmonary reactance than Q- subjects (p < 0.01 for all comparisons). The same pattern was seen both in G+ subjects ((Q+/Q-) R5 0.39/0.32, R5–R20 0.10/0.07, X5 0.13/0.09, AX 0.55/0.27, p < 0.05 for all) and G- subjects ((Q+/Q-) R5 0.35/0.29, R5–R20 0.08/0.06, X5 0.10/0.08, AX 0.31/0.19 p < 0.05 for all) except for R20 (adjusted for gender and age).

Conclusions: Self-reported chronic bronchitis or emphysema or COPD was associated with higher pulmonary resistance and lower pulmonary reactance measured by IOS, both among subjects with and without COPD according to GOLD criteria. IOS may have the potential to detect pathology associated with COPD earlier than spirometry.

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Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by chronic, progressive airway obstruction and can be diagnosed by spirometry.¹ Major treatment guidelines, e.g. the global initiative for obstructive lung disease (GOLD 2010) recommend that COPD should be identified as early as possible in order to increase the opportunity to affect disease progression.¹ Most COPD patients experience respiratory symptoms such as dyspnoea, cough and sputum production, but the role of symptoms in early detection of COPD is unclear. Studies by de Marco et al.² and Lindberg et al.³ suggest that the presence of chronic cough/phlegm identifies subjects at risk of COPD independent of smoking habits. However, longitudinal data from the Copenhagen Heart Study did not show that productive cough was a predictor of COPD development.⁴ Furthermore, respiratory symptoms may not always be adequately reported and there is also a poor relationship between respiratory symptoms and spirometric variables.^{1,5}

Respiratory mechanics can also be measured by forced oscillation measurements.^{6–8} A commercially available method is provided by the Impulse Oscillometry System (IOS) (MasterScreen IOS, Viasys GmbH, Hoechst, Germany). IOS assesses pulmonary resistance (R_s) and reactance (X_r), which are the real and imaginary part of respiratory impedance (Z_r), at various frequencies. Oscillometry has been proposed to be able to reflect more distal airway function⁹ compared to spirometry^{10,11} and distal airways are a major site for airway obstruction in COPD.^{12–15} A clinical advantage of this method is that it is performed during tidal breathing and requires no forced manoeuvres. The relevance of this method in COPD is still somewhat unclear, but an association between reactance and FEV₁ in COPD patients has been shown.¹⁶ Another study showed a correlation between IOS measurements and health status and dyspnoea among COPD patients.¹⁷ These studies did not include control groups, and it is not clear what information IOS contributes in subjects with symptoms of COPD but normal spirometry.

The objective of this study was to examine pulmonary resistance and reactance measured by IOS in subjects with or without self-reported chronic bronchitis or emphysema or COPD (according to a questionnaire on respiratory diseases and symptoms, Q+/Q–) and subjects with or without COPD diagnosed according to the GOLD criteria (G+/G–). Our primary hypothesis was that pulmonary resistance is higher and reactance lower among subjects reporting respiratory symptoms in a group of subjects not fulfilling the spirometric criteria for COPD according to GOLD and that this may indicate early manifestations of COPD.

Methods

This was a cross sectional study including one clinic visit at the Department of Clinical Physiology, Malmö University Hospital (UMAS) in Malmö.

Study population

The invited study population consisted of 870 invited subjects, who comprised a sub-population to a postal respiratory questionnaire survey ("Questions about the

lungs") performed in a population-based cohort in 1992 as well as 2000.^{18,19} Three different groups of subjects all residing in the area of Malmö, Southern Sweden were recruited; (1) Lung healthy never-smokers = subjects who did not report any respiratory symptoms and never had smoked (2) Lung healthy current smokers = subjects who did not report any respiratory symptoms and were current smokers and (3) Subjects with COPD = subjects who responded that they had or had had a diagnosis of chronic bronchitis or emphysema or COPD.

At the study visit, subjects were classified according to their current smoking habits (current smokers, ex-smokers and never-smokers), their answers to the repeated questionnaire and their spirometry results according to the GOLD 2010 criteria.

Ethic approval

The study was approved by the Ethics Committee of Lund University and participants signed an informed consent before any study related procedure.

Questionnaire

Questions about the lungs

This questionnaire was used both for study recruitment purposes and for classification of several respiratory symptoms at the study visit. "Questions about the lungs" is a questionnaire about respiratory diseases and symptoms that has been used in several previous surveys and is described elsewhere.¹⁸

Self-reported chronic bronchitis or emphysema or COPD

Self-reported chronic bronchitis or emphysema or COPD (Q+) was defined as a positive answer to any of the questions "Do you have or have had chronic bronchitis or emphysema or chronic obstructive pulmonary disease (COPD)?" and "Did you get the diagnosis chronic bronchitis or emphysema or chronic obstructive pulmonary disease (COPD) by a physician?"

Chronic bronchitis, long-standing cough and use of pulmonary medication

Symptoms of chronic bronchitis (CB) were defined as a positive answer to "Have you had periods of 3 months with cough with phlegm during 2 years in a row during the last years?". Long-standing cough was defined as a positive answer to "Have you had long-standing cough during the last 12 months?". Any use of pulmonary medication was defined as a positive answer to "Do you regularly take or have taken any pulmonary medication?".

Smoking habits

Subjects who currently smoked or had stopped smoking within the last 12 months prior to the study visit were classified as smokers. Subjects who had stopped smoking more than 12 months prior to the study visit were classified as ex-smokers. Subjects who had never been smoking daily for more than one month were classified as never-smokers. The total tobacco consumption was calculated in pack-

years (one pack year = 20 cigarettes smoked per day for one year).

Lung function tests

All lung function measurements were made 15–45 min after inhalation of 1.0 mg of terbutaline (Bricanyl Turbuhaler).

Spirometry

Spirometry was performed according to the standards of the European Respiratory Society (ERS)²⁰ and European reference values were used.²⁰ A spirometer (Master Screen, Viasys GmbH – Erich Jaeger, Hoechberg, Germany) was used to measure forced expiratory volume in one second (FEV₁) and vital capacity (VC).

IOS

The pulmonary resistance and reactance was measured using IOS (MasterScreen IOS, Viasys GmbH, Hoechberg, Germany). The device consists of a loudspeaker that generates pressure oscillations composed of multiple frequencies, which are superimposed during 30 s of normal tidal breathing. This allows the assessment of resistance and reactance at several frequencies simultaneously, ranging from 5 to 35 Hz. Subjects sat upright, had a nose clip and firmly supported their cheeks with their hands. A minimum of three trials were performed. The following variables were evaluated: I. Respiratory resistance at 5 Hz (R5), which is assumed to reflect total airways resistance, II. Respiratory resistance at 20 Hz (R20), which is assumed to reflect central airways resistance, III. The fall in resistance from R5 to R20 (R5–R20) was used as a surrogate for the frequency dependence of respiratory resistance, which increases with increasing inhomogeneity of peripheral airways,^{10,11} IV. Distal capacitive reactance at 5 Hz (X5), which is assumed to reflect peripheral airways function and V. An area index of low frequent reactance (AX). This parameter incorporates the area, which ranges from the negative reactance between 5 Hz and resonant frequency to the zero line.

Classification and staging of COPD

COPD was diagnosed according to the GOLD (Global initiative for chronic Obstructive Lung Disease) criteria (www.goldcopd.org, version 2010). Also staging of COPD severity was performed according to the GOLD criteria with stage I (mild): FEV₁ ≥ 80% of predicted normal (PN), stage II (moderate): 50% ≤ FEV₁ < 80% PN, stage III (severe): 30% ≤ FEV₁ < 50% PN, and stage IV (very severe COPD): FEV₁ < 30% PN.

Statistical analyses

IBM SPSS Statistics 18 (SPSS Inc., Chicago, Illinois 60606) was used for the statistical analyses. One-way ANOVA was used for comparisons of continuous variables. Continuous variables not normally distributed (IOS variables) were log-transformed before the analyses. The mean values of IOS variables in the four study groups were adjusted for age and

gender and compared in a general linear model. As the IOS variables had skewed distributions the variables were log-transformed before the general linear model analysis. Log-transformed values were back transformed after the statistical analyses and presented as geometric means.

Categorical variables were compared using Chi-squared analysis. A *p*-value of <0.05 was considered statistically significant.

Results

Participation

Of the 870 invited subjects 450 subjects (52%) participated. The participation rates for lung healthy never-smokers, lung healthy current smokers and subjects with self-reported COPD were 50% (*n* = 60), 50% (*n* = 282) and 57% (*n* = 108), respectively.

Characteristics of the study population

At the study visit subjects were classified into four different groups based on spirometry results and current answers to the questionnaire: Q–/G–, Q–/G+, Q+/G– and Q+/G+ (Fig. 1). Thirty-one participants were excluded from the analyses due to incomplete answers to the questionnaire. The characteristics of the study population are shown in Table 1. Overall more women than men participated.

Subjects with COPD according to the GOLD criteria (G+) were older than subjects with no COPD according to the GOLD criteria (G–), regardless of concomitant self-reported chronic bronchitis or emphysema or COPD (Q+) or not (Q–) (*p* < 0.01).

Q–/G+ subjects had smoked more in terms of pack-years compared with Q+/G– (*p* < 0.01). The proportion of ex-smokers was significantly higher in both of the groups of

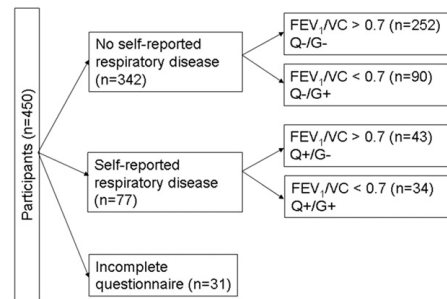


Figure 1 Flow chart demonstrating the classification of participants. Among subjects with no self-reported respiratory disease (*n* = 342) 252 subjects did not fulfil the spirometric criteria for COPD according to GOLD (Q–/G–) and 90 subjects did (Q–/G+). Among subjects with self-reported respiratory disease (*n* = 77) 43 subjects did not fulfil the spirometric criteria for COPD according to GOLD (Q+/G–) and 34 subjects did (Q+/G+).

Table 1 General characteristics of the study population. Numbers and percentages are shown for categorical variables and means with standard deviations are shown for continuous variables. (Q+/- = self-reported chronic bronchitis or emphysema or COPD or not, G+/- = COPD according to GOLD or not).

Subjects	Q-/G- (n = 252)	Q-/G+ (n = 90)	Q+/G- (n = 43)	Q+/G+ (n = 34)	Total (n = 419)
Male gender	99 (39.3%)	49 (54.4%)	12 (27.9%)	13 (38.2%)	173 (41.3%)
Female gender	153 (60.7%)	41 (45.6%)	31 (72.1%)	21 (61.8%)	246 (58.7%)
Age, years	60.1 (7.5)	64.1 (6.9)	60.0 (7.5)	65.5 (6.2)	61.4 (7.6)
Height, cm	169.4 (8.8)	171.3 (8.6)	167.7 (8.4)	168.3 (8.6)	169.5 (8.8)
BMI, kg/m ²	26.2 (5.0)	26.0 (4.3)	29.3 (6.4)	27.2 (5.6)	26.6 (5.1)
Never-smokers	65 (25.8%)	3 (3.3%)	10 (23.3%)	1 (2.9%)	79 (18.9%)
Ex-smokers	58 (23.0%)	30 (33.3%)	25 (58.1%)	20 (58.8%)	133 (31.7%)
Current smokers	129 (51.2%)	57 (63.3%)	8 (18.6%)	13 (38.2%)	207 (49.4%)
Pack-years	19 (17)	32 (23)	19 (18)	30 (15)	23 (19)
COPD stage I	0	64 (71.1%)	0	18 (52.9%)	82 (19.6%)
COPD stage II	0	26 (28.9%)	0	10 (29.4%)	36 (8.6%)
COPD stage III-IV	0	0	0	6 (17.6%)	6 (1.4%)

Q+ subjects compared to any of the groups of subjects with Q- ($p < 0.05$).

A diagnosis of COPD according to the GOLD criteria was confirmed in 30% of the study population most subjects at GOLD stage I.

Spirometry and IOS results and report of respiratory symptoms in the study population

The crude mean levels of resistance and reactance over the full frequency range are plotted in Fig. 2 for the four different study groups. Especially for the measurement of resistance, both of the curves representing subjects reporting respiratory symptoms (Q+) are clearly separated from both of the curves representing symptom-free subjects (Q-). We wanted further to analyse these differences, comparing subjects with and without respiratory symptoms and with and without COPD according to GOLD.

Symptomatic subjects (Q+) had statistically significantly lower spirometry values (FEV₁, VC, FEV₁/VC) than non-symptomatic subjects (Q-) (Table 2a). Q+ subjects also had statistically significantly higher pulmonary resistance and lower (i.e. abnormal) reactance than Q- subjects.

Subjects fulfilling the spirometric criteria for COPD according to GOLD (G+) had significantly higher pulmonary resistance and lower pulmonary reactance than subjects not fulfilling the GOLD criteria (G-), except for unadjusted R20 (Table 2b). A higher proportion of G+ subjects reported use of any pulmonary medication, but no difference was seen between the groups regarding report of long-standing cough and symptoms characteristic of chronic bronchitis. As expected, G+ subjects had significantly lower FEV₁ and lower FEV₁/VC than G- subjects.

In Table 3 all 4 study groups are compared. This shows that among all G+ subjects, lung function variables according to spirometry were lower and pulmonary resistance was higher and pulmonary reactance was lower for Q+ subjects compared to Q- subjects (except for VC% PN, $p = 0.25$ and R20, $p = 0.11$) The same pattern was seen among G- subjects (except for FEV₁/VC% PN, $p = 0.84$).

G+ subjects had lower FEV₁ and FEV₁/VC than G- subjects, irrespective of symptoms.

While pulmonary resistance did not differ significantly between Q+/G+ subjects and Q+/G- subjects, pulmonary reactance was significantly lower among Q+/G+ subjects than Q+/G- subjects ($p < 0.05$). The measurements with forced oscillation thus identified differences between

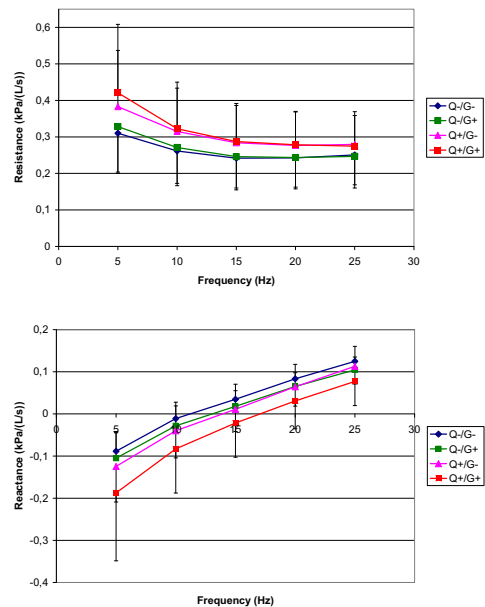


Figure 2 The crude mean values of resistance and reactance at different frequencies in the different study groups. (Q+/- = questionnaire positive or negative, G+/- = fulfilling the spirometric criteria for COPD according to GOLD or not).

Table 2a Comparison of spirometry and IOS results between Q+ and Q- subjects (subjects with or without self-reported chronic bronchitis or emphysema or COPD). Means with standard deviations are shown for continuous variables and numbers and percentages are shown for categorical variables. Continuous variables not normally distributed (all unadjusted IOS values) are presented as medians and 80% central ranges. Geometric means are adjusted for gender and age. PN = predicted normal value. (Q+/- = self-reported chronic bronchitis or emphysema or COPD or not).

	Q+ (n = 77)	Q- (n = 342)	p-value
FEV ₁ % PN	90 (23)	101 (15)	<0.001
VC% PN	106 (19)	111 (14)	0.015
FEV ₁ /VC	0.70	0.74	0.003
FEV ₁ /VC% PN	90 (17)	97 (10)	<0.001
R5 (kPa/(L/s))	0.37 (0.23–0.57)	0.30 (0.19–0.47)	<0.001
R5 geometric mean	0.36	0.30	<0.001
R20 (kPa/(L/s))	0.26 (0.19–0.38)	0.23 (0.16–0.35)	0.004
R20 geometric mean	0.26	0.23	0.004
R5–R20 (kPa/(L/s))	0.11 (0.04–0.23)	0.06 (0.02–0.14)	<0.001
R5–R20 geometric mean	0.09	0.06	<0.001
X5 (kPa/(L/s))	-0.12 (-0.31 to -0.05)	-0.08 (-0.16 to -0.04)	<0.001
X5 geometric mean	-0.11	-0.08	<0.001
AX	0.49 (0.08–1.88)	0.20 (0.06–0.83)	<0.001
AX geometric mean	0.40	0.22	<0.001
Symptoms of CB	24 (31.2%)	30 (8.8%)	<0.001
Long-standing cough	30 (39.0%)	49 (14.3%)	<0.001
Any use of pulmonary medication	29 (37.7%)	12 (3.5%)	<0.001

symptomatic and asymptomatic subjects not revealed by spirometry. For pulmonary resistance and reactance, higher values for unadjusted R5 and R20 were observed for Q+/-G- subjects than for Q-/G+ subjects ($p < 0.05$). After adjustments for gender and age, no differences were observed.

Discussion

Results from this study suggest that subjects with self-reported chronic bronchitis or emphysema or COPD (Q+) have a higher pulmonary resistance and lower pulmonary

Table 2b Comparison of spirometry and IOS results between G+ and G- subjects (subjects with or without COPD according to the GOLD criteria). Means with standard deviations are shown for continuous variables and numbers and percentages are shown for categorical variables. Continuous variables not normally distributed (all unadjusted IOS values) are presented as medians and 80% central ranges. Geometric means are adjusted for gender and age. PN = predicted normal value. (G+/- = COPD according to GOLD or not).

	G+ (n = 124)	G- (n = 295)	p-value
FEV ₁ % PN	85 (18)	104 (14)	<0.001
VC% PN	110 (18)	110 (14)	0.629
FEV ₁ /VC	0.62	0.78	<0.001
FEV ₁ /VC% PN	81 (11)	102 (6)	<0.001
R5 (kPa/(L/s))	0.32 (0.20–0.57)	0.30 (0.19–0.49)	0.029
R5 geometric mean	0.34	0.30	0.002
R20 (kPa/(L/s))	0.23 (0.16–0.36)	0.24 (0.16–0.35)	0.505
R20 geometric mean	0.25	0.23	0.049
R5–R20 (kPa/(L/s))	0.08 (0.03–0.21)	0.06 (0.02–0.15)	0.013
R5–R20 geometric mean	0.07	0.06	0.023
X5 (kPa/(L/s))	-0.10 (-0.25 to -0.05)	-0.08 (-0.16 to -0.04)	<0.001
X5 geometric mean	-0.10	-0.08	<0.001
AX	0.34 (0.09–1.54)	0.20 (0.06–0.86)	<0.001
AX geometric mean	0.33	0.21	<0.001
Symptoms of CB	21 (16.9%)	33 (11.1%)	0.098
Long-standing cough	26 (21.0%)	53 (18.0%)	0.433
Any use of pulmonary medication	21 (16.9%)	20 (6.8%)	<0.001

Table 3 Spirometry, IOS and questionnaire results at the study visit. Means with standard deviations are shown for continuous variables and numbers and percentages are shown for categorical variables. For IOS variables geometric means (adjusted for gender and age) are shown. PN = predicted normal value. Letters a, b, c and d show pair wise significant comparisons between indicated groups ($p < 0.05$). (Q+/– = self-reported chronic bronchitis or emphysema or COPD or not, G+/– = COPD according to GOLD or not).

	Q–/G– (a) (n = 252)	Q–/G+ (b) (n = 90)	Q+/G– (c) (n = 43)	Q+/G+ (d) (n = 34)
FEV ₁ ,% PN	105 ^{b,c,d} (13)	87 ^{a,c,d} (13)	100 ^{a,b,d} (15)	78 ^{a,b,c} (25)
VC% PN	110 ^c (14)	111 ^c (16)	105 ^{a,b} (16)	108 (22)
FEV ₁ /VC	0.78 ^{b,d}	0.64 ^{a,c,d}	0.79 ^{b,d}	0.58 ^{a,b,c}
FEV ₁ /VC% PN	102 ^{b,d} (6)	83 ^{a,c,d} (7)	102 ^{b,d} (7)	76 ^{a,b,c} (16)
R5 (kPa/(L/s))	0.29 ^{b,c,d}	0.32 ^{a,d}	0.35 ^a	0.39 ^{a,b}
R20 (kPa/(L/s))	0.23 ^{c,d}	0.24	0.25	0.27 ^a
R5–R20 (kPa/(L/s))	0.06 ^{c,d}	0.07 ^d	0.08 ^a	0.10 ^{a,b}
X5 (kPa/(L/s))	0.08 ^{b,c,d}	0.09 ^{a,d}	0.10 ^{a,d}	0.13 ^{a,b,c}
AX	0.19 ^{b,c,d}	0.27 ^{a,d}	0.31 ^{a,d}	0.55 ^{a,b,c}
Symptoms of CB	18 ^{c,d} (7.1%)	12 ^c (13.3%)	15 ^{a,b} (34.9%)	9 ^a (26.5%)
Long-standing cough	36 ^{c,d} (14.3%)	13 ^{c,d} (14.4%)	17 ^{a,b} (39.5%)	13 ^{a,b} (38.2%)
Any use of pulmonary medication	5 ^{b,c,d} (2.0%)	7 ^{a,c,d} (7.8%)	15 ^{a,b} (34.9%)	14 ^{a,b} (41.2%)

reactance than subjects without self-reported chronic bronchitis or emphysema or COPD (Q–), both in subjects with and without spirometry diagnosed COPD according to the GOLD criteria (G+ and G–). In addition, characteristics of Q+ subjects were associated with more frequent reports of symptoms characteristic of chronic bronchitis, long-standing cough and use of pulmonary medication compared with G+ subjects. Together these findings suggest that IOS could be of interest for getting increased understanding of relationships between underlying disease mechanisms of COPD, for example small airway disease and clinically important symptoms. IOS may be complementary to spirometry for this purpose. Furthermore, it seems conceivable that IOS would be of value for detection of individuals that already have developed typical pathological airway changes (i.e. small airway disease) for COPD, even though not yet meeting the GOLD diagnosis criteria.

Worldwide, COPD is underdiagnosed as well as detected too late in its course, due to factors such as underuse of spirometry and patient delay.^{1,21,22} Early detection is of importance to get the best possibility to intervene pharmacologically and with other treatments. IOS seems to have the potential to evaluate further the occurrence of distal airway pathology, an important component of COPD,^{12–14,23} by measuring resistance and reactance. Since IOS is a non-invasive method that requires minimal active engagement by the patient it could perhaps also be used routinely with the purpose of facilitate early detection of COPD. Since forced oscillations measurements are made during quiet breathing at functional residual capacity (FRC), forced breathing manoeuvres which may affect bronchial tone are avoided. In theory, elastic properties of the lung are reflected in the low frequencies of reactance (distal airways) and inertial properties are reflected in the high frequencies of reactance (central airways).²⁴ For resistance, the theory is that resistance at low frequencies represents total airway resistance, and resistance at high frequencies represents central airway resistance, proposing that the difference between these two is a surrogate for

peripheral airway resistance.⁹ Already in the 1980s Clément et al.²⁵ could separate healthy subjects from subjects with respiratory complaints, associated or not with a reduced FEV₁. This group also studied differences between smokers and non-smokers with normal spirometry without finding any significant differences in forced oscillation.²⁶ A previous study of respiratory impedance in a large sample of subjects referred for routine spirometry has shown that the impedance measurements contributed significantly to the discrimination between subjects with and without marked respiratory complaints.²⁷ A recent study has also shown that IOS measurements can be used to define different subgroups of COPD patients.²⁸

Symptoms of long-standing cough and symptoms characteristic of chronic bronchitis were more frequent among Q+ subjects than G+ subjects. There was no significant difference concerning the report of long-standing cough and symptoms characteristic of chronic bronchitis comparing G+ and G– subjects.

It has been shown that subjects with respiratory symptoms have a high risk of developing COPD^{2,3,29} although there are conflicting results.⁴ Symptoms also have a predictive value among COPD patients, for example it has been shown that dyspnoea better predict 5-year survival than FEV₁ in this group.³⁰ Cough and sputum production may be symptoms of particular importance, since they may be independent risk factors for FEV₁ decline,³¹ COPD hospitalisation and also overall mortality.^{32,33}

The fact that there is a poor relationship between respiratory symptoms and spirometric variables^{1,5} may be a further reason to use additional methods such as for example IOS for characterisation of lung physiology, particularly if these methods are validated, certified, non-invasive and user friendly. Concerning IOS, there is a need for more extensive studies of reference values.

A strength of this study is that the original study group is population-based. In a representative population-based sample, the prevalence of COPD is very low, and we therefore performed a stratified sampling to increase the

proportion of subjects with COPD. Interpretation of the information obtained by questionnaires always involves uncertainties, which is a limitation that this study shares with many other studies. Screening subjects for symptoms is difficult; therefore there is a need for sensitive and simple objective screening methods for future studies in this field.

The GOLD criteria for a COPD diagnosis are based on a fixed cut-off FEV₁/VC ratio of 0.7. This leads to a risk of overestimation of COPD especially in elderly men.³⁴ Still, we preferred to apply the fixed value since also subjects with an FEV₁/VC below 0.7 but above the lower limit of normal for the age-adjusted predictions appear to risk an unfavourable outcome.³⁴

In conclusion, this study shows that subjects reporting respiratory symptoms differ in lung mechanics measured by the forced oscillation technique, whether they fulfil the GOLD criteria of COPD or not. This may indicate that IOS better captures changes in respiratory mechanics than spirometry. As forced oscillation measurements are considered better to reflect distal airway function than spirometry, IOS may be useful for early detection of COPD. Further research is, however, needed to establish this.

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Conflict of interest statement

Ulf Nihlén and Gunnar Engström are full-time employees at AstraZeneca. The other authors report no conflicts of interest.

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Paper III

Atherosclerotic plaques in the internal carotid artery and associations with lung function assessed by different methods

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Summary

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Background: Previous studies on associations between reduced lung function and cardiovascular disease (CVD) have mainly been based on forced expiratory volume in 1-s (FEV₁) and vital capacity (VC). This study examined potential associations between five different lung function variables and plaques in the internal carotid artery (ICA).

Methods: Subjects (n = 450) from a previous population-based respiratory questionnaire survey [current smokers without lower respiratory symptoms, subjects with a self-reported diagnosis of chronic obstructive pulmonary disease (COPD) and never-smokers without lower respiratory symptoms] were examined using spirometry, body plethysmography and measurements of diffusing capacity for CO (D_{L,CO}). Plaques in the ICA were assessed by ultrasonography.

Results: Two hundred and twenty subjects were current smokers, 139 ex-smokers and 89 never-smokers. COPD was diagnosed in 130 subjects (GOLD criteria). Plaques in the ICA were present in 231 subjects (52%). General linear analysis with adjustment for established risk factors for atherosclerosis, including C-reactive protein, showed that D_{L,CO} was lower [77.4% versus 83.7% of predicted normal (PN), P = 0.014] and residual volume (RV) was higher (110.3% versus 104.8% of PN, P = 0.020) in subjects with than without plaques in the ICA. This analysis did not show any statistically significant association between plaques and FEV₁ or VC.

Conclusion: The occurrence of plaques in the ICA was associated with low D_{L,CO} and high RV, but not significantly with FEV₁ or COPD status. The results suggest that the relationships between reduced lung function, COPD and CVD are complex and not only linked to bronchial obstruction and low-grade systemic inflammation.

Introduction

Studies have shown that airflow obstruction measured as a decrease in forced expiratory volume in 1-s (FEV₁) as well as a diagnosis of chronic obstructive pulmonary disease (COPD) are factors associated with atherosclerosis and cardiovascular disease (CVD) (Hole et al., 1996; Engstrom et al., 2000, 2006; Sin & Man, 2003; Sin et al., 2005; Maclay et al., 2007; Johnston et al., 2008; Mannino et al., 2008). The mechanisms behind these relationships are unclear. It has been suggested that a low-grade systemic inflammation that is present in both COPD and CVD could be one partial explanation (Garcia-Rio et al., 2010).

Tobacco smoking, an important risk factor for both COPD and CVD, causes in itself an increase in inflammatory markers such as C-reactive protein (CRP) (Gan et al., 2005). Both smoking and low FEV₁ are associated with an increased prevalence of carotid atherosclerosis (Ebrahim et al., 1999; Engstrom et al., 2001), which in turn is related to increased risk of stroke and myocardial infarction (Hollander et al., 2002). The relationship between decreased lung function and atherosclerosis thus remains poorly understood.

Previous studies on associations between reduced lung function and CVD have mainly been based on FEV₁ and vital capacity (VC). This gives a limited insight into the pathophysiology of the lungs,

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and the objective of this study was to examine a broader spectrum of lung function variables and their potential associations with CVD expressed as ultrasound diagnosed atherosclerotic plaques in the internal carotid artery (ICA).

Methods

This was a cross-sectional study performed at the Department of Clinical Physiology, Malmö University Hospital (UMAS) in Malmö between June 2004 and May 2007.

The study was approved by the Ethics Committee of Lund University, and all participants signed an informed consent form before any study-related procedure.

Study population

In 2000, a postal respiratory questionnaire survey was performed in a randomly selected adult population in southern Sweden, ($n = 11\,933$, response rate 78.1%) (Nihlen et al., 2004). On the basis of the questionnaire results, subjects from three independent groups were recruited to this study including clinical examinations: (i) never-smokers without any report of lung disease or lower respiratory symptoms, (ii) current smokers without any report of lung disease or lower respiratory symptoms and (iii) subjects with self-reported diagnosis of chronic bronchitis and/or emphysema and/or COPD at any of two surveys. A total of 870 adult subjects who participated in the survey 2000 were invited to participate in this study. The participation rate of subjects invited to this study was 52% [for different groups: (i) 50% ($n = 60$), (ii) 50% ($n = 282$) and (iii) 57% ($n = 108$)], and the final study population comprised of 450 subjects (185 men/265 women), aged 46–78 years (mean age, 61.5 years). Study participants were re-classified at the study visit according to their current smoking habits and spirometry results. Two participants, both male ex-smokers diagnosed with COPD stage 1 and stage 2, were excluded from the statistical analyses. One of them was not examined using ultrasound, and the other subject had a stent in the left internal carotid artery and in addition occlusion of the right internal carotid artery.

Lung function tests

Both spirometry and body plethysmography were performed according to ERS recommendations (Quanjer et al., 1993), and European reference values were used (Quanjer et al., 1993). A spirometer (Master Screen; Viasys GmbH – Erich Jaeger, Hoehberg, Germany) was used to measure FEV₁ and vital capacity (VC), while TLC and RV were measured using a body plethysmograph (Master Screen, Viasys GmbH – Erich Jaeger). All measurements were taken 15–45 min after inhalation of 1.0 mg of terbutaline (Bricanyl® Turbuhaler®; AstraZeneca, Lund, Sweden). Diffusing capacity for carbon monoxide (D_{LCO}) was measured using the single-breath technique (Cotes et al., 1993) (Master Screen, Viasys GmbH – Erich Jaeger), and

the reference values for D_{LCO} were corrected for haemoglobin values according to established procedures (Macintyre et al., 2005).

Diagnosis and staging of COPD

Diagnosis of COPD and severity staging was performed according to recommendations by GOLD (Global initiative for chronic Obstructive Lung Disease) criteria (<http://www.goldcopd.org>, 2010). In brief, these recommendations state that a postbronchodilator FEV₁/VC value <0.7 is diagnostic for COPD. For staging purposes, postbronchodilator FEV₁ is used: stage I (mild) FEV₁ ≥ 80% of predicted normal (PN), stage II (moderate) FEV₁ < 80% and ≥50% PN, stage III (severe) FEV₁ < 50% and ≥30% PN and stage IV (very severe) FEV₁ < 30% PN.

Ultrasonography of atherosclerotic plaques in the internal carotid artery

A 7.5-MHz linear ultrasound probe was used to screen the ICA, the bulb and the distal portion of the common carotid artery bilaterally for plaques. Plaques were defined as absent or present. There is no uniform worldwide definition of carotid plaque (Wyman et al., 2006). There are no well-known reference values for thickness of the total vessel wall in this region. In this study, a plaque was defined as a focal thickening of the total vessel wall (of 2 mm or more) relative to adjacent segments protruding into the lumen in at least one side, which is estimated to correspond well to the definition of plaque in this region as the presence of focal thickening at least 50% greater than that of the surrounding vessel wall used in several other studies (Salonen & Salonen, 1991; Persson et al., 1992; Hollander et al., 2002; North et al., 2002; Prabhakaran et al., 2006; Roman et al., 2006). All examiners were experienced and not presented to any of other study data prior to examination.

Smoking habits

Subjects who were currently smoking or had stopped within the last 12 months prior to the study were classified as current smokers. Subjects who stopped smoking more than 12 months prior to the study were classified as ex-smokers. Subjects who reported smoking daily for a period <1 month were classified as never-smokers. Total tobacco consumption was calculated in pack years (one pack year = smoking of 20 cigarettes per day for 1 year).

hsCRP and HbA1c

High-sensitivity CRP (hsCRP) was measured in serum by an IMMAGE rate nephelometer, Beckman-Coulter, Brea, CA, USA. The detection limit was 0.2 mg l⁻¹ with imprecision 9% (CV) at 1.0 mg l⁻¹, 7% at 13.0 mg l⁻¹ and 7% at 75.0 mg l⁻¹. Glycated haemoglobin (HbA1c) was measured by high-pressure liquid chromatography.

Blood lipids

Total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides were measured in plasma on Beckman Coulter LX20 (Beckman Coulter), a routine method in Malmö University Hospital since 1999.

Blood pressure

Blood pressure was measured in the right upper arm with subjects in supine position, after 5–10 mins of rest. Pressure was measured to the nearest 5 mmHg. The mean of two measurements was calculated.

Statistics

Lung function variables and potential confounding factors for atherosclerosis were compared in subjects with and without atherosclerotic plaques in the ICA using one-way ANOVA. A multivariate logistic regression equation with gender, age, systolic blood pressure, pack years, hsCRP, HbA1c, total cholesterol, HDL and a diagnosis of COPD according to the GOLD criteria 2010 as covariates was used to calculate odds ratios (ORs) and 95% confidence intervals (CI) for associations between these factors and the occurrence of atherosclerotic ICA plaques. A general linear model was used for the analyses of potential associations between obtained lung function results (for all subjects and for subgroups based on smoking habits) and occurrence of ICA plaques (as a fixed factor). Adjustments were made for potential confounders (gender, age, systolic blood pressure, pack years, hsCRP, HbA1c, total cholesterol and HDL) that according to ANOVA between subjects with and without ICA plaques showed a *P*-value of <0.1, except for LDL (*P* = 0.097). For variables not normally distributed (hsCRP and triglycerides), log-transformed values were used in the statistical analyses. The Statistic Package for the Social Sciences (SPSS)

version 16.0 (SPSS Inc. Chicago, IL, USA) was used for the statistical analyses.

Results

Characteristics of the study population

Basic characteristics of the study population included in the statistical analyses (*n* = 448) are shown in Table 1. Information about pack years was missing in one subject. Body plethysmography was not performed in one subject. Diffusing capacity for carbon monoxide ($D_{L,CO}$) was not measured in eight subjects. COPD was diagnosed in 130 subjects, 29% of the study population. Eighty-seven subjects (77%) had mild COPD (stage I), 36 subjects had moderate COPD (stage II) and seven subjects had severe to very severe COPD (stages III–IV).

Comparisons between subjects with and without plaques in the ICA

Atherosclerotic plaque in at least one of the ICA was detected in 231 (52%) of the subjects (Table 1). Comparisons between subjects with and without plaques in the ICA are shown in Table 2. HsCRP and HbA1c were missing in two and three subjects, respectively. Total cholesterol, HDL and triglycerides were missing in 11 subjects and LDL in 16 subjects. As shown in Table 2, subjects with plaques were older and had higher systolic blood pressure, heavier smoking history and in addition, higher hsCRP and HbA1c than subjects without plaques in the ICA. Total cholesterol and LDL were numerically lower in subjects with plaques than without plaques (*P* = 0.051 and 0.097, respectively). Subjects with plaques had statistically significant lower HDL values than subjects without plaques (*P* = 0.042).

Multivariate logistic regression analysis showed statistically significant associations between age (OR per 1 year = 1.08; 95% CI: 1.04–1.11, *P* < 0.001), systolic blood pressure (OR per

Table 1 Basic characteristics of the study population. For continuous variables, values are presented as mean and standard deviation. For categorical variables, values are presented as numbers and percentages.

	Never-smokers <i>n</i> = 89	Ex-smokers <i>n</i> = 139	Current smokers <i>n</i> = 220	All subjects <i>n</i> = 448
Sex (Men/women)	31 (35%)/58 (65%)	56 (40%)/83 (60%)	96 (44%)/124 (56%)	183 (41%)/265 (59%)
Age (years)	62.3 (8.2)	62.1 (7.3)	60.7 (7.5)	61.4 (7.6)
Pack years	0 (0)	24.4 (17.1)	30.1 (17.6)	22.3 (19.3)
COPD	7 (8%)	49 (35%)	74 (34%)	130 (29%)
COPD stage I	5 (6%)	34 (24%)	48 (22%)	87 (19%)
COPD stage II–IV	2 (2%)	15 (11%)	26 (12%)	43 (10%)
FEV ₁ (% PN)	108.9 (17)	97.4 (19)	96.6 (16)	99.3 (18)
VC (% PN)	114.5 (16)	110.0 (17)	108.5 (14)	110.2 (16)
FEV ₁ /VC (% PN)	101.3 (8.6)	94.2 (14)	94.5 (11)	95.8 (12)
TLC (% PN)	104.9 (12)	102.9 (13)	103.1 (12)	103.4 (12)
RV (% PN)	106.3 (18)	106.5 (25)	108.6 (23)	107.4 (23)
$D_{L,CO}$ (% PN)	88.8 (16)	83.1 (19)	77.6 (16)	81.5 (17)
Subjects with ICA plaques	29 (32%)	68 (49%)	134 (61%)	231 (52%)

COPD, chronic obstructive pulmonary disease; ICA, internal carotid artery; PN, predicted normal value.

Table 2 Comparisons between subjects with and without ultrasound diagnosed atherosclerotic plaques in the internal carotid artery (ICA) according to ANOVA analysis (continuous variables) and Chi-squared analysis (categorical variables). For continuous variables, values are presented as mean and standard deviation. For categorical variables, values are presented as numbers and percentages. For variables not normally distributed (hsCRP and triglycerides), values are presented as medians and ranges.

	Subjects with ICA Plaque (n = 231)	Subjects without ICA plaque (n = 217)	P-value
Sex (Men/women)	115 (50%)/116 (50%)	68 (31%)/149 (69%)	<0.001
Age (years)	63.6 (7.03)	59.2 (7.56)	<0.001
Body mass index (kg m ⁻²)	26.4 (4.9)	26.7 (5.2)	0.461
Systolic blood pressure (mmHg)	141 (18)	135 (17)	<0.001
Pack years	28.4 (20.8)	15.9 (15.2)	<0.001
hsCRP (mg l ⁻¹)	2.8 (0.1–92.1)	2.0 (0.1–25.6)	<0.001
HbA1c (%)	4.81 (0.82)	4.65 (0.41)	0.014
Cholesterol (mmol l ⁻¹)	5.67 (1.14)	5.87 (1.00)	0.051
LDL (mmol l ⁻¹)	3.69 (1.05)	3.84 (0.87)	0.097
HDL (mmol l ⁻¹)	1.27 (0.41)	1.35 (0.39)	0.042
Triglycerides (mmol l ⁻¹)	1.4 (0.4–4.6)	1.3 (0.4–10.8)	0.283
COPD diagnosis	83 (36%)	47 (22%)	0.001
FEV ₁ (% PN)	95.7 (18)	103.1 (17)	<0.001
VC (% PN)	108.1 (16)	112.4 (15)	0.004
FEV ₁ /VC (% PN)	93.7 (13)	98.0 (11)	<0.001
TLC (% PN)	103.1 (12)	103.7 (13)	0.564
RV (% PN)	110.1 (24)	104.7 (20)	0.012
D _{LCO} (% PN)	78.2 (17)	85.2 (17)	<0.001

HDL, high-density lipoprotein; PN, predicted normal value.

1 mmHg = 1.02 (95% CI: 1.01–1.03, P = 0.003) and pack years (OR per one pack year = 1.04 (95% CI: 1.03–1.06, P < 0.001) and the occurrence of ICA plaques. However, no

statistically significant associations were found for gender, hsCRP, HbA1c, total cholesterol, HDL or a diagnosis of COPD and the occurrence of ICA plaques.

Lung function variables in relation to plaques in the ICA

COPD was more frequently diagnosed in subjects with than without plaques in the ICA (Table 2). Of subjects with plaques, 50 (22%) subjects had mild COPD and 33 (14%) subjects had moderate to very severe COPD, while corresponding figures for subjects without plaques were 37 (17%) and 10 (5%), respectively. FEV₁, VC and D_{LCO} were statistically significant lower and RV higher in subjects with plaques in the ICA than in subjects without plaques (Table 2). The relationships between ICA plaques and RV and D_{LCO}, respectively, remained statistically significant according to general linear analyses with adjustments for potential confounding factors (Table 3). The relationship between RV and plaques was largely consistent in different categories of smoking. The relationship between D_{LCO} and plaques was most prominent in ex-smokers. The general linear analysis did not show any statistically significant differences in FEV₁, VC or TLC between subjects with and without plaques in the ICA (Table 3).

Discussion

To our knowledge, this is the first study on potential associations between reduced lung function and atherosclerotic manifestations that included other lung function assessments in addition to spirometry and measurements of peak expiratory flow. The results in a selected subset from a previous questionnaire survey indicate that D_{LCO} is lower and RV is higher in subjects with ultrasound detected atherosclerotic plaques in the ICA independently of established risk factors for atherosclerosis such as older age or increase in hsCRP, HbA1c, systolic blood pressure, total cholesterol and pack years of smoking. In line with the results from others (Hole et al., 1996; Engstrom et al., 2001; Zureik et al., 2001),

Table 3 Pairwise comparisons of different lung function measures between subjects with and without ultrasound diagnosed atherosclerotic plaque in the ICA (as a fixed factor) grouped by smoking habits and in all subjects according to general linear analyses with adjustments for potential confounders. Adjusted mean values and P-values are given.

	ICA plaque							
	Never-smokers (n = 84)		Ex-smokers (n = 136)		Smokers (n = 215)		All subjects (n = 435)	
	Yes (n = 29)	No (n = 55)	Yes (n = 67)	No (n = 69)	Yes (n = 130)	No (n = 85)	Yes (n = 226)	No (n = 209)
COPD, n	3 (10%)	4 (7.3%)	29 (43%)	18 (26%)	51 (39%)	23 (27%)	83 (37%)	45 (22%)
FEV ₁ (% PN)	106.4 (P = 0.331)	110.9	96.0 (P = 0.424)	98.7	95.7 (P = 0.521)	97.3	97.9 (P = 0.145)	100.5
VC (% PN)	115.8 (P = 0.732)	114.4	110.2 (P = 0.873)	109.7	108.0 (P = 0.493)	109.5	109.8 (P = 0.572)	110.7
TLC (% PN)	107.5 (P = 0.258)	103.8	104.1 (P = 0.358)	101.8	103.8 (P = 0.375)	102.2	104.3 (P = 0.200)	102.6
RV (% PN)	112.7 (P = 0.049)	102.9	109.5 (P = 0.231)	104.0	111.0 (P = 0.121)	105.5	110.3 (P = 0.020)	104.8
D _{LCO} (% PN)	88.5 (P = 0.886)	89.1	79.8 (P = 0.028)	86.4	76.7 (P = 0.346)	78.9	79.5 (P = 0.014)	83.7

COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; ICA, internal carotid artery; PN, predicted normal value.

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FEV₁ and VC were lower in subjects with than without plaques in the ICA. However, after adjustments for risk factors, no statistically significant associations between FEV₁, VC or a diagnosis of COPD and the occurrence of ICA plaques were found.

Taken together, the present study results seem to suggest that also other pathophysiological mechanisms than bronchial disease could be associated with increased risk of atherosclerotic disease. Differences regarding study designs and assessment methods may explain why the present study found that neither decrease in FEV₁ nor a diagnosis of COPD was independent risk factor for ICA as has been observed by others (Hole et al., 1996; Engstrom et al., 2001; Zureik et al., 2001). For instance, the present study population included a very high proportion of current smokers (49%) and of subjects with COPD (29%). However, the results of the present study are in line with another study that did not find an independent association between a reduction in FEV₁ and atherosclerotic plaques in the internal carotid arteries (Schroeder et al., 2005). It has been proposed that an underlying low-grade systemic inflammation could contribute to the relationship between reduced lung function and CVD (Engstrom et al., 2002; Sin & Man, 2003; Eickhoff et al., 2008). The results of this study are not in conflict with such an explanation as shown by e.g. a statistically significant higher hsCRP in subjects with ICA plaques than in subjects without plaques. However, similar to the results for e.g. FEV₁, there was no statistically significant association between increase in hsCRP and ICA plaques in the multivariate logistic regression analysis.

The relationships between CRP and lung function are complex, and there are several possible causal links between them. For example, both are strongly related to smoking and airway infections. Inflammation could also be an intermediate link between low lung function and carotid plaque. Including CRP in the multivariate analysis could be overadjustment in this situation. However, all multivariate analyses were also performed without CRP (data not shown), and the results remained essentially the same.

The ultrasonographic definition of an atherosclerotic plaque in the carotid arteries in this study was carefully considered. There is no uniform definition (Wyman et al., 2006), but most definitions used have clear similarities. Ultrasonography requires considerable experience, especially regarding measurements of minor objects. Our aim was to make a stable assessment of the presence or absence of plaque, which was why we chose to use our since decades routinely used definition, corresponding well to definitions used in other studies (Salonen & Salonen, 1991; Persson et al., 1992; Hollander et al., 2002; North et al., 2002; Prabhakaran et al., 2006; Roman et al., 2006).

One may speculate whether the observed associations between a lower D_{L,CO} and a higher RV in subjects with ICA plaques could reflect that also other mechanisms than a

low-grade systemic inflammation contribute to the known association between reduced lung function and CVD. One conceivable mechanism could be degradation of elastic tissue, a central pathological mechanism in the development of lung emphysema. Petersen et al., (2002) showed that serum elastin-derived peptides are elevated in patients with symptomatic carotid stenosis, which supports that elastin degradation is also involved in the development of atherosclerotic disease. The same study showed similar findings in patients with abdominal aortic aneurysm (AAA). In addition, it has been shown that smoking causes changes in carotid plaque composition including decreased elastin content (Kangavari et al., 2004).

As the study is cross-sectional and is of moderate size, the findings have to be interpreted with some caution and it is not possible to draw any conclusions about causal relationships. It should also be noted that the vast majority of subjects diagnosed as having COPD had only mild disease, which makes it difficult to conclude whether the main findings are of clinical importance in COPD or may rather represent some other aspects, e.g. normal ageing of the lungs.

Total cholesterol was slightly higher in subjects without than with plaques in the ICA. The reason is unclear but the finding could, perhaps, be explained by differences regarding statin use. Use of statins was not recorded, and therefore, it is unknown whether statins were more commonly used in subjects with than without ICA plaques. Furthermore, no associations between blood lipids and lung function were found, which is in line with other studies (Zureik et al., 2001; Engstrom & Janzon, 2002; Selvaraj et al., 2005; Engstrom et al., 2006).

In conclusion, this study has shown that diffusing capacity for carbon monoxide is lower and residual volume is higher in subjects with atherosclerotic plaques in the ICA when influence of established risk factors for atherosclerotic disease was adjusted for. The results suggest that the underlying mechanisms behind the associations between reduced lung function, COPD and CVD are complex and not only related to processes causing bronchial disease and low-grade systemic inflammation. Further studies including thorough lung physiological characterization are warranted to get further understanding of the mechanisms behind the associations between reduced lung function, COPD and cardiovascular disease.

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Conflict of interest

Ulf Nihlén and Gunnar Engström are full-time employees at AstraZeneca. Other authors report no conflicts of interest.

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Paper IV



Associations between lung function and alcohol consumption – Assessed by both a questionnaire and a blood marker



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Summary

Background: Studies on the influence of alcohol consumption on lung function have shown conflicting results. Self-reported alcohol consumption may be inaccurate. This study used both a validated alcohol questionnaire and a blood marker of heavy alcohol consumption, and examined potential associations with different lung physiological variables.

Methods: The study population (450 subjects) answered an alcohol questionnaire (AUDIT-C) and performed spirometry, body plethysmography and a test for diffusing capacity for CO ($D_{L,CO}$). Carbohydrate deficient transferrin (CDT), a clinically used blood marker for identifying heavy alcohol consumption, and C-reactive protein (CRP), a marker of systemic inflammation were analysed.

Results: Using AUDIT-C, 407 subjects were alcohol drinkers and 29 non-drinkers. Of the alcohol drinkers, 224 subjects were “hazardous drinkers” and 183 “moderate drinkers”. Thirty-four subjects had a CDT $\geq 2.0\%$ (=heavy drinkers). There was no difference in lung function between hazardous and moderate drinkers. Heavy drinkers had lower $D_{L,CO}$ (74% vs 83% PN, $p = 0.003$), more symptoms of chronic bronchitis ($p = 0.001$) and higher AUDIT-C scores ($p < 0.001$) than non-heavy drinkers. After adjustments (pack years and CRP) the difference in $D_{L,CO}$ ($p = 0.037$) remained. Multiple regression showed an association between CDT and both FEV₁ ($p = 0.004$) and $D_{L,CO}$ ($p = 0.012$) in all alcohol drinkers, but not in never-

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smokers. The AUDIT-C score was associated with CDT (also after adjustments, $p < 0.001$) but not with any lung function variable.

Conclusion: The results from this study suggest that alcohol and particularly heavy drinking has an independent additive negative effect on lung function in smokers.

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Introduction

The effects of alcohol consumption on lung function are still not completely clarified, even though several studies have been performed over the last decades [1–5]. Large, population-based studies have shown no effect at all of alcohol on lung function [6,7] or COPD (chronic obstructive pulmonary disease) mortality [4] as well as a negative effect of high alcohol consumption on lung function [5,8]. Also positive effects on lung function by light to moderate drinking, compared to abstaining from alcohol has been reported [9]. Some studies showed abnormal lung function among high consumers of alcohol recruited in hospital settings [1,2,10]. Most studies have measured forced expiratory volume in one second (FEV₁) and vital capacity (VC) by spirometry as the only tests of lung function [5,7,8].

For alcohol consumption, most studies in this field have used self-reported information [5,11], which could be subject to recall bias [12]. A previous study used γ -glutamyltranspeptidase (GGT) instead, a biological marker of drinking, and found a negative association between FEV₁ and alcohol consumption [13]. Carbohydrate deficient transferrin (CDT) is another, and compared with GGT a more sensitive and specific serum marker of elevated alcohol consumption [14,15]. A previous study in a smaller group of COPD patients showed an inverse correlation between CDT and FEV₁ [16]. That study did not include any questions about alcohol consumption, however.

The objective of this study was to examine potential effects of alcohol consumption on lung function, after adjustments for potential confounders including pack years and C-reactive protein (CRP) in a population-based group of subjects by using both a validated questionnaire (AUDIT-C) and a clinically used specific alcohol blood marker and conducting lung physiological assessments at a specialised centre.

Methods

This was a cross sectional study including one clinic visit performed at the Department of Clinical Physiology, Malmö University Hospital (UMAS) in Malmö between June 2004 and May 2007.

Study population

The invited study population consisted of 870 subjects, who comprised a sub-population from a postal respiratory questionnaire survey ("Questions about the lungs") performed in a random population sample in 1992 as well as 2000 [17]. For the current study, three different groups of subjects, all residing in the area of Malmö, southern Sweden, were recruited from the previous study; (1) subjects

who did not report any respiratory symptoms and never had smoked ($n = 120$) (2) subjects who did not report any respiratory symptoms and were current smokers ($n = 560$) (3) subjects who responded that they had or had a diagnosis of chronic bronchitis or emphysema or COPD ($n = 190$). At the study visit, subjects were classified according to their current smoking habits (current smokers, ex-smokers and never smokers) and spirometry results according to the GOLD 2010 criteria. The recruitment process is described in more detail elsewhere [18].

Ethics approval

The study was approved by the Ethics Committee of Lund University and participants signed an informed consent before any study related procedure.

Questionnaires

Questions about the lungs

This questionnaire was used both for study recruitment purposes and at the study visit. "Questions about the lungs" is a questionnaire about respiratory diseases and symptoms that has been used in several previous surveys and is described elsewhere [19].

Chronic bronchitis, chronic cough and pulmonary medication

A positive answer to "Have you had periods of 3 months with cough with phlegm during 2 years in a row sometimes during the last years?" was interpreted as a report of chronic bronchitis. The question "Have you had longstanding cough during the last 12 months?" was used to identify longstanding cough. Use of pulmonary medication was assessed by the item "Do you regularly take or have taken any pulmonary medication?".

AUDIT-C

The Alcohol Use Disorders Identification Test (AUDIT) is a validated questionnaire for examining drinking habits [20]. The three alcohol consumption questions (AUDIT-C) from this test is a brief screening test for problem drinking [20]. The AUDIT-C questions and their scores are:

1. How often do you have a drink containing alcohol?
(Never (0 points), Monthly or less (1 points), Two to four times a month (2 points), Two to three times a week (3 points), Four or more times a week (4points)).
2. How many drinks containing alcohol do you have on a typical day when you are drinking?
(1 or 2 (0 points), 3 or 4 (1 points), 5 or 6 (2 points), 7 to 9 (3 points), 10 or more (4 points)).
3. How often do you have six or more drinks on one occasion?

(Never (0 points), Less than monthly (1 points), Monthly (2 points), Weekly (3 points), Daily or almost daily (4 points)).

The total AUDIT-C score is the sum of scores for the 3 questions resulting in possible total AUDIT-C scores from 0 to 12.

The first question in AUDIT-C "How often do you have a drink containing alcohol?" was used to identify non-drinkers. Subjects that had not answered the first question in AUDIT-C were excluded from further analyses. The total score of AUDIT-C (ranging from 0 to 12) was used to discriminate between moderate and hazardous drinking. For women a total score on AUDIT-C ≥ 3 was regarded as hazardous drinking and for men ≥ 4 [21].

Carbohydrate deficient transferrin (CDT)

CDT was analyzed in serum by the high-performance liquid chromatography (HPLC) method [22]. CDT is expressed as the percentage of transferrin being carbohydrate deficient, a share that is increased by heavy alcohol consumption. The level of CDT reflects the alcohol consumption of the last 1–3 weeks. CDT was used to identify subjects with heavy alcohol consumption. Similarly to clinical practice a value of $\geq 2.0\%$ was used for identifying subjects with heavy alcohol consumption in the study [23] hsCRP.

High-sensitivity C-reactive protein (hsCRP) was measured in serum by an IMMAGE rate nephelometer, Beckman–Coulter, Brea, CA, USA. The detection limit was 0.2 mg/l with imprecision 9% (coefficient of variation) at 1.0 mg/l, 7% at 13.0 mg/l and 7% at 75.0 mg/l.

Smoking habits

Subjects who currently smoked or had stopped within the last 12 months prior to the investigation were classified as current smokers. Ex-smokers were subjects who had stopped smoking more than 12 months prior to the investigation. Subjects who never had been smoking daily for at least one month were classified as never-smokers. For subjects being current smokers or ex-smokers, the term ever-smokers were used. The total tobacco consumption was calculated in pack years.

Lung function testing

Both spirometry and body plethysmography were performed according to European Respiratory Society (ERS) recommendations [24], and European reference values were used [24] as previously described [18]. Diffusing capacity for CO ($D_{L,CO}$) was measured using the single-breath technique [25], Master Screen, Viasys GmbH – Erich Jaeger) also as previously described [18].

Classification and staging of COPD

COPD was diagnosed according to the GOLD (Global initiative for chronic Obstructive Lung Disease) criteria (<http://www.goldcopd.org>, version 2010), based on a post-bronchodilator FEV_1/VC ratio < 0.7 . Also staging of COPD severity was performed according to the GOLD criteria

(version 2010) with stage I (mild): $FEV_1 \geq 80\%$ of predicted normal (PN), stage II (moderate): $50\% \leq FEV_1 \leq 80\%$ PN, stage III (severe): $30\% \leq FEV_1 \leq 50\%$ PN, and stage IV (very severe COPD): $FEV_1 < 30\%$ PN.

Statistical analyses

IBM SPSS Statistics 20 (SPSS Inc. Chicago, Illinois 60606) was used for the statistical analyses. One-way ANOVA was used for comparisons of continuous variables between groups. Continuous variables not normally distributed (CDT, CRP and current daily tobacco consumption) were log-transformed before the analyses. Categorical variables were compared using Chi-square analysis. Multivariate linear regression analyses of crude lung function variables with adjustments for age, sex, weight and height in the first step and addition of pack years and CRP in a second step was used to analyse the influence of the different alcohol drinking categories as well as CDT on lung function variables. Adjustments were made for age, sex, weight and height in the first step and then also for pack years and CRP. A p -value of < 0.05 was considered statistically significant.

Results

Characteristics of the study population

Of the 870 invited subjects, 450 subjects (52%) participated. Participation rates for never-smokers not reporting COPD, current smokers not reporting COPD and subjects reporting COPD were 50% ($n = 60$), 50% ($n = 282$) and 57% ($n = 108$), respectively.

Fourteen subjects were excluded from further analyses because they had not answered AUDIT-C and then could not be classified as non-drinkers or alcohol drinkers. The final study groups are described in Fig. 1a and b.

Table 1 shows the general characteristics and Table 2 shows the lung function and respiratory symptoms of the alcohol drinking part of the study population divided in two different ways – into moderate and hazardous alcohol drinkers according to AUDIT-C ($n = 407$) (Fig. 1a) and into non-heavy and heavy alcohol drinkers according to CDT ($n = 401$) (Fig. 1b).

Comparisons of moderate and hazardous drinkers vs non-heavy and heavy drinkers

Comparisons between moderate and hazardous drinkers (according to the AUDIT-C definitions) showed statistically significant differences in age and smoking habits (Table 1). Hazardous drinkers were younger, had more pack years, and in addition were more often current smokers. There were no significant differences between hazardous and moderate drinking regarding any of the lung function variables or respiratory symptoms. Levels of CDT were higher among hazardous compared with moderate drinkers (1.48, vs 1.11, $p < 0.001$).

Comparisons between non-heavy and heavy drinkers (according to CDT; Tables 1 and 2) showed that heavy drinking was associated with more pack years and current

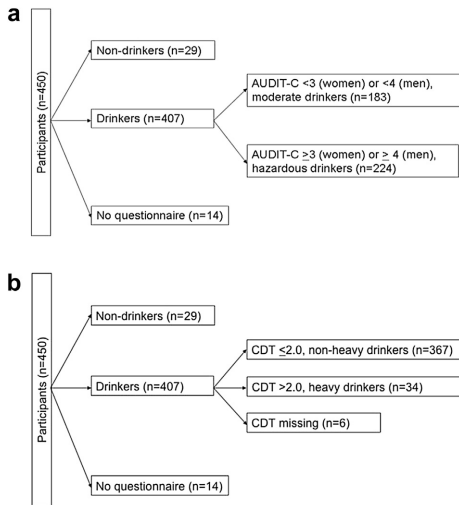


Figure 1 (a). Flow chart demonstrating the classification of participants depending on their alcohol consumption in non-drinkers, moderate and hazardous drinkers according to AUDIT-C. (b). Flow chart demonstrating the classification of participants depending on their alcohol consumption in non-drinkers according to AUDIT-C, and non-heavy and heavy alcohol drinkers according to CDT.

smoking. Not seen for hazardous drinkers though, heavy drinking was associated with male gender and lower BMI as well as with lower FEV_1/VC and $D_{L,CO}$. Heavy drinkers more often had COPD according to the GOLD criteria and more often reported respiratory symptoms.

In Table 3 the different alcohol drinking groups are compared concerning lung function. For hazardous and moderate drinkers, no differences were seen, neither with nor without adjustments for pack years and CRP. For heavy and non-heavy drinkers a significant difference was seen for $D_{L,CO}$, both when adjusted for age, sex, height and weight and after additional adjustments for pack years and CRP.

Then the continuous associations between CDT and lung function and other potential influencing factors were investigated. After adjusting the crude lung function variables for age, sex, height and weight, a higher CDT was associated with lower FEV_1 , VC , FEV_1/VC and $D_{L,CO}$. The associations between CDT and FEV_1 and $D_{L,CO}$ remained also after additional adjustments for pack years and CRP. All analyses of $D_{L,CO}$ were made with and without adjustment for haemoglobin, and no significances were changed (not shown).

Results of the multiple linear regression analyses with FEV_1 and $D_{L,CO}$ as target variables for each of the four alcohol drinking categories are shown in Table 4. This was also performed in drinkers who were never-smokers and in ever-smokers, respectively. In all groups except never-smoking drinkers, CDT was statistically significantly associated with a decrease in FEV_1 and in ever-smoking drinkers, hazardous drinkers and heavy drinkers also with a decrease in $D_{L,CO}$.

AUDIT-C was associated with CDT (also after adjustment for confounders, $r = 0.344$ and $p < 0.001$) but not with any lung function variable (not shown).

Discussion

This study found an association between higher level of the blood marker CDT and lower FEV_1 and $D_{L,CO}$ among subjects reporting any alcohol consumption on AUDIT-C, also after adjustments for potential confounders including tobacco consumption (pack years) and systemic inflammation (CRP). These results may suggest that alcohol and particularly heavy drinking has an independent negative effect on lung function.

To our knowledge there is no previously published study on associations between lung function and alcohol consumption that has assessed alcohol consumption by both self-report, using the validated questionnaire AUDIT-C as well as by the blood marker, CDT, considered to be specific for identifying heavy alcohol consumption. CDT is likely to provide a more reliable identification of subjects with chronic heavy alcohol consumption than a questionnaire [12]. Questionnaires are an established tool especially in primary care to identify subjects with hazardous alcohol consumption. In this study, four subjects with elevated CDT illustrate the difference between self-reported consumption as retrieved from a questionnaire and a biomarker, in giving information in AUDIT-C classifying them as moderate drinkers. CDT is a biomarker rather reflecting biological effects of alcohol consumption. With AUDIT-C, subjects are categorised as hazardous or moderate drinkers on the basis of amount and frequency of alcohol intake. The alcohol consumption required to raise CDT above 2% appears to be considerably larger than that to be classified as hazardous drinker by AUDIT-C [26]. It is therefore expected that the number of heavy drinkers identified with CDT will be smaller than the number of hazardous drinkers identified by AUDIT-C [27].

The most common and important risk factor for decreased lung function is smoking, and smoking is more common among high consumers of alcohol [10]. Therefore it is an important pre-requisite to obtain detailed information about smoking habits in studies on alcohol and lung function [4,5]. The population in this study was recruited based on their smoking habits, explaining the large number of ever-smokers (see Methods).

There were no significant differences in lung function between hazardous and moderate drinking according to AUDIT-C, but lung function was lower in subjects with elevated CDT compared with the rest of subjects who reported any alcohol consumption (non-heavy drinkers). A multivariate linear regression also showed a significant association between increase in CDT and decrease in lung function. Nihlén et al. [16] found an association between a diagnosis of COPD and a higher level of CDT and among COPD patients. In that study, a lower FEV_1 was associated with higher CDT. The focus of that study was not the relationship between alcohol consumption and pulmonary function, and no self-reported information on alcohol consumption was available. The reason behind the association between a higher CDT and lower lung function

Table 1 Characteristics of the alcohol drinking part of the study population, divided into moderate drinkers and hazardous drinkers based on their total score of AUDIT-C, and into non-heavy drinkers and heavy drinkers based on their CDT levels. The selection of the final study groups is described in Fig. 1a and b.

	AUDIT-C classification (n = 407)			CDT classification (n = 401)		
	Moderate drinkers (n = 183)	Hazardous drinkers (n = 224)	p-Value	Non-heavy drinkers (CDT ≤ 2.0%) (n = 367)	Heavy drinkers (CDT > 2.0%) (n = 34)	p-Value
Women	62% (113)	54% (122)	0.168	60% (219)	29% (10)	0.001
Age (years)	62.9 (46–78)	60.1 (46–77)	<0.001	61.2 (46–78)	63.4 (53–77)	0.097
BMI (kg/m ²)	26.6 (18–44)	26.1 (17–45)	0.341	26.5 (17–45)	24.1 (18–31)	0.007
CRP (mg/l)	3.66 (0.1–25.6)	3.06 (0.1–21.5)	0.083	3.25 (0.1–26)	4.04 (0.5–22)	0.076
Ever-smokers	73% (134)	87% (194)	0.001	79% (291)	94% (32)	0.062
Current smokers	39% (72)	55% (124)	0.002	45% (165)	85% (29)	<0.001
Pack years	19.6 (0–110)	24.7 (0–135)	0.008	21.3 (0–115)	34.7 (0–135)	<0.001
Current daily tobacco consumption (number of cigarettes)	4.7 (0–54)	8.0 (0–55)	0.029	5.9 (0–55)	14.0 (0–35)	0.057
CDT (%)	1.11 (0.3–3.8)	1.48 (0.6–8.0)	<0.001	1.16 (0.3–1.9)	3.02 (2.0–8.0)	<0.001
AUDIT-C total score	1.75 (1–3)	4.63 (3–12)	<0.001	3.14 (1–9)	5.59 (1–12)	<0.001
AUDIT Q1: How often do you have a drink containing alcohol?	1.54 (1–3)	2.77 (1–4)	<0.001	2.15 (1–4)	3.03 (1–4)	<0.001
AUDIT Q2: How many drinks containing alcohol do you have on a typical day when you are drinking?	0.08 (0–1)	0.72 (1–4)	<0.001	0.40 (0–4)	0.79 (0–4)	0.001
AUDIT Q3: How often do you have six or more drinks on one occasion?	0.14 (0–1)	1.14 (1–4)	<0.001	0.59 (0–3)	1.76 (0–4)	<0.001

Continuous values are presented as means and ranges. Categorical variables are presented as percentage (n). BMI = body mass index. Q = question. For more information on scoring in AUDIT-C, see Methods.

remains to be clarified but a possible explanation could be that there are dose-dependent, detrimental effects of alcohol on lung function. Only one of the heavy drinkers reported treatment with inhaled medications. This could be due to the fact that heavy drinkers are visiting the health care system more seldom despite respiratory disease or poor adherence to prescribed medications, or both. It may also be that heavy drinkers in our study did not adequately report their use of medications. Lung function was measured as a post-bronchodilator value, which would minimize the effect of the low frequency of inhaled medications among heavy drinkers in this study.

Recall bias and inaccuracy in the reporting of alcohol consumption may partly explain why no associations were observed between lung function and AUDIT-C. This can perhaps also explain why some previous studies [6,7] did not find any association between high alcohol consumption and decrease in lung function after adjustment for smoking habits. These studies have used self-reported information on alcohol consumption only and lung function measurements were restricted to spirometry. There are, however, also large, community-based studies that indicate an independent, negative effect of alcohol intake on lung function

[5,13,28]. Case-control studies have also shown heavy drinkers to have lower lung function than moderate drinkers [1,2,10].

Our study adds new aspects on the relation between alcohol consumption and lung function. To the best of our knowledge, this is the first large, community-based study that has included measurement of $D_{L,CO}$. The difference in $D_{L,CO}$ between the groups of heavy and non-heavy drinkers was significant, which was not the case for FEV_1 . This suggests that $D_{L,CO}$ is a more sensitive indicator of the effects of alcohol on the lungs. Peavy et al. showed in a previous study [29] an acute, negative effect on $D_{L,CO}$ 60 min after alcohol ingestion. Another study [2] showed that former alcoholics had higher $D_{L,CO}$ than current alcoholics, indicating that a low $D_{L,CO}$ because of heavy alcohol consumption is something that can be affected positively by abstinence. The mechanism remains unknown, but appears not to be caused by a difference in haemoglobin concentration. The relation between alcohol consumption and CRP has been found to be U-shaped with the lowest values among moderate drinkers [26]. In our study we did not observe such a U-shaped relationship. In addition CRP is well known to be increased in smokers and also in COPD,

Table 2 Lung function and respiratory symptoms of the alcohol drinking part of the study population, divided into moderate drinkers and hazardous drinkers based on their total score of AUDIT-C, and into non-heavy drinkers and heavy drinkers based on their CDT levels. The selection of the final study groups is described in Fig. 1a and b.

	AUDIT-C classification (n = 407)			CDT classification (n = 401)		
	Moderate drinkers (n = 183)	Hazardous drinkers (n = 224)	p-Value	Non-heavy drinkers (CDT ≤ 2.0%) (n = 367)	Heavy drinkers (CDT > 2.0%) (n = 34)	p-Value
FEV ₁ (% of PN)	100 (19)	100 (16)	0.996	100(17)	96(20)	0.113
VC (% of PN)	111 (16)	110 (15)	0.562	111 (15)	109(18)	0.392
FEV ₁ /VC (% of PN)	96 (12)	96 (11)	0.686	96 (11)	92(13)	0.033
TLC (% of PN)	104 (12)	103 (12)	0.477	104 (12)	103(13)	0.648
RV (% of PN)	108 (21)	106 (21)	0.391	107 (21)	108(30)	0.778
D _{L,CO} (% of PN)	82 (17)	83 (16)	0.596	83 (16)	74(20)	0.003
COPD (n)	30% (54)	29% (64)	0.922	27% (99)	50% (17)	0.008
COPD stage I (n)	21% (38)	19% (42)	0.701	19% (68)	29% (10)	0.191
COPD stage II-IV (n)	9% (16)	10% (22)	0.841	8% (31)	23% (7)	0.045
Symptoms of CB	10% (18)	16% (33)	0.152	11% (39)	37% (11)	0.001
Long-standing cough	18% (32)	19% (42)	0.765	17% (61)	40% (12)	0.012
Any use of pulmonary medication	8% (15)	10% (21)	0.782	10% (35)	3% (1)	0.339

Continuous values are presented as means and standard deviations. Categorical variables are presented as percentage (n). TLC = total lung capacity, RV = residual volume, CB = chronic bronchitis.

probably due to a systemic inflammation [30]. CRP was used in our analyses to adjust for potential residual confounding effects of smoking.

A practical clinical consequence of the results of this study could be that lung function testing, including D_{L,CO}, may be recommended in subjects with increased CDT. Further studies may be valuable to examine whether there likewise would be a threshold or not also for the AUDIT-C score above which lung function testing should be considered.

An interesting question, not addressed in this study, is whether there may be any negative effect of abstaining from alcohol, which has been suggested in some studies [9,31]. In this study, the non-drinkers were few (n = 29) and not included in the statistical analyses. For this reason this study can not evaluate whether moderate alcohol consumption may be beneficial compared to abstaining. A larger, well defined sample of non-drinkers would be necessary to study this potential phenomenon.

Table 3 Relationship between alcohol consumption and lung function variables according to multivariate linear regression analyses.

	FEV ₁ (l)	VC (l)	FEV ₁ /VC	TLC (l)	RV (l)	D _{L,CO} (mmol/kPa/min)
Hazardous vs moderate ^a	-0.008 (0.050) p = 0.875	0.004 (0.052) p = 0.933	-0.034 (0.890) p = 0.970	-0.036 (0.072) p = 0.621	-0.036 (0.046) p = 0.436	-0.070 (0.141) p = 0.621
Hazardous vs moderate ^b	0.023 (0.047) p = 0.628	0.012 (0.052) p = 0.816	0.608 (0.868) p = 0.484	-0.050 (0.072) p = 0.490	-0.059 (0.046) p = 0.199	0.002 (0.133) p = 0.985
Heavy vs non-heavy ^a	-0.170 (0.089) p = 0.058	-0.038 (0.094) p = 0.686	-2.963 (1.594) p = 0.064	-0.011 (0.130) p = 0.929	0.045 (0.083) p = 0.587	-0.833 (0.251) p = 0.001
Heavy vs non-heavy ^b	-0.065 (0.086) p = 0.450	0.036 (0.094) p = 0.698	-1.756 (1.567) p = 0.263	0.052 (0.130) p = 0.693	0.034(0.083) p = 0.686	-0.501 (0.239) p = 0.037
CDT ^a	-0.287 (0.071) p < 0.001	-0.205 (0.075) p = 0.007	-3.231 (1.281) p = 0.012	-0.185 (0.104) p = 0.077	0.027 (0.067) p = 0.684	-0.780 (0.202) p < 0.001
CDT ^b	-0.201 (0.069) p = 0.004	-0.145 (0.076) p = 0.057	-2.259 (1.267) p = 0.075	-0.131 (0.106) p = 0.214	0.020 (0.068) p = 0.764	-0.489 (0.193) p = 0.012

Values are unstandardized B coefficients (standard error, SE). For the continuous relationship between CDT and lung function variables, the B coefficient represents the change after increase of 1 standard deviation of lnCDT.

^a Adjusted for sex, age, height and weight.

^b In addition adjusted for pack-years and CRP.

Table 4 Summary of relationships between CDT and FEV₁ and D_{L,CO} respectively, in different alcohol consumption categories according to multivariate linear regression analyses. Adjustments have been made for sex, age, height, weight, pack years and CRP. Unstandardized B coefficients and *p*-values are shown.

Target variable	Alcohol variable	Moderate drinkers (<i>n</i> = 183)	Hazardous drinkers (<i>n</i> = 224)	Non-heavy drinkers (<i>n</i> = 367)	Heavy drinkers (<i>n</i> = 34)	Never-smoking drinkers (<i>n</i> = 79)	Ever-smoking drinkers (<i>n</i> = 328)
FEV ₁	CDT	-0.373	-0.221	-0.261	-0.837	0.015	-0.218
		<i>p</i> = 0.015	<i>p</i> = 0.020	<i>p</i> = 0.029	<i>p</i> = 0.020	<i>p</i> = 0.944	<i>p</i> = 0.003
D _{L,CO}	CDT	-0.211	-0.759	-0.233	-2.149	0.741	-0.568
		<i>p</i> = 0.614	<i>p</i> = 0.006	<i>p</i> = 0.484	<i>p</i> = 0.041	<i>p</i> = 0.180	<i>p</i> = 0.007

This study has some other important limitations. Elevated alcohol consumption is closely associated with smoking [32]. It can not be excluded that residual confounding by smoking may have influenced at least partly the results despite adjustments for smoking in the statistical analyses. Additional analyses did not show any associations between CDT (or AUDIT-C total score) and lung function variables in never-smokers (*n* = 79). This observation indicates that the found associations are applicable in ever-smokers only and may be attributable to additive effects of alcohol and smoking. The background to such effects is unknown, but it is known that alcohol has a lot of different detrimental effects also affecting lung tissue [33]. In comparison with most epidemiological studies, the study population is limited. The number of heavy drinkers was small why the results should be interpreted with some caution. Another limitation is that because of this we have not had the possibility to compare the results for alcohol drinkers with a large, well-defined group of non-drinkers.

In conclusion, this study showed an association between higher CDT and lower FEV₁, as well as D_{L,CO}, in subjects drinking alcohol, also after adjustments for potential confounders. No such associations were observed in never-smokers, however. Together with the observed statistically significant relationship between the AUDIT-C score and the level of CDT this result suggests that increasing alcohol consumption has a negative effect on lung function at least in smokers and that the negative effects of alcohol is additive to that of smoking. Further studies are needed to examine the mechanisms behind these results.

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Competing interests

Ulf Nihlén is a current full-time employee at AstraZeneca and own stocks from AstraZeneca as a part of a corporate bonus program. Gunnar Engström was employed at AstraZeneca when the paper was initiated.

The other authors report no conflicts of interest.

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