

Diffusing capacity of the lung for carbon monoxide

Variability and longitudinal changes over nine years



Michael L. Storebø

Thesis for the Degree of Philosophiae Doctor (PhD)
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Abbreviations

ATS	American Thoracic Society
CMBC	Concentration of moving blood cells
CO	Carbon monoxide
COHb	Carboxyhaemoglobin
COPD	Chronic obstructive pulmonary disease
CVC	Cutaneous vascular conductance
DALY	Disability-adjusted life-year
D_L	Diffusing capacity of the lung
DL_{CO}	Diffusing capacity of the lung for carbon monoxide
D_M	Membrane conductivity
ECG	Electrocardiography
ERS	European Respiratory Society
EVF	Erythrocyte volume fraction
FEV₁	Forced expiratory volume in 1 second
FVC	Forced vital capacity
GEE	Generalised estimating equations
Hb	Haemoglobin
ILD	Interstitial lung disease
IVC	Inspiratory vital capacity
K_{CO}	Carbon monoxide transfer coefficient
LDF	Laser Doppler flowmetry
MSP	Mean skin perfusion
MST	Mean surface temperature
NICE	The National Institute for Health and Care Excellence
PFT	Pulmonary function testing
PU	Perfusion units
RV	Residual volume
SD	Standard deviation
θ	CO-Hb chemical reaction rate
TLC	Total lung capacity

V_A	Alveolar volume
V_C	Volume of blood in alveolar capillaries

Introduction

Pulmonary diseases are major causes of death and disability on a global scale. In 2016, chronic obstructive pulmonary disease (COPD) was the 3rd most frequent cause of death in the world (1). It was the cause of approximately 2.9 million deaths, which was an increase of 5.5% since 2006. 63.4 million disability-adjusted life-years (DALYs) were lost to COPD in 2016, an increase of 6.5% over the last ten years (2). Cancers of the lower respiratory tract were the cause of 1.7 million deaths in 2016 (1). This made it the 6th most frequent cause of death globally, with an increase in deaths of 18% since 2006. Respiratory cancers caused 36.4 million DALYs to be lost, an increase of 13.7% (2). Interstitial pulmonary diseases (ILDs) are not as prevalent as COPD and respiratory cancers, causing 127.500 deaths and 2.7 million DALYs lost in 2016, but they have an increasing impact on the global burden of disease, with a 40.4% increase in deaths and 32.6% increase in DALYs lost due to ILDs in the 2006-2016-period.

In order to better prevent, treat and manage respiratory diseases, we need improved tools for assessing the state of the lung in both epidemiological research, clinical trials and clinical settings. We need to know that these tools are reliable, and that they give us results that are valid in the setting in which they are used. We also need to have reference materials consisting of healthy samples, in order to evaluate the results from those with disease or suspected disease. In thoracic medicine, tests of pulmonary function play a key role in diagnosis and management, together with radiological examinations, nuclear medicine examinations and invasive procedures such as bronchoscopy.

Exchange of oxygen from the surrounding atmosphere to the blood and of carbon dioxide the other way, is the role of the lung. In order to achieve this, they have to conduct air from outside the organism through the conducting airways, into the parts of the lung where gas exchange between alveolar gas and blood can take place. Lung function can be divided into gas exchange, which is a passive process facilitated by means of diffusion, and ventilation of the regions of the lung where gas exchange can take place. Ventilation is an active process, regulated by the central nervous system, and performed by the respiratory muscles which are contracting to expand the thorax

and the lung during inhalation, and relaxing during exhalation. Although the respiratory muscles are what performs the ventilation of the lung, the state of the conducting airways is the main limiter of effective ventilation. Gas exchange therefore relies on lung ventilation and is affected by diseases which hamper air movement through the conducting airways. But gas exchange is also dependent on conditions only affecting the diffusion of gas molecules between alveolar gas and blood without interfering with ventilation.

A number of different tests are in use to evaluate lung function in patients with respiratory symptom, to clarify which aspects of lung function is causing the symptoms and impairments, and to determine the severity of disease. These tests have different strengths and limitations, and they differ also in how well defined their normal values are.

Better quality of clinical tests and understanding of the normal variation of their values are important to improve diagnostics and management of diseases of the lung, and in that way also improving future lung health care.

A key pulmonary function test (PFT) is the measurement of the diffusing capacity of the lung. However, as compared to the most commonly used PFT, spirometry, there are far less data available as to the change of gas diffusing capacity throughout life, factors influencing its level and its relationship to other clinical data.

Abstract

Pulmonary gas exchange oxygenates our blood and facilitates transfer of carbon dioxide produced out of the body. Measurement of pulmonary gas exchange by diffusing capacity of the lung for carbon monoxide (DL_{CO}) shows a relatively large variability compared to other lung function measurements.

DL_{CO} is reduced by about 10 % in male test subjects 2-6 hours after exercise, which can contribute to measurement variability if it is not taken into consideration. The mechanisms behind post-exercise reduction in DL_{CO} are not fully understood.

We hypothesized that cutaneous vasodilation due to thermoregulation contributes to reducing pulmonary capillary blood volume after exercise, and thus reduction in DL_{CO} due to less haemoglobin being able to bind oxygen in the lung.

12 subjects, 6 women, went through an experimental protocol of baseline measurements of DL_{CO} , mean surface temperature and cutaneous vascular conductance, and then a bout of exercise on a stationary bike to exhaustion. DL_{CO} -measurements were repeated after 90 minutes. They were then exposed to cold air to induce vasoconstriction, after which measurements were repeated. The participants acted as their own controls by going through the entire experiment except the cold exposure, on a different day.

DL_{CO} was reduced by 10% in the men, and 5% in the women, 90 minutes post-exercise. Mean surface temperature and cutaneous vascular conductance were at the same level as at baseline. Exposure to cold air induced a cutaneous vasoconstriction, but DL_{CO} remained at the same level.

Post-exercise cutaneous hemodynamics and thermoregulation does not seem to contribute to the reduction in DL_{CO} in the late recovery phase after exercise.

In addition to challenges due to the relatively large measurement variability, little is known about the normal trajectory of DL_{CO} -values throughout life, what causes change in DL_{CO} over time, and what impact change in DL_{CO} has on respiratory symptoms.

We wanted to model the change in DL_{CO} over time in a general population sample and investigate possible predictors of different trajectories and we wanted to investigate whether the change in DL_{CO} has any impact on dyspnoea in a general population sample.

830 participants in the Hordaland County Cohort Study provided two measurements of DL_{CO} and forced spirometry 9 years apart. Blood samples were analysed for haemoglobin and carboxyhaemoglobin. We also recorded age, height, weight, smoking status, accumulated tobacco smoke exposure, occupational exposure to dust and gas, education level and level of dyspnoea.

Mean change in DL_{CO} was $-0.025 \text{ mmol} \cdot \text{min}^{-1} \cdot \text{kPa}^{-1} \cdot \text{year}^{-1}$. We found that the decline accelerated with higher age. Smoking was a predictor for a more rapid decline in DL_{CO} , and there was a dose-response-relationship between accumulated tobacco smoke exposure and rate of decline in DL_{CO} .

The decline in DL_{CO} was associated with an increase in dyspnoea score in men. We found no such association for women. An interaction between age and change in DL_{CO} was observed in both men and women, with a more severe increase in dyspnoea per unit of decline in DL_{CO} with higher age.

In a general population sample observed over 9 years, the rate of decline in DL_{CO} accelerated with higher age. Smoking was associated with a more rapid decline. An association between decline in DL_{CO} and increase in dyspnoea was observed in the men, but not in the women.

List of Publications

1. Storebo ML, Hope A, Vaagbo G, Thorsen E. Postexercise reduction in lung diffusion capacity is not attenuated by skin cooling. *Clin Physiol Funct Imaging*. 2008;28(6):403-8.
2. Storebo ML, Eagan TM, Eide GE, Gulsvik A, Thorsen E, Bakke PS. Change in pulmonary diffusion capacity in a general population sample over 9 years. *Eur Clin Respir J*. 2016;3:31265.
3. Storebo ML, Eagan TM, Eide GE, Gulsvik A, Thorsen E, Bakke PS. Does change in gas diffusing capacity relate to change in dyspnoea score independently of spirometry? Manuscript.

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1. Background

1.1 Definition of diffusing capacity

Diffusing capacity of the lung (D_L) is a measurement of how many gas molecules are transported from the alveolar gas to the blood per unit of time per unit of driving pressure. Diffusing capacity is measured in $\text{mmol} \cdot \text{min}^{-1} \cdot \text{kPa}^{-1}$ or in $\text{mL} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$. The driving pressure is the partial pressure gradient across the alveolocapillary membrane for the gas in question.

Capacity may be a somewhat imprecise term in this regard, as we are measuring the rate of gas exchange at rest in standardised test conditions, and not the maximum capacity. The term transfer factor has also been used for the measurement of gas exchange, but the American Thoracic Society (ATS) and the European Respiratory Society (ERS) task force for standardisation of lung function testing have agreed upon using the term diffusing capacity in their reports (3).

1.2 Physiology in normal conditions

Oxygen rich air is inhaled, and the oxygen molecules reaches the alveoli in the lung. The alveolocapillary membrane is permeable to gas molecules, and consists of only two layers of cells, with a common basal membrane, making the distance between the alveolar air and capillary blood small enough for effective diffusion. Oxygen molecules diffuse along the partial pressure gradient, from the oxygen rich inhaled air, to the oxygen poor blood in the pulmonary capillaries. The oxygen is dissolved in the blood plasma and is then quickly bound to haemoglobin molecules in the erythrocytes. Almost all of the oxygen in the blood is bound to haemoglobin, but the pressure gradient is between the alveolar air and the dissolved oxygen in plasma. One could say that haemoglobin is a sink to the oxygen in plasma and keeps the gradient high, until all the haemoglobin molecules are saturated and equilibrium is reached.

The surface area of the alveolocapillary membrane is also a key to the effectiveness of pulmonary gas exchange. The lung consists of hundreds of millions of alveoli (4). Together they provide a large surface area over which gas exchange can take place.

Carbon dioxide produced in the tissues, is transported back to the lung in the blood. It also is also mostly bound to haemoglobin, with only a small fraction of about 5-10% dissolved freely in plasma. In the pulmonary capillaries, carbon dioxide diffuses the opposite way of oxygen, because of the lower partial pressure of carbon dioxide in the alveolar gas. It then leaves the organism during exhalation.

1.3 Measuring diffusing capacity

Oxygen is the gas of interest in regard to lung-blood gas exchange. As stated above, the driving pressure is part of the unit of gas exchange, and therefore, in order to calculate diffusing capacity, the driving pressure has to be calculated. To do that calculation, partial pressure in alveolar gas and lung capillary blood has to be known. Oxygen levels in the blood returning to the lung from the tissues around the body show large variability (5). Carbon monoxide (CO) is therefore used as a substitute for oxygen, as CO can be assumed to be absent in blood, and because CO is bound by haemoglobin in the same way as oxygen. Diffusing capacity of the lung for carbon monoxide (DL_{CO}) has become a standard measurement of pulmonary gas exchange in thoracic medicine, and the single breath-method is the most commonly used method to measure DL_{CO} (3).

Single breath DL_{CO} is measured with the test subject breathing through the test apparatus, with the following procedure:

1. Tidal breathing
2. Exhalation to residual volume (RV)
3. A valve in the testing apparatus switches to allow inhalation of the test gas
4. Inhalation to total lung capacity (TLC)
5. 10 seconds breath hold
6. Exhalation
7. Exhaled gas is analysed for concentration of the test gases

The test gas contains a known concentration of CO. However, inhaled test gas mixes with the air left in the lung after full exhalation, the residual volume, and is diluted into a lower partial pressure. In order to calculate the diffusing constant for carbon

monoxide, K_{CO} , we need to calculate the volume of gas containing CO in the lung, termed alveolar volume (V_A), to find the partial pressure of CO after dilution which will also be the driving pressure. This is done by adding a biologically and chemically inert tracer gas, which also has to be relatively insoluble, to the test gas. Helium and methane (CH_4) are the most frequently used tracer gases. They are diluted in the RV, but stay in the lung, and as we know the concentration of the tracer gas in the inhaled test gas ($P_{I,Tr}$), and measure the inspired volume (V_I) and concentration in the expired alveolar gas ($P_{A,Tr}$), after discarding gas from the dead space where no gas exchange takes place, V_A can be calculated by the following formula after taking the volume of the dead space (V_D), where no gas exchange takes place, into account:

$$V_A = (V_I - V_D) \times \frac{P_{I,Tr}}{P_{A,Tr}}$$

$P_{A,CO}$ can then be calculated:

$$P_{A,CO} = \frac{P_{I,CO} \times V_I}{V_A}$$

K_{CO} is calculated as the fall in concentration of CO per unit of time per unit of driving pressure, with CO concentration in blood assumed to be zero:

$$K_{CO} = \frac{\Delta[CO]}{\Delta t \times P_{A,CO}}$$

In order to calculate lung diffusing capacity in terms of carbon monoxide uptake, K_{CO} has to be multiplied by V_A :

$$DL_{CO} = K_{CO} \times V_A$$

DL_{CO} can be partitioned into two conductance components. Membrane conductivity (D_M) represents the effectiveness of the alveolocapillary membrane in gas exchange. The vascular component represents the effectiveness of the pulmonary vascular system in binding CO to haemoglobin in blood and transporting it away. The vascular component is a product of the rate of chemical binding between CO and haemoglobin (θ) and the amount of haemoglobin in alveolar capillary blood (V_C). The relationship

between DL_{CO} , the membrane component and vascular component can be expressed in this way:

$$\frac{1}{DL_{CO}} = \frac{1}{D_M} + \frac{1}{\theta \times V_C}$$

Measurement of the membrane and vascular components of DL_{CO} can be done by measuring DL_{CO} twice, using two test gases with different partial pressures of oxygen. It is not done routinely.

1.4 Pathophysiology of gas exchange

Gas exchange can be reduced by several different mechanisms in disease. These are some examples, and some of the diseases of the lung impact gas exchange by several mechanisms.

In obstructive pulmonary diseases and neuromuscular diseases, ventilation of the alveoli is reduced. Consequently, the driving pressure for gas exchange is reduced. Due to destruction of alveoli, the alveolar surface available for gas exchange is also reduced.

Pulmonary embolism obstructs the pulmonary blood vessels, reducing lung perfusion and making less blood available to absorb inhaled oxygen.

Alpha-1 antitrypsin deficiency causes destruction of alveoli by the enzyme neutrophil elastase, leading to a reduced surface area over which gas exchange can take place.

Left-sided heart failure causes a chronic increase in pulmonary capillary wedge pressure, leading to a thickening of the alveolocapillary membrane. A longer distance between the alveolar air molecules and capillary blood results in a decrease in the rate of gas exchange.

Reduced haemoglobin concentration in anaemia reduces the amount of oxygen that can be taken up per unit of blood volume, and in turn the rate of gas exchange.

Reduction in total lung volume, as can be seen after tuberculosis and lung cancer surgery, will of course also cause a reduction in diffusion capacity.

1.5 DL_{CO} in clinical use

Measurement of DL_{CO} is routinely used in thoracic clinics. Clinical guidelines recommend measuring DL_{CO} in assessing and managing several diseases.

National Institute for Health and Care Excellence (NICE) in the United Kingdom recommends measuring DL_{CO} to assess severity and prognosis of COPD, as forced spirometry alone poorly reflects disability in patients with COPD (6). In patients with COPD and chronic respiratory failure, DL_{CO} has been shown to be a prognostic marker independent of forced spirometry (7).

DL_{CO} has been found to be an important prognostic factor in idiopathic pulmonary fibrosis (8). NICE guidelines for diagnosis and management of idiopathic pulmonary fibrosis in adults recommend measuring DL_{CO} at the time of diagnosis and at follow-ups 6 and 12 months after diagnosis to assess the prognosis for these patients (9).

In those undergoing lung resection, mainly due to lung cancer, DL_{CO} has been shown to be a strong predictor of pulmonary complications after surgery (10), and measurement of DL_{CO} is recommended to evaluate the risk of the procedure (11).

1.6 Variability in DL_{CO}

1.6.1 Magnitude of variability

Current guidelines on measurement on DL_{CO} (3) states that the mean of two efforts with measured values no more than 10% apart should be reported as the subject's DL_{CO}. This means that variability of 10% is considered to be acceptable.

Punjabi *et al.* (12) observed that 98% of a sample of over 6.000 patients who visited a general pulmonary function laboratory were able to meet the criteria of two efforts with values within 10% of each other. In healthy subjects, they observed a coefficient of variability between efforts of 3%.

Jensen *et al.* (13) performed repeated measurements of DL_{CO} on healthy subjects over a 6-month period, using several of the apparatuses that were available on the market. They also performed repeated DL_{CO} -measurements using a simulator in order to estimate instrument variability. Estimated coefficients of variability per instrument ranged from 5% to 10%, and instrument variability accounted for 36% to 70% of the observed variability.

1.6.2 Sources of variability

Physiological and pathological variability in available haemoglobin in the pulmonary capillaries influence the vascular component of DL_{CO} . Total available haemoglobin per unit of time is dependent on blood haemoglobin concentration and cardiac output.

Carbon monoxide is, as mentioned above, assumed not to be present in the blood when calculating DL_{CO} , and the pressure gradient is assumed to be equivalent to the partial pressure of CO in the alveoli. This is not always the case. Cigarette smoking is the major source of CO in human blood. It binds with haemoglobin to form carboxyhaemoglobin, and causes a reduction in measured DL_{CO} values (14). There is also a small endogenous production of CO in the body, mainly from catabolism of haeme groups of haemoglobin, which Coburn *et al.* estimated to $0.28-0.46\text{mL} \cdot \text{hour}^{-1}$ (15). With carbon monoxide density of about 40mmol/L at 1000hPa , endogenous production of CO amounts to about $0.01-0.02\text{mmol} \cdot \text{hour}^{-1}$. Norwegian reference values for carboxyhaemoglobin state a carboxyhaemoglobin fraction of 0.018 as the upper limit of normal in non-smokers (16).

Corrections for levels of haemoglobin and carboxyhaemoglobin can be made if they are measured (3).

Diurnal variation in DL_{CO} has been observed, but is attributed to diurnal variations in blood haemoglobin concentration and carboxyhaemoglobin and not how the lung function per se (17).

Menstrual cycle variation of DL_{CO} has been observed by Sansores *et al.* (18), with a 9% difference between peak before menses, and nadir on day three of menstruation. Pulmonary capillary blood volume and haemoglobin concentrations were found to be

unchanged and could not explain the observed variability in that study. Farha *et al.* in contrast found a 25% decrease in pulmonary capillary blood volume, and also found a correlation between pulmonary capillary blood volume and proangiogenic factors related to the menstrual cycle (19).

1.6.3 Impact of variability in DL_{CO}

In the study mentioned above Jensen *et al.* found a marked difference in the magnitude of variability between measurements of forced expiratory volume in 1 second (FEV₁) and DL_{CO}, with FEV₁ coefficients of variance of 2.56 % to 4.24%. This makes it easier to detect changes and differences in FEV₁ than in DL_{CO}. In clinical settings, this means that a pulmonary fibrosis patient has to have a more severe worsening in her lung function in terms of DL_{CO} before it can be identified, compared to the worsening in FEV₁ for a COPD patient. In a research setting, it means that larger sample size is required to detect the same relative change or difference in DL_{CO} than in FEV₁, making it more demanding in terms of resources and more difficult in terms of recruiting participants to do research on gas exchange.

1.7 Post-exercise reduction in DL_{CO}

Physical exercise induces a transient reduction in DL_{CO} and can be a cause of day to day variability in DL_{CO}. Sheel *et al.* (20) found a 10% reduction in DL_{CO} 1-6 hours after maximal exercise. DL_{CO} was back to baseline values after 24 hours. Submaximal exercise also has been found to induce a reduction in DL_{CO} (21, 22), but of less magnitude than maximal exercise.

In elite marathon runners, cyclists and triathletes, it has been found that high intensity exercise can cause a subclinical pulmonary oedema (23-25). This could reduce DL_{CO} due to a thickening of the alveolocapillary membrane.

Pulmonary oedema has however not been found post-exercise in moderately trained individuals (26), or after submaximal exercise (27, 28), even though a post-exercise reduction in DL_{CO} also is present in those cases.

Pulmonary capillary volume has been found to be decreased by 12 % one hour after exercise (25), and this significantly contributes to reduced DL_{CO} . Hanel *et al.* also found a post-exercise reduction in intrathoracic blood volume by using transthoracic bioimpedance and technetium labelled erythrocytes (29). An increased number of erythrocytes in skeletal muscle was detected in that study, but not enough to account for the entire reduction in DL_{CO} .

None of the prior studies on post-exercise reduction in DL_{CO} have included women.

In thermoneutral conditions, skin blood volume amounts to about 2% of total blood volume (30). The skin plays a major role in thermoregulation of the body, and cutaneous blood volume increases when the body is heated (31). DL_{CO} is influenced by the thermal status of the body (32).

One could hypothesize that increased cutaneous blood volume due to elevated body temperature post-exercise could contribute to a reduction in the intrathoracic blood volume.

1.8 DL_{CO} in general population studies

Several cross-sectional studies on DL_{CO} in general population studies have been published. Some have studied factors associated with DL_{CO} (33-35). Several studies have also been published to established reference values for DL_{CO} in healthy subject, to be used to interpret observed values of patients in clinical settings.

1.8.1 Normal trajectory of DL_{CO}

Reference values for DL_{CO} are based on population studies with measurements of DL_{CO} , and using regression models with several variables, such as sex and height, to estimate predicted values for each patient who is being examined at thoracic medical clinics. Reference equations for calculating predicted DL_{CO} contain a coefficient for age, showing an estimated decrease in DL_{CO} with ageing (36-40). A cross-sectional design is however inferior to a longitudinal design when trying to model change in DL_{CO} with ageing. A cross-sectional study would in this situation be prone to generation effects. It could for instance be that older generations have different

trajectories in DL_{CO} than the younger, due to changes in environmental and occupational exposure. This would lead to errors in interpretations of change in DL_{CO} over time in a patient using extrapolated cross-sectional data, as is the situation today.

Some longitudinal studies on trajectories of DL_{CO} have been made with samples from specific populations, such as firefighters (41), middle aged men in London (42, 43), divers (44), patients with pulmonary fibrosis (45), pigeon breeders (46) and shipyard workers (47). The trajectories observed in these studies can however not be used to estimate trajectories in a general population.

To our knowledge data from only two longitudinal studies on DL_{CO} in general populations samples have been published.

The Tucson Epidemiology Study of Obstructive Lung Disease observed 543 subjects with a mean observation time of 8 years, between 1982-1983 and 1990-1991. Sherrill *et al.* (48) found an acceleration in decline in DL_{CO} with higher age. Smokers had a lower DL_{CO} at baseline, but not a more rapid decline than non-smokers during the observation period.

The Po River Delta Epidemiologic Study followed 928 subjects with a mean observation time of 8 years. Similarly to the Tucson study, Viegi *et al.* (49) found an accelerated decline in DL_{CO} with higher age, and no association between smoking and rate of decline in DL_{CO} .

None of the prior longitudinal studies based on general population samples have examined if the change in DL_{CO} was associated with any change in respiratory symptoms.

2. Aims

The aims of this thesis were:

1. To examine whether redistribution of blood from the thorax to the skin could be part of what causes the post-exercise reduction in DL_{CO} .
2. To describe the trajectory of change in DL_{CO} in a general population sample, and to identify variables that predict different trajectories.
3. To examine whether change in DL_{CO} over time influences change in dyspnoea score.

3. Materials and Methods

3.1 Physiological experiment

3.1.1 Study design

The study was approved by the regional ethics committee. It was designed as a controlled trial of a crossover design. Each participant went through the experiment twice, with and without the intervention, and were their own controls.

The experiment consisted of baseline measurements, a bout of physical exercise on a cycle ergometer, 90 minutes of rest, post-exercise measurements, a cold exposure intervention to induce cutaneous vasoconstriction, and post-intervention measurements. In the control setting, the cold exposure was replaced by further resting for 30 minutes in thermoneutral conditions (figure 1).

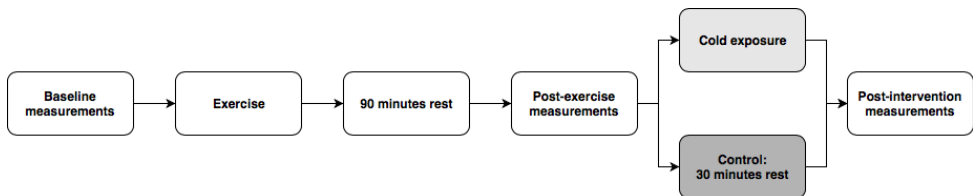


Figure 1. Study procedures overview

3.1.2 Study population

A sample of 12 healthy subjects, six women, were recruited for the study. They were aged 20 to 27 years, exercised regularly, and were never-smokers.

3.1.3 Pulmonary function testing

Forced spirometry and measurement of single breath DL_{CO} were performed on a Morgan Benchmark (PK Morgan Ltd, Kent, UK) lung function testing apparatus, with helium as a tracer gas. Measurements of DL_{CO} were performed at baseline, 90 minutes post-exercise and post-intervention. Forced spirometry was only performed at baseline. Measurements were done in accordance with current guidelines (3).

3.1.4 Skin temperature measurements

Skin temperature was used to assess the thermal state of the subjects, and to validate the effect of the intervention. To estimate mean skin temperature (MST), the probes were placed in accordance with the method developed by Ramanathan (50), on the lateral part of the right calf, over the medial head of the right quadriceps, on the lateral part of the right biceps brachii and in the right mid-clavicular line 2.5cm below the clavicle. Rectal temperature was also recorded to make certain that the cold exposure did not affect the core temperature of the subjects, possibly inducing a general pressor response.

3.1.5 Laser Doppler flowmetry

Laser Doppler flowmetry was used to estimate cutaneous blood flow. The probes used for estimating mean skin temperature, also contained a laser emitter. We used one additional probe for the flowmetry, and this was placed 2cm below the right processus zygomaticus. The laser light penetrates 0.5-1.0mm into the skin. The probe detects the amount of light reflected from erythrocytes to give a representation of the amount of erythrocytes in the sampled skin volume, and the frequency shift in the light gives a representation of the velocity of the erythrocytes in the cutaneous blood vessels (51, 52). Perfusion was monitored for 5 minutes to be certain that we were observing a steady state, but only the last minute was used for the analyses. The instrument reports perfusion in perfusion unit (PU), which is an arbitrary unit. Mean skin perfusion (MSP) was recorded as the mean of the values from all five probes. Heart rate and arterial blood pressure were measured along with the perfusion measurements. Cutaneous vascular conductance (CVC) was calculated as perfusion per mmHg of mean arterial blood pressure.

3.1.6 Exercise protocol

Exercise to induce the post-exercise reduction in DL_{CO} was performed on a cycle ergometer, while monitoring heart rate with ECG and oxygen uptake by a SensorMedics Vmax Spectra 229 (Viasys Healthcare Inc., Conshohocken, PA, USA). Exercise started with a 5-minute warm up period, with a workload of 50 W for women and 70 W for men. After 5 minutes, the workload increased with 15 W per minute for

the women, and 20 W per minute for the men, and the subjects were asked to continue until exhaustion.

3.1.7 Intervention

After 90 minutes of rest in room temperature (21-22°C), and post-exercise measurements as detailed above, the subjects were exposed to cold air of 3-9°C outside the laboratory in order to induce surface cooling. We did not want to induce general hypothermia and shivering, so the cold exposure ended when the first uncontrolled muscle twitch was observed or reported by the subject.

3.1.8 Statistical analyses

Mean values from the intervention and control setting were compared using paired Student's t-test, with the Bonferroni method to correct for multiple comparisons. A significance level of 5% was selected a priori.

3.2 Hordaland County Cohort Study

3.2.1 Study design

The Hordaland County Cohort Study was an epidemiologic, prospective cohort study based on a general population sample. Recruiting started in 1985, with baseline data collection in 1987/1988, and follow-up in 1996/1997.

3.2.2 Study population

A random sample of 4,992 individuals from the Hordaland County, which had a total population of 267,304, were invited to answer a postal questionnaire in 1985. 3,370 people responded. From the responders, a stratified sample of 1,512 subjects aged 18-73 years, were invited to a baseline clinical examination. Stratification was done to ensure that the sample held a number of subjects with obstructive pulmonary disease, occupational exposure and asymptomatic non-smokers. The response rate was 84%, with 1,275 people attending baseline examination.

DL_{CO} measurements were obtained from 1,152 (90%) of those who attended the baseline study visit. 881 (76%) of those with DL_{CO} measurements from the baseline visit, attended the follow-up visit in 1996/1997. 81 were lost to follow up because they

had moved out of the county, 63 withdrew consent, 23 had to withdraw due to serious illness, 43 were dead, and we were unable to establish contact with 61. DL_{CO} values were obtained from 830 (94%) of those who attended the follow-up visit. Mean observation time was 9 years.

3.2.3 Pulmonary function testing

A Sensormedics Gould 2100 automated system (Sensormedics BV, Bilthoven, the Netherlands) was used for PFT. The instrument used at follow-up was the same that had been used at baseline, with the same calibration procedures, and biological controls were used throughout the observation time to ensure that measurements were not drifting.

DL_{CO}, along with K_{CO} and V_A, were measured using the single breath method, described above, with a breath-holding time of 10 seconds, a 750mL washout and a 750mL sample volume. Helium was used as a tracer gas to calculate V_A. Norsk Hydro A/S (Rjukan, Norway) delivered the test gas with certified concentrations of the gas mixture.

Guidelines for measurement of DL_{CO} require that subjects are able to achieve an inspiratory vital capacity (IVC) during the measurement that is at least 85% of their forced vital capacity (FVC). In our sample only 531 subjects (64%) were able to achieve this. Reducing the required IVC/FVC ratio to 0.7, meant that 750 subjects (90%) could be included. Analyses were performed both for those with an IVC/FVC ratio ≥ 0.85 and those with a ratio >0.7 , and the results were not significantly altered. It was therefore decided to use the analyses of subjects who were able to achieve a IVC/FVC ratio above 0.7.

DL_{CO} values were reported as the mean of two measurements, with no more than 10% variability. Norwegian reference equations for DL_{CO} are based on the same population included in this study (36), and it would therefore not make sense to use those to calculate percent predicted values for DL_{CO}. European reference values were used instead (53).

Forced spirometry with measurements of FEV₁ and FVC were performed on the same apparatus as the DL_{CO} measurements. Each subject had to perform three technically satisfactory efforts, with no more than 300mL difference between the two measurements with two values. Percent predicted FEV₁ was calculated using Norwegian reference equations (54).

All lung function measurements were performed in accordance with current guidelines at the time (53, 55-59)

3.2.4 Additional measurements

Height and weight were also recorded at each visit. Additionally, blood samples were drawn and analysed for haemoglobin concentration and fraction of carboxyhaemoglobin.

3.2.5 Questionnaires

Questionnaires were used to record smoking habits, including smoking status and cumulative tobacco smoke exposure, educational level and occupational exposure to dust or gas. The questionnaires have been described in detail by Bakke *et al.*, Aanerud *et al.* and Welle *et al.* (60-62).

3.2.6 Dyspnoea score

Subjects were asked if they experienced dyspnoea, and if so if it occurred during rest, walking on level ground, walking two flights of stairs or walking uphill. The responses were translated into a dyspnoea score with a value of 0 being no dyspnoea, and 4 being dyspnoea at rest.

3.2.7 Statistical analyses

Independent samples t-test and exact chi-square test were used to compare those in the study to those lost to follow up. Independent samples t-test was also utilised in testing for cohort effects. Comparison of mean values from baseline and follow up was performed using paired samples t-test. Normal distribution testing was done using Kolmogorov-Smirnov and Shapiro-Wilk methods. A model for change in DL_{CO} as a function of age was made using curve estimation. Multiple linear regression was used

to model baseline DL_{CO} as a function of the same baseline variables as in the longitudinal analysis described below.

Generalised estimating equations (GEE) was used to analyse change in DL_{CO} as a function of baseline variables, including age, sex, height, weight, smoking habits, cumulated tobacco exposure in terms of pack years, occupational exposure to dust or gas, socioeconomic status represented by educational level, and lastly baseline FEV_1 . We adjusted for baseline DL_{CO} in order to get results based on relative change in DL_{CO} instead of absolute change, as one would expect those with higher DL_{CO} values at baseline to have larger absolute change in DL_{CO} during follow-up. Continuous independent variables were centred around their means. We also decided to investigate whether there was an interaction between baseline age and sex, age and smoking habits, and sex and smoking habits. Our analysis assumed an exchangeable correlation structure.

Ordinal regression was used to examine whether there was an association between change in DL_{CO} and change in dyspnoea, with adjustments for change in weight, age at baseline, change in FEV_1 change in smoking habits and accumulated pack years of cigarettes during the observation time. As in the GEE analysis described above, we centred age around the mean value, which was 45 years at the midpoint of the study. We also used ordinal regression to investigate if there were baseline predictors for baseline dyspnoea score.

4. Synopsis of Results

4.1 Paper I

DL_{CO} is reduced by approximately 10 % 1-6 hrs after maximal exercise. Mechanisms may be interstitial alveolar oedema or reduced pulmonary capillary blood volume, or a combination thereof.

It was hypothesized that thermal stress following exercise contributes to the reduction in DL_{CO}, and that skin cooling would attenuate the post-exercise reduction in DL_{CO}.

Cutaneous vascular conductance (CVC), mean surface temperature (MST), rectal temperature and DL_{CO} were measured before and 90 min after maximal incremental cycle exercise. Thereafter the subjects were exposed to cold air without eliciting shivering one day and another day served as control. The measurements were repeated 120 min after exercise. Twelve healthy subjects (6 male) aged 20-27 years were studied.

Exercise load, both in terms of peak work load and peak oxygen uptake, were the same during the intervention and control settings. DL_{CO} was reduced by 7.1 % (SD=6.3 %, p=0.003) and 7.6 % (SD=5.3 %, p < 0.001) 90 and 120 min after exercise in the control experiment. It was reduced by 5.6 % (SD=5.5 %, p=0.014) 90 min after exercise and remained reduced by 6.1 % (SD=6.1 %, p=0.012) after cooling despite a significant reduction in CVC from 0.25 PU · mmHg⁻¹ (SD=0.10) to 0.15 PU · mmHg⁻¹ (SD=0.11) and in MST from 31.9 (SD=0.6) °C to 27.4 (SD=1.9) °C. Rectal temperature was not affected. In the control setting, no variables changed from 90 minutes post-exercise to final measurements.

We observed a 10 % reduction in DL_{CO} 90 minutes post-exercise in the men, similarly to prior studies. Among the women, observed post-exercise reduction in DL_{CO} was only about 5 %.

We conclude that the post-exercise reduction in DL_{CO} is present when thermal status is restored after exercise, and that it is not influenced by further skin surface cooling.

4.2 Paper II

Data on the change in diffusion capacity of the lung for carbon monoxide (DL_{CO}) over time is limited. We aimed to examine change in DL_{CO} (ΔDL_{CO}) over a 9-year period and its predictors.

A Norwegian community sample comprising 1152 subjects aged 18-72 years was examined in 1987/88. Of the 1109 subjects still alive, 830 (75%) were re-examined in 1996/97. DL_{CO} was measured with the single breath-holding technique. Co-variables recorded at baseline included gender, age, height, weight, smoking status, pack years, occupational exposure, educational level and spirometry. Generalized estimating equations analyses were used to examine relations between ΔDL_{CO} and the co-variables.

At baseline mean (standard deviation: SD) DL_{CO} was 10.8 (2.4) and 7.8 (1.6) $\text{mmol} \cdot \text{min}^{-1} \cdot \text{kPa}^{-1}$ in men and women, respectively. In multiple linear regression, men were found to have higher baseline DL_{CO} than women. Higher age, current or ever-smoking, and accumulated tobacco smoke exposure were negatively associated with baseline DL_{CO} . Positive associations with baseline DL_{CO} were observed for body height, body weight and FEV_1 . Socioeconomic status, in terms of educational level, was also found to be associated with baseline DL_{CO} , as those with higher education were found to have higher baseline DL_{CO} as compared to those with secondary school in the multivariate model. We found no association between occupational exposure to airborne agents and baseline DL_{CO} .

Large variations in ΔDL_{CO} were observed, but with a normal distribution. Mean (SD) ΔDL_{CO} was -0.24 (1.31) $\text{mmol} \cdot \text{min}^{-1} \cdot \text{kPa}^{-1}$. ΔDL_{CO} was negatively related to baseline age, DL_{CO} , current smoking and pack years, and positively related to FEV_1 and weight. Gender, occupational exposure and educational level were not related to ΔDL_{CO} .

Percent predicted DL_{CO} increased on average 3% during follow-up, while average percent predicted FEV_1 values were reduced by 3%.

Mean V_A was significantly reduced from 6.49 L (1.30) at baseline to 6.29 L (1.38) at follow-up. No significant change in mean K_{CO} was observed during the study. Women and those with higher V_A at baseline were found to have a more rapid decline in V_A . Male sex, higher baseline K_{CO} , higher age, current smoking and pack years were associated a more rapid decline in K_{CO} , as was lower body weight and lower educational level.

In a community sample, more rapid decline in DL_{CO} during 9 years of observation time was related to higher age, baseline current smoking, more pack years, larger weight and lower FEV_1 .

4.3 Paper III

Data on how diffusion capacity of the lung for carbon monoxide (DL_{CO}) influences respiratory symptoms is limited. Even more so data on how change in DL_{CO} influences change in respiratory symptoms over time. We aimed to examine if there was an association between change in DL_{CO} and change in dyspnoea in a community sample observed over a period of 9 years.

A Norwegian community sample comprising 1152 subjects aged 18-73 years was examined in 1987 and 1988. Of the 1109 subjects still alive, 830 (75%) were re-examined in 1996/97. DL_{CO} was measured with the single breath-holding technique. Self-reported dyspnoea was recorded using four categories from no dyspnoea to dyspnoea at rest. Co-variables recorded included sex, age, height, weight, smoking status, pack years, and spirometry. Ordinal regression was used to examine the relationship between change in dyspnoea and change in DL_{CO} , with adjustment for other co-variables.

Higher baseline dyspnoea score was associated with lower baseline DL_{CO} , lower FEV_1 , higher age, higher weight. Current smokers and ex-smokers had a significantly higher dyspnoea score than never-smokers at baseline. A significant, positive correlation between pack years smoked and dyspnoea score at baseline was also found.

About 77% of the participants had no change in dyspnoea during the observation time. About 6% had a decrease in dyspnoea, and about 17% had an increase. ΔDL_{CO} was $-0.37 \text{ mmol} \cdot \text{min}^{-1} \cdot \text{kPa}^{-1}$ for men (95% CI: -0.51 to -0.23) and $-0.09 \text{ mmol} \cdot \text{min}^{-1} \cdot \text{kPa}^{-1}$ for women (95% CI: -0.20 to 0.01).

We observed an association between reduction in DL_{CO} and increase in dyspnoea score in the male part of our sample. In addition, we observed an interaction between change in DL_{CO} and baseline age, with a more severe increase in dyspnoea score per unit reduction in DL_{CO} with higher age. This interaction was observed in both men and women.

In a community sample with observations over a 9-year period, decline in DL_{CO} was associated with an increase in dyspnoea for men, but not for women. The effect accelerated with higher age.

5. Methodological Discussion

5.1 Physiological experiment

5.1.1 Study design

This study of paper I was designed as a randomised controlled trial, with a variant of the crossover design, where all participants went through the experiment with and without the intervention, and thus could serve as their own controls. The order of which the participants went through the two experiment settings was randomised.

Participants were recruited from various sports organizations in Bergen, as opposed to some prior studies, which have focused on single sport athletes, such as cyclists, rowers and runners (21, 25, 63-66). This ensured some heterogeneity in the group, but our participants were still a selected sample not representative of the general population. One could hypothesise that an even more homogenic group would give less variability in measurements and thus higher statistical power.

Participants were never-smokers with no history of pulmonary, cardiovascular or any other severe illness. Before inclusion, they went through a screening process, and were found to have normal vital signs and clinical examination findings.

The randomised controlled trial is considered gold standard when examining a response to an intervention. Using a crossover design takes away risk of significant differences in the intervention and control arm. In a small study like the present one, this is more advantageous than in a larger one with higher statistical power.

The crossover design requires that the effects of one part of the trial does not carry over into the other part of it. Sheel *et al.* have studied the time course of post-exercise reduction in DL_{CO} (20), and found that DL_{CO} values were back to baseline after 24 hours. In our study, the two parts of the experiment were spaced 5 to 10 days apart, and there should be no carry over effect.

Randomised controlled trials should preferably be blinded when possible, to prevent bias in measurements in the intervention and control settings. Ideally, both participants and investigators should be blinded to whether they are taking part in the intervention

or control setting. With a cold exposure intervention, such as in the present study, blinding the participants seems impossible. Blinding of the investigators could however have been done. The cold exposure induced a significant reduction in surface temperature, which probably would have been detectable upon touch when placing the skin probes post intervention. Gloves worn by the investigator could maybe have prevented that.

If the hypothesis had been confirmed, one might argue that the lack of blinded observers and participants could have worked to explain the result. However, as the hypothesis was not confirmed, we think that the impact on the results of unblinded observers and participants was minor.

5.1.2 Pulmonary function testing

Single breath measurement of DL_{CO} is the most widely used measurement of pulmonary gas exchange and was performed to standards recommended by the ATS and ERS. Observed values should therefore be comparable to those found in prior studies.

Measurements of D_M and V_C by performing DL_{CO} measurements twice with different partial pressures of oxygen could have given some clues to the mechanisms behind the observed DL_{CO} -values in this study. Unfortunately, we did not have a setup with the additional test gas available. However, with the observed results of no significant change in DL_{CO} after vasoconstriction, observations of D_M and V_C would not have had any value.

5.1.3 Exercise protocol

Several different modalities of exercise have been used in prior studies, including marathon running, triathlon, row ergometers and arm cranking (21, 23, 63, 67, 68). An incremental work load bike ergometer was used in the present study, and it was designed to bring participants to exhaustion in 15-20 minutes. Similar exercise protocols have also been used in several other studies on post exercise reduction in DL_{CO} (69, 70).

The exercise protocol induced a reduction in DL_{CO} of similar magnitude as has been observed by others, at least for the male part of the sample. In this regard, it must be considered to have been adequate. For the sake of comparability, we could have used exactly the same protocol as some of the prior studies, as they are well described in the published articles.

5.1.4 Measurement of skin perfusion

Direct measurement of skin blood volume in live specimens is not available. We chose to use laser Doppler flowmetry (LDF) due to availability. This method was developed to measure blood flow and not volume, but flow is a product of the cross-sectional area of the vessel and the blood velocity. Perfusion unit values acquired through LDF is a product of two factors, the amount laser light reflected from red blood cells, concentration of moving blood cells (CMBC), and the velocity of the red blood cells, represented by the frequency shift in reflected light.

The number of blood cells in a given sample of skin, is proportional to the blood volume in the sample, as long as the erythrocyte volume fraction (EVF) is constant. We did not draw blood samples to measure EVF, but subjects drank 500mL of water post exercise, to compensate for fluid loss. EVF measurements parallel to flowmetry would have added to the validity of the measurements.

LDF shows a large variability, with coefficients of variance with repeated measurements estimated to 20%-58% (71-74). However, arterial blood pressure affects blood flow, and the above variance estimates are for unadjusted perfusion units. We chose to use LDF and mean arterial pressure to calculate cutaneous vascular conductance CVC in order to be certain that an observed change in blood flow was not only due to change in blood pressure, and at the same time reduce the variability somewhat. Conductance is regulated through vasodilation and -constriction, which correlates with the volume of blood that can be accommodated in the vessel. It can be thus used as an indirect measurement of blood volume. This is the method also used in several prior studies on skin hemodynamics in relation to exercise (75-77).

LDF only samples about 1mm^3 per probe. Using five probes placed on different regions of the skin, alleviates some of the problems with this small sampling volume,

but we are still measuring only a very small fraction of the whole skin tissue. Additionally, it was not possible to wear the probes during the entire experiment. We drew the outline around each probe at baseline, but the accuracy of that method was not enough to reproduce probe placement within 1mm², and therefore we were not measuring the exact same skin tissue sample at each point.

Skin photoplethysmography is a method that also uses reflected light, and can be used to measure changes in blood volume (78). Using light of different wavelength, it can measure changes deeper into the skin (79), and it can also be used in conjunction with LDF (80). Unfortunately, this method was not available to us.

Although LDF has its limitations, using it to calculate cutaneous vascular conductance is well known, as it has been used in several studies on skin hemodynamics.

5.1.5 Cold exposure intervention

We utilised exposure to ambient outside temperature to reduce skin temperature and induce cutaneous vasoconstriction. The experiment was performed during the winter months, in the western part of Norway, and the air temperature ranged from 3°C to 9°C. The exposure was terminated at the first observed involuntary muscle twitch, which took place after 8 to 15 minutes in our sample.

The skin cooling protocol could have been standardised better using a climate chamber or liquid cooling garment, but neither was available to us.

Cutaneous vasoconstriction was the goal of the cold exposure. Vasoconstriction can be induced both by reduced skin temperature (81) and reduced core temperature with normal skin temperature (82). After heat retention by means of vasoconstriction, shivering is the next autonomic response to prevent further body temperature reduction. Shivering starts at a core temperature threshold about 1°C lower than that of vasoconstriction (82). Circulating norepinephrine becomes dramatically elevated even by a small reduction in core temperature, with dramatic effects on systemic hemodynamics (83). By terminating the exposure when we observed the first uncontrolled muscle twitch, we could be fairly certain that vasoconstriction had occurred, but without a significant reduction in core temperature. Our results showed a

significant reduction in CVC, without any change in heart rate, blood pressure or core temperature, suggesting that cutaneous vasoconstriction was achieved without any systemic sympathetic response.

5.1.6 Statistical methods

Student's t-test is the most widely used statistical method for comparing mean values. We utilised the variation of this test for analysing paired samples. With testing of multiple pairs, the risk of type I errors increases. We therefore used Bonferroni corrections to adjust for that.

A regression analysis might have been used to model change in post-exercise to post-intervention DL_{CO} as a function of change in cutaneous vascular conductance or using the underlying measurements of LDF and adjusting for blood pressure, but just comparing the means seemed the most intuitive to us.

5.1.7 Validity of the study

We used thoroughly tested and widely used methods for measurement of DL_{CO} , and observed results similar to others, and are fairly certain that our observations represent pulmonary gas exchange.

As discussed above, there is no plausible method for direct measurement of skin blood volume in live specimens, and we cannot be completely certain that we actually measured changes in blood volume in the skin. We did however utilise both flowmetry, conductance calculations and skin temperature measurements, which are all associated with vasodilation and vasoconstriction, which results in changes in skin blood volume.

5.2 Hordaland County Cohort Study

5.2.1 Study design

This was a prospective cohort study of a general population sample. It is a design well suited for modelling normal trajectories in lung function. It has a sample size and observation time which are comparable to prior studies (48, 49). The response rates

were high both at baseline and follow-up. A 9-year follow-up is adequate to detect changes in DL_{CO} in healthy individuals without requiring an even larger sample population.

Additional observation points would have strengthened the study and made modelling more precise and less vulnerable to regression towards the mean. An even longer observation time would also have added to the study. However, a longer observation time would be a trade off versus increased loss to follow-up and survival bias

Stratified sampling made analyses possible on subsamples of the study population which would probably have been too low in numbers with ordinary randomised sampling, as our analyses of occupational exposure. Stratification in this way does however make the study population less representative of the general population.

5.2.2 Data collection

Pulmonary function testing was performed in accordance with guidelines, with robust routines for calibration and monitoring with biological controls. Using the exact same apparatus for pulmonary function testing at both baseline and follow-up is also a strength of this study. Furthermore, we had measurements of haemoglobin and carboxyhaemoglobin in blood, which could be confounders when studying changes in DL_{CO} .

A significant proportion of the participants were not able to achieve an IVC/FVC-ratio of at least 0.85. Not meeting this criterium could lead to an underestimation of V_A , and consequently DL_{CO} . However, after performing further analyses of the dataset, we found that including also those with a ratio between 0.7 and 0.85 did not alter our results significantly and chose to include them in the final results.

Data on dyspnoea was self-reported and thus dependent on variability in the perception of dyspnoea. It is not an objectively quantifiable symptom. Dyspnoea score was an ordinal variable with five possible values, which gives a somewhat low resolution in the collected data but makes reporting easier for the participants than choosing from a large number of possible values. We might have exposed the participants to a physical challenge test, such as a ramp protocol on a treadmill, and

asked them to rate their level of dyspnoea on a visual analogue scale at specified time points, to get more resolution to the dyspnoea score. However, this would have been very expensive with such a large sample.

5.2.3 Statistical methods

Generalised estimating equations is a robust method for analysing data sets from epidemiological surveys with repeated measurements of the outcome variable. Prior studies (48, 49) have utilised random effects models, which also is used for analysis of longitudinal, epidemiological data. The two methods differ in how they are interpreted but are both valid methods in this setting.

Longitudinal data with only two data points will be susceptible to regression towards the mean. We adjusted for baseline DL_{CO}, which compensates for that to a degree.

Change in dyspnoea score, which was the outcome variable in paper III, is an ordinal variable, with possible values from -4 to 4. This made ordinal regression the obvious method to analyse the data.

5.2.4 Validity of the study

This is a relatively large epidemiological survey with a 9-year follow-up and high response rates. The main weaknesses are the ratio of participants who were not able to fulfil the criteria for a technically acceptable DL_{CO}-measurement, and that the survey only has two points of observation.

External validity

External validity is a measure of how well the sample population represents the reference population, and thus to which degree the observed results in the sample can be generalized. The sample included in the Hordaland County Cohort Study has been found to representative of the population it was sampled from with regards to age, gender and smoking habits (60, 84, 85).

Internal validity

Internal validity is a measure of how well the results and conclusions in a study actually represent phenomena in the study population. The validity of pulmonary

function testing procedures and statistical methods have been discussed above. Considerations regarding bias and confounding does warrant further discussion.

Selection bias occurs when those who respond to an invitation to take part in a survey, are significantly different from the non-responders. Selection bias has been assessed for the Hordaland County Cohort Study before (86, 87), and it was found that there were more smokers among the non-responders.

In a longitudinal survey, systematic differences between those who were lost to follow-up as compared to those who stayed in the survey, will lead to attrition bias. In the current study, we observed that those who were lost to follow-up were significantly older and had significantly lower lung function than those who remained in the study. One could hypothesise that this would be due to higher morbidity and mortality in the former group. If those who were lost to follow-up had remained in the study, we would probably have observed an even stronger association between accelerated decline in DL_{CO} with higher age, and a stronger association between smoking and rate of decline in DL_{CO} .

Information bias occurs when there is a skewness in how different subgroups on the sample population report data. In the present study, smoking habits, occupational exposure and level of dyspnoea were probably the data most susceptible to information bias.

Social conventions may cause smokers to underreport their smoking habits, which could cause the observed association between smoking and change in DL_{CO} to be weaker than it actually was. Additionally, smokers have been observed to also underreport respiratory symptoms (88), which could lead to an association between change in smoking habits and change in dyspnoea not being detected.

Recall bias could cause underreporting of occupational exposure to dust or gas due to the fact that not everybody would recall such exposure. It could also lead to skewness in reporting, as those who get respiratory symptoms or disease, might have had better recollection of occupational exposure, than those who did not have any respiratory problems.

Differences in physiology and psychology may cause men and women to perceive dyspnoea differently, contributing to the fact that an association between change in DL_{CO} and change in dyspnoea among women was not observed in the current study (89). Social conventions causing a higher threshold for men to report symptoms associated with lower levels of fitness and work capacity have also been discussed (90). Our findings of a gender difference are similar to observations from cross-sectional studies on both DL_{CO} (35, 90) and FEV_1 (54).

Confounding takes place when one finds an association between an independent and dependent variable, but the reality is that there is a third variable, which is associated with the independent variable, which is the cause of the observed association. One could argue that the observed association between smoking and rate of decline in DL_{CO} , was due to smoking causing the airways to have less conductance, which could lead to air trapping, and not the gas exchange over the alveolocapillary membrane itself. In our analyses, we adjusted for FEV_1 to compensate for this possible confounder. Additionally, men tend to have a taller and heavier body stature but adding height and weight to our analyses adjusted for that. Other possible confounders we have taken account for are: Occupational exposure and smoking, occupational exposure and socioeconomic status, socioeconomic status and smoking, sex and smoking habits, weight gain which could possibly cause an increase in dyspnoea.

By using standardised data collection and utilising validated methods, as well as including potential confounders in our multivariate analyses, we can be fairly certain our conclusions are valid, which is in line with the conclusion of previous discussions by Aanerud regarding the validity of this survey (62).

6. Discussion of the Results

6.1 Physiological experiment

6.1.1 A negative study

We observed a reduction in DL_{CO} of about 10% 90 minutes after a bike ergometer ramp protocol until exhaustion in the men in this study. This finding is in line with prior studies (20). A post-exercise reduction of 5% was observed in the women.

Mean surface temperature and cutaneous vascular conductance were not significantly different from baseline 90 minutes post-exercise. This observation in itself suggests that changes in skin hemodynamics does not play a major role in the reduction of intrathoracic blood volume after exercise.

Skin cooling by exposure to cold air significantly reduced mean surface temperature and cutaneous vascular conductance. Even though this suggests cutaneous vasoconstriction had occurred, no further change in DL_{CO} was observed. Heart rate, blood pressure and core temperature were not affected by skin cooling, giving no evidence of a systemic sympathetic response.

6.1.2 First study on women

A reduction of about 5% 90 minutes after exercise was observed in the women in this study. No prior studies on post-exercise reduction in DL_{CO} in women have been published to our knowledge.

There are gender differences in post-exercise hemodynamics among endurance trained men and women (91, 92). Oestrogen and progesterone have been shown to be vasoactive (93). Lynn *et al.* did not find a pattern of variation in post-exercise hemodynamics through the menstrual cycle, although resting hemodynamics was observed to vary significantly (94). It is currently not known whether this affects intrathoracic blood volume to an extent which influences DL_{CO} .

6.1.3 False negative?

A false negative conclusion occurs when one makes the error of rejecting a hypothesis which is in fact true. This can be due to poor statistical power caused by high

variability, insufficient sample size, or a combination of the two. It can also be due to application of inadequate methods of low validity to the study.

Sheel *et al.* examined the time course of post-exercise reduction in DL_{CO} , and found an ongoing reduction until 6 hours, after which they had no observation point until 24 hours post-exercise. Our measurements took place 90 minutes and 120 minutes post-exercise, and one could hypothesise that skin cooling attenuated an ongoing reduction. We did however not observe any further reduction in DL_{CO} from 90 to 120 minutes post-exercise in the control setting. Additionally, skin hemodynamics were not different from baseline after exercise, as mentioned above. With all observations pointing in the same direction, we assume the risk of this being a false negative study to be low.

6.1.4 Blood redistribution elsewhere?

Post-exercise hemodynamics is characterised by increased systemic vascular conductance and reduced arterial blood pressure (95). A reduction in intrathoracic blood volume has been observed (29), and it has been hypothesised that it is caused by redistribution to organs recovering after the physical effort. An increase in blood volume in the muscles in the thigh was observed by Hanel *et al.* (29), but not to an amount which could explain the entire reduction in DL_{CO} . The gut delivers nutrients needed in the recovery phase after exercise, and pooling of blood in the gut could play a role in the post-exercise depletion of intrathoracic blood volume. A diffuse pooling of blood in the peripheral venous system, not large enough to be easily detected at each individual site, could also be the underlying mechanism of redistribution of blood from the central organs.

6.2 Hordaland County Cohort Study

6.2.1 Decline in DL_{CO} accelerates with higher age

We observed an average yearly change in DL_{CO} of $-0.025 \text{ mmol} \cdot \text{min}^{-1} \cdot \text{kPa}^{-1}$, with an accelerated decline with higher age in our multivariate model. These findings are comparable to prior studies (48, 49). We found that age squared gave the best estimate for change in DL_{CO} as a function of age, which supports the results from the

multivariate analysis. The association between age and the rate of decline in DL_{CO} was independent of the other baseline variables included in the multivariate model.

Age-related reduction in alveolar ventilation, impaired cardiac function, increased emphysema and elevated pulmonary blood pressure might be explanations of the accelerated decline in DL_{CO} with higher age (96).

6.2.2 Smoking associated with accelerated decline in DL_{CO}

Smoking and accumulated tobacco exposure were predictors of both lower baseline DL_{CO} and a more rapid decline during the 9-year follow-up in the present study. Prior studies have also found smoking to be associated with lower baseline DL_{CO} (48, 49). Others have found an association between smoking and rate of decline in DL_{CO} in firefighters (41) and a sample of 84 middle-aged men (42), but we are the first to observe an association between smoking and decline in DL_{CO} in a general population sample. By including cumulative tobacco smoke exposure measured by pack years, we found a dose-response relationship between smoking and rate of decline in DL_{CO} .

DL_{CO} may be reduced due to airflow limitation caused by smoking. However, our observed associations between smoking status and change in DL_{CO} and pack years and DL_{CO} were independent of change in FEV_1 .

Smoking is associated with amount of emphysema (97), which reduces the area of the alveolocapillary membrane, and thus DL_{CO} . An association between level of emphysema and level of DL_{CO} , after adjusting for FEV_1 , has also been observed (98). Together with our data, this may suggest that smoking causes a more rapid decline in DL_{CO} at least partly due to development of emphysema.

Smokers have a higher risk of developing anaemia than non-smokers (99), which may lead to lower DL_{CO} -values. Smokers also have higher levels of carboxyhaemoglobin in blood, which further reduces observed DL_{CO} -values (14). Our findings did however persist after adjusting DL_{CO} -values for haemoglobin-concentrations and fraction of carboxyhaemoglobin in blood.

Of the other comparable studies, the Po-delta survey had a lower response rate than the current study. Smokers have been found to be lost to follow-up more often than non-

smokers (87). The Tucson survey had fewer participants and did not include any participants over the age of 59 at baseline. This can explain why these two studies did not find an association between smoking and the rate of decline in DL_{CO} .

6.2.3 Comparison to cross-sectional surveys

We observed a significant increase in percent of predicted DL_{CO} of 3% using European reference equations (53), while absolute values were reduced. This suggests that the equations, which are based on cross-sectional data, may overestimate the age-coefficient in their model. The discrepancy may be due to a cohort-effect, confounders such as smoking and occupational exposure, or regression towards the mean in the longitudinal data. We adjusted for baseline DL_{CO} to compensate for the latter.

6.2.4 Dyspnoea- DL_{CO} -association

An association between DL_{CO} and respiratory symptoms has been observed in cross-sectional studies before (35, 90). This is however the first study where an association between change in dyspnoea and change in DL_{CO} has been found. The association was only found in the male participants. The same results were found for K_{CO} , but there was not observed any association between change in V_A and change in dyspnoea.

As for the association between smoking and rate of decline in DL_{CO} discussed above, level of emphysema may be the underlying link between change in DL_{CO} and change in dyspnoea, as Grydeland *et al.* have observed both an association between amount of emphysema and respiratory symptoms (100) and amount of emphysema and level of DL_{CO} (98). Reduced DL_{CO} may also have extrapulmonary causes, such as cardiac insufficiency, which may cause an increase in dyspnoea. Fatigue due to systemic inflammation, which has been found to be associated with impaired gas exchange, may also contribute to our findings.

6.2.5 DL_{CO} has stronger impact on dyspnoea with higher age

An interaction between age and change in DL_{CO} was observed in both the men and women in this study, suggesting a larger increase in dyspnoea per unit of decline in DL_{CO} with higher age. One hypothesis to the cause of this, could be reduced reserve capacity in the cardiovascular and respiratory systems with higher age, making older

people for instance less able to compensate for reduced gas exchange due to impairments in the lung by increasing cardiac output.

7. Summary

We have confirmed the finding of others of a post-exercise reduction in DL_{CO} . Change in skin hemodynamics related to thermoregulation does not seem to be a contributing factor to the reduction in intrathoracic blood volume which others have observed.

We are the first to study post-exercise reduction in DL_{CO} in women and found a significantly lower reduction in DL_{CO} for the women compared to the men.

Our findings confirm the observations of others of an accelerated decline in DL_{CO} with higher age. We are the first to find an association between smoking and decline in DL_{CO} in a longitudinal survey of a general population sample. The association showed a dose-response-relationship between accumulated tobacco smoke exposure and rate of decline in DL_{CO} .

Finally, we found an association between decline in DL_{CO} and increased dyspnoea score among the men in our study, but no such association among the women. A significant interaction between change in DL_{CO} and baseline age was present, with a more severe increase in dyspnoea per unit of change in DL_{CO} with higher age.

8. Conclusions

1. Redistribution of blood from the intrathoracic space to the skin does not seem to be a contributing mechanism of post-exercise reduction in DL_{CO} .
2. In a general population sample of 830 subjects and a follow-up of 9 years, we observed a mean change in DL_{CO} of $-0.025 \text{ mmol} \cdot \text{min}^{-1} \cdot \text{kPa}^{-1} \cdot \text{year}^{-1}$. Higher age, smoking, lower FEV_1 and lower weight were associated with a more rapid decline in DL_{CO} .
3. Decline in DL_{CO} was associated with increasing dyspnoea in the male part of our sample. No such association was observed among the women. An interaction between change in DL_{CO} and age at baseline was observed, with a more severe increase in dyspnoea per unit of decline in DL_{CO} with higher age. The interaction was significant for both men and women.

9. Perspectives

A better understanding of the variability of DL_{CO}-measurements, will make us able to take precautions to reduce that variability, and give clinicians in pulmonology a more reliable tool to diagnose and monitor disease. This may lead to detection of some pulmonary diseases at an earlier stage and more accurate staging of severity, with possibilities of early intervention and improved prognosis. In addition, researchers will gain a higher statistical power in studies involving DL_{CO}-measurements, and thus requiring less participants and resources to perform analyses with robust results.

Even though Hanel *et al.* were able to describe and quantify depletion of the central blood volume, and an increase in the blood volume in the muscles of the thigh (29), the mechanisms of post-exercise are not fully understood. Modern imaging techniques, such as SPECT, may give deeper insight into post-exercise redistribution of blood if they are applied to this field of research.

Differences between post-exercise systemic hemodynamics have been observed by others (94), but we are the first to observe an association between sex and magnitude of post-exercise reduction in DL_{CO}. Further studies which also take menstrual cycle and vasoactive sex hormones into account are needed to confirm our findings and shed light on possible mechanisms.

Decline in DL_{CO} accelerates with higher age, and a better understanding of the ageing of the pulmonary and cardiovascular system is needed to understand why. With the coming age wave in Europe and Northern America, we will see an increasing proportion of the population in the higher age groups. A better understanding of the lung health in the elderly, might give us measures to provide better health and quality of life for this group, and give more life to the years.

A better understanding of the lung health in the elderly includes a characterisation of the trajectories of DL_{CO} in the elderly and what genetic and environmental risk factors that influences these trajectories.

As stated above, predicted values for DL_{CO} using reference equations based on cross-sectional surveys, seem to overestimate the age coefficient when compared to

longitudinal data, which may lead to erroneous conclusions when diagnosing or monitoring patients in clinical settings. Our data suggest that the existing reference equations should be reconsidered, which they actually recently have been, using novel approaches and statistical methods (101).

Smoking is a well-known risk factor for a host of diseases and health issues. The majority of research on smoking and lung health has been focused on lung cancer, and impact on airway obstruction and lung function in terms of FEV₁. Less is known about smoking and its effect on pulmonary gas exchange, beyond that related to airway obstruction. Further longitudinal studies on smoking and DL_{CO}, coupled with modern methods for describing pulmonary structure, biochemistry and cell biology may give us an even better understanding of the decremental impact of smoking on lung health, and provide more background information for policy makers.

Source of data

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Postexercise reduction in lung diffusion capacity is not attenuated by skin cooling

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Summary

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Pulmonary diffusion capacity for carbon monoxide (DL_{CO}) is reduced by approximately 10% 1–6 h after maximal exercise. The mechanisms may be interstitial alveolar oedema and reduced pulmonary capillary blood volume. It was hypothesized that thermal stress following exercise contributes to the reduction in DL_{CO} , and that skin cooling would attenuate the postexercise reduction in DL_{CO} . Cutaneous vascular conductance (CVC), mean surface temperature (MST), rectal temperature and DL_{CO} were measured before and 90 min after maximal incremental cycle exercise. Thereafter, the subjects were exposed to cold air without eliciting shivering one day and another day served as control. The measurements were repeated 120 min after exercise. Twelve healthy subjects (six male) aged 20–27 years were studied. DL_{CO} was reduced by 7.1% (SD = 6.3%, $P = 0.003$) and 7.6% (SD = 5.3%, $P < 0.001$) 90 and 120 min after exercise in the control experiment. It was reduced by 5.6% (SD = 5.5%, $P = 0.014$) 90 min after exercise and remained reduced by 6.1% (SD = 6.1%, $P = 0.012$) after cooling despite a significant reduction in CVC and in MST from 31.9 (SD = 0.6)°C to 27.4 (SD = 1.9)°C. We conclude that the postexercise reduction in DL_{CO} is present when thermal status is restored after exercise, and that it is not influenced by further skin surface cooling.

Introduction

Diffusion capacity of the lung for carbon monoxide (DL_{CO}) is reduced by approximately 10% 1–6 h after maximal exercise in men, with complete recovery within 24 h (Sheel *et al.*, 1998). The reduction in DL_{CO} in this late recovery phase after exercise is lower with submaximal exercise (Hanel *et al.*, 1993), and has been demonstrated after running, cycling and rowing (Rasmussen *et al.*, 1986; Hanel *et al.*, 1997; McKenzie *et al.*, 2005). There is no significant difference in the postexercise reduction in DL_{CO} in untrained, moderately trained and highly trained individuals (Sheel *et al.*, 1998). Variability is of major concern with measurements of DL_{CO} in clinical practice and epidemiological studies (Welle *et al.*, 1999; Jensen *et al.*, 2007), and some of this variability could be attributed to the physical activity level during the last 24 h before the measurement.

Subclinical pulmonary oedema owing to elevated pulmonary capillary pressure was first suspected to be the major cause of the reduction in DL_{CO} (Rasmussen *et al.*, 1986; Manier *et al.*, 1991), as it would increase the thickness of the blood–gas barrier. This has been observed in elite marathon runners, cyclists and triathletes (Caillaud *et al.*, 1995; Hopkins *et al.*, 1998; McKenzie *et al.*, 2005), but not in less-trained subjects (Gallagher *et al.*, 1988) and not after moderate-intensity exercise (Hodges *et al.*,

2007). The functional significance of the reduction in DL_{CO} is minimal. Exercise can be continued to the same peak oxygen uptake 4 h after a preceding maximal exercise test despite a lower pre-exercise DL_{CO} (Hanel *et al.*, 1994).

Partition of DL_{CO} into the membrane and blood components indicates a reduced pulmonary capillary blood volume as the major cause for the reduction in DL_{CO} (McKenzie *et al.*, 2005), and a reduced intrathoracic blood volume has been demonstrated by transthoracic bioimpedance measurements and radioactively labelled erythrocytes (Hanel *et al.*, 1997). There is a general reduction in systemic vascular resistance after exercise (Halliwill, 2001), and increased blood flow and volume in skeletal muscle recovering after exercise (Hanel *et al.*, 1997).

The skin blood volume in thermoneutral conditions is estimated to be about 2% of the total blood volume (Pang, 2001), and indirect evidence suggests 500–600 ml of blood may be pooled in the cutaneous circulation with whole body heating (Rowell, 1986). Skin blood flow and volume is increased during exercise, and in the recovery phase owing to the thermal stress of exercise could then contribute to the redistribution of blood volume away from the intrathoracic circulation after exercise. Thermal status influences the measurement of DL_{CO} (Cotes *et al.*, 2006), but it is not known whether elevated body temperature and cutaneous vasodilation

Table 1 Subject characteristics.

	Male (n = 6)	Female (n = 6)
Age	24 (1)	23 (3)
Height (m)	1.79 (0.05)	1.72 (0.03)
Body mass (kg)	72.5 (6.3)	67.2 (4.1)
BMI ($\text{kg}\cdot\text{m}^{-2}$)	22.7 (2.0)	22.8 (1.3)
FVC (% pred.)	107 (9)	105 (15)
FEV ₁ (% pred.)	100 (9)	101 (16)
Exercise ($\text{h}\cdot\text{week}^{-1}$)	8 (4)	8 (3)
VO _{2peak} ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	50.3 (4.6)	43.4 (5.5)

Values are mean (SD). BMI, body mass index; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; VO_{2peak}, peak oxygen uptake.

contributes to the postexercise reduction in DL_{CO} . If so, it would be expected that skin surface temperature increased in the late recovery phase after exercise, and that skin surface cooling might attenuate the reduction in DL_{CO} .

Methods

Twelve healthy well-trained subjects aged 20–27 years participated in the study (six men) and all were never-smokers. They exercised an average 8 h a week (range 2–13 h). Their anthropometric characteristics, dynamic lung volumes and peak oxygen uptake by cycle ergometry are given in Table 1. The study was approved by the regional ethics review committee and all subjects gave written informed consent.

Protocol

Each subject performed progressive exercise until exhaustion on a cycle ergometer on 2 days 5–10 days apart, and they had not involved in exercise during the last 24 h. Before exercise, baseline measurements of skin perfusion, skin temperature, rectal temperature, arterial blood pressure, heart rate and DL_{CO} were carried out and repeated 90 min after the end of exercise. This recovery was indoors at a room temperature of 21–22°C on both days. The subjects were requested to drink 500 ml water to compensate for fluid loss during this period. Thereafter, the subjects either remained indoors at the same temperature for another 30 min as control, or they were subjected to skin surface cooling outdoor at ambient temperature of 3–9°C wearing shorts and t-shirts. Cooling was continued until the first uncontrolled muscle twitch appeared, but without eliciting shivering. The duration of the cooling procedure ranged from 8 to 15 min. All measurements were repeated after the control and cooling exposure, which were in random order on the 2 days. All measurements were done in the same order each time.

Cycle ergometry

The exercise protocol included a 5-min warm-up period with a workload of 70 W for men and 50 W for women. After this, the

workload was increased by 20 or 15 W per min for women, and oxygen uptake was recorded by a Sensormedics Vmax Spectra 229 (Viasys Healthcare Inc., Conshohocken, PA, USA) using a mouthpiece and nose clip.

Lung function measurements

Measurements of dynamic lung volumes and DL_{CO} were performed in accordance with the American Thoracic Society and European Respiratory Society guidelines (MacIntyre et al., 2005; Miller et al., 2005) on a Morgan Benchmark (PK Morgan Ltd, Kent, UK) lung function testing apparatus. All measurements were done with the subjects seated wearing a nose clip. Measurement of DL_{CO} was done with the single-breath-holding method, with helium added to the test gas to calculate effective alveolar volume (V_A). The subjects first exhaled to residual volume, then inhaled the test gas to total lung capacity and held their breath for 10 s before exhalation. Upon exhalation, the first 1 l of gas was disregarded, while the next 700 ml, assumed to be alveolar gas was analysed for CO and He concentrations. The mean of two technically satisfactory tests, with values no more than 10% apart, was recorded. Diffusion coefficient of the lung for CO (K_{CO}) was calculated as $DL_{CO} \times V_A^{-1}$.

Surface temperature and cutaneous perfusion

Cutaneous perfusion and surface temperature were measured by means of laser Doppler flowmetry and integrated thermostatic probes. The PeriFlux System 5000 equipped with five PF 5010 laser Doppler units with wavelength 780 nm, and four PF 5020 heating units (Perimed AB, Stockholm, Sweden) was used. The angled thermostatic laser Doppler probes (Perimed AB) had a fibre separation of 0.25 mm. Given the aforementioned wavelength and fibre separation, the laser light is conducted by optical fibres to the skin where it penetrates 0.5–1.0 mm and is partly reflected. When the light is backscattered by moving erythrocytes, there will be a frequency shift which is proportional to the velocity of moving erythrocytes. The amount of backscattered light is dependent on the number of erythrocytes. Based on this, skin perfusion can be calculated, and is expressed in arbitrary perfusion units (PU) (Bonner, 1981; Gush et al., 1984).

Surface temperatures were recorded on the lateral part of the right calf, over the medial head of the right quadriceps, on the lateral part of the right biceps brachii and in the right mid-clavicular line, 2.5 cm below the clavicle. To calculate mean surface temperature (MST), temperatures from the four locations were weighted according to Ramanathan (1964). Cutaneous perfusion was measured by the same probes and in the same locations as for measurements of surface temperature. An additional probe was placed 2 cm below the right processus zygomaticus. For recording of surface temperature and perfusion, the subjects rested supine while measurements were performed for 5 min. The first 4 min were disregarded, and the mean over the last minute was used for analysis. The mean skin

perfusion (MSP) was calculated using mean values over the last minute from all five probes without any weighting.

Rectal temperature was measured immediately before the skin temperature and perfusion measurements, and blood pressure was measured by sphygmomanometry immediately after the cutaneous perfusion and temperature recordings, with the subjects in the supine position. The mean blood pressure was calculated as diastolic pressure plus one-third of pulse pressure, and mean cutaneous vascular conductance (CVC) was calculated as mean skin perfusion divided by mean blood pressure.

Statistics

All data are given as mean (standard deviation). Differences from baseline and differences between postexercise and post-intervention measurements were tested using paired Student's *t*-test. The Bonferroni method was used to correct for multiple comparisons. A *P*-value less than 0.05 was considered significant.

Results

The subjects exercised to the same peak work load and oxygen uptake both days. The peak oxygen uptake was 3.01 (0.54) l min⁻¹ and 3.16 (0.64) l min⁻¹. The duration of the exercise bout was 15–20 min in both men and women, including the 5-min warm-up period. There were no differences in the baseline and 90-min postexercise MST and mean CVC (Table 2 and Fig. 1). There was a minimal reduction in rectal

temperature. The mean arterial pressure did not differ from baseline, but the heart rate was reduced 90-min postexercise.

There was a decrease in MST from 31.9 (0.6)°C to 27.4 (1.9)°C after skin cooling (*P* < 0.001), but no change from 90- to 120-min postexercise in the control experiment. There was no further reduction in the rectal temperature. CVC showed the same pattern of change as MST, with a drop from 0.25 (0.10) to 0.15 (0.11) PU·mmHg⁻¹ after skin cooling (*P* = 0.001). During the control, CVC did not change. Blood pressure did not change, but heart rate remained slightly reduced compared with baseline measurements.

The DL_{CO} was reduced 90-min postexercise in both experiment and control, by 5.6 (5.5)% (*P* = 0.014) and 7.1 (6.3)% (*P* = 0.003), respectively. After skin cooling, DL_{CO} remained reduced by 6.1 (6.1)%, and after the control DL_{CO} was still reduced by 7.6 (5.3)% (Fig. 1).

The 90-min postexercise reduction in DL_{CO} was 7.6 (6.7)% and 10.7 (4.0)% on the days for experiment and control in men, and 3.6 (3.6)% and 3.5 (6.3)% for women. There were no changes in *V*_A, and the pattern of change in *K*_{CO} was the same as for DL_{CO}.

Discussion

The men in this study had a mean postexercise reduction in DL_{CO} of about 10% after symptom-limited progressive exercise on a cycle ergometer, whereas the women had a reduction of less than 5%. At least in the men, the reduction in DL_{CO} in this study was comparable with other studies of young moderately and well-trained men (Sheel et al., 1998). Whether there are

Table 2 Diffusion capacity of the lung for carbon monoxide (DL_{CO}), temperature and haemodynamics at baseline, postexercise and postintervention.

	Baseline	90 min postexercise	Postintervention
Experiment with skin cooling			
DL _{CO} (mmol·min ⁻¹ ·kPa ⁻¹)	10.6 (2.4)	9.9 (1.8)*	9.9 (1.9)*
<i>K</i> _{CO} (mmol·min ⁻¹ ·kPa ⁻¹ ·l ⁻¹)	1.8 (0.2)	1.7 (0.2)*	1.7 (0.2)*
Rectal temperature (°C)	37.3 (0.4)	36.9 (0.5)*	37.0 (0.6)*
MAP (mm Hg)	85.9 (3.9)	85.4 (5.5)	87.6 (6.7)
MST (°C)	31.5 (0.3)	31.9 (0.6)*	27.4 (1.9)**†
Mean skin perfusion (PU)	18.0 (4.8)	20.9 (7.0)	12.9 (7.8)**†
Conductance (PU·mmHg ⁻¹)	0.21 (0.06)	0.25 (0.10)	0.15 (0.11)†
Heart rate (BPM)	64 (11)	58 (12)*	54 (7)*
Control			
DL _{CO} (mmol·min ⁻¹ ·kPa ⁻¹)	10.6 (2.6)	9.8 (2.0)*	9.8 (2.1)*
<i>K</i> _{CO} (mmol·min ⁻¹ ·kPa ⁻¹ ·l ⁻¹)	1.8 (0.3)	1.7 (0.3)*	1.7 (0.3)*
Rectal temperature (°C)	37.1 (0.2)	36.9 (0.2)*	36.9 (0.2)*
MAP (mm Hg)	87.8 (6.0)	84.3 (5.8)*	85.6 (5.1)
MST (°C)	32.0 (0.6)	32.4 (1.1)*	32.6 (1.0)*
Mean skin perfusion (PU)	21.5 (7.8)	22.2 (7.6)	25.5 (12.5)
Conductance (PU·mmHg ⁻¹)	0.24 (0.09)	0.26 (0.10)	0.30 (0.17)
Heart rate (BPM)	64 (12)	60 (10)	57 (8)*

Values are mean (SD).

DL_{CO}, pulmonary diffusion capacity for carbon monoxide; *K*_{CO}: Transfer coefficient for carbon monoxide; MAP, mean arterial pressure; MST, mean surface temperature.

*Significantly different from baseline.

†Significantly different from postexercise.

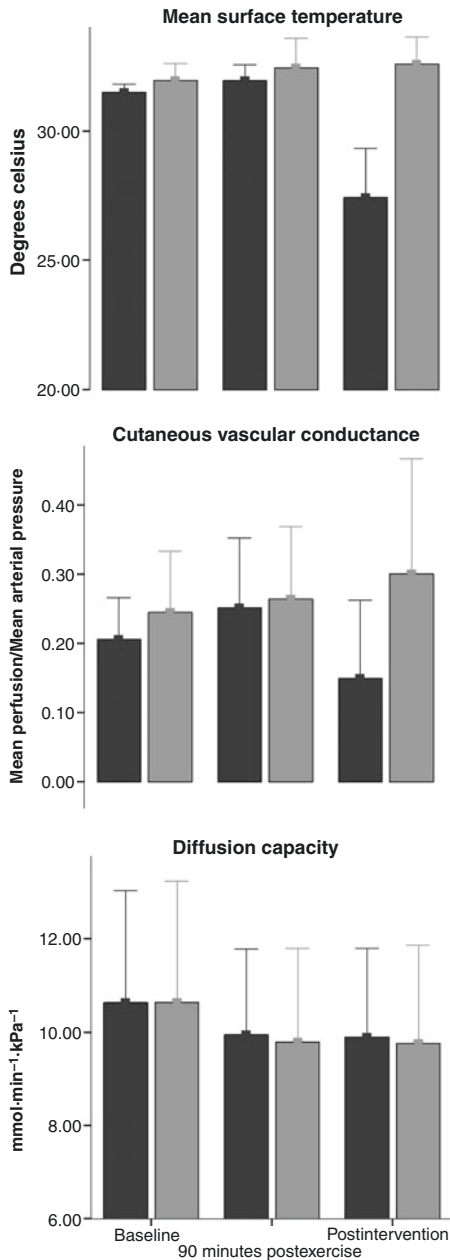


Figure 1 Changes in mean surface temperature, cutaneous vascular conductance and diffusion capacity of the lung for carbon monoxide. Bars represent means, error bars show 1 SD. Filled bars show results from experiment with skin surface cooling and shaded bars show results from control.

gender differences in this response is not known. There was no difference in the baseline DL_{CO}, MST or CVC before exercise on the 2 days. The reduction in DL_{CO} 90 min after exercise was the same on the 2 days, without any changes in thermal status or cutaneous conductivity. This observation itself, before the cooling procedure, indicates no influence of skin surface temperature and blood flow on the post-exercise reduction in DL_{CO} in this experiment.

The cooling procedure resulted in a reduction in skin temperatures and perfusion without changes in blood pressure, rectal temperature or heart rate 105–120 min after exercise. In the control experiment, thermal status and skin perfusion remained unchanged 120 min after exercise. There was no effect of the cooling procedure on DL_{CO}. To lower the surface temperature, the subjects were exposed to air cooling. The cooling procedure was not standardized with respect to ambient temperature and time as a climate chamber was not available. Other procedures that can more easily be standardized like immersion of hands and feet in cold water is associated with sympathetic activation and a reduction in DL_{CO} possibly owing to increased pulmonary vascular resistance (Frans et al., 1994). There was no indication of sympathetic activation with increased heart rate and blood pressure after the cooling procedure in this study.

The interval between exercise and the first postexercise measurement of DL_{CO} was 90 min. Most of the postexercise reduction in DL_{CO} takes place between 60 and 120 min after exercise and remains for at least 6 h (Sheel et al., 1998). The cooling procedure could then have attenuated an ongoing further reduction in DL_{CO}, but there was no further reduction in DL_{CO} between 90 and 120 min postexercise in the control situation. DL_{CO} is also influenced by haemoglobin concentration and repeated tests increasing the CO concentration in the blood. Fluid intake was standardized during the postexercise period, and the number of DL_{CO} tests was the same in the control experiment as in the cooling experiment.

Skin cooling induced a decrease in CVC. As the resistance in cutaneous blood vessels increases, a smaller proportion of cardiac output will be distributed to the skin, and it could be hypothesized that decreased CVC would lead to redistribution of blood volume and increased central blood volume that would influence DL_{CO}. It could be that the blood volume in cutaneous vessels already is small under thermoneutral conditions, and that cooling would not influence pulmonary capillary blood volume to an extent that would have an effect on DL_{CO}. A reduced intrathoracic blood volume could be attributed to the redistribution of blood volume to organs recovering after exercise. Increased blood volume has been demonstrated in recovering thigh muscle by bioimpedance measurements and radioactively labelled erythrocytes, but not to an extent that could explain the whole reduction in intrathoracic blood volume (Hanel et al., 1997). Wilson et al. (2007) have shown that whole body skin cooling induces a visceral vasoconstriction. This should then add to the effect of skin cooling on intrathoracic blood volume.

The laser-Doppler method used for cutaneous flowmetry can only measure perfusion in about 1 mm³ skin tissue per probe (Braverman, 1997). The regional differences in cutaneous perfusion and temperature are large (Wardell et al., 1994; Hafner et al., 2007). Ramanathan (1964) showed that the four probes placed on the chest and extremities are adequate for evaluating MST by weighting contribution from each site. Skin temperature is closely related to cutaneous perfusion (Nilsson, 1987) and both measurements were integrated in the same probe. Whether the same weighting procedure is valid for the estimation of mean cutaneous perfusion is not known. We calculated the mean cutaneous perfusion and conductance as the mean of the registrations including the probe placed in the face. Capillary blood flow is related to arterial blood pressure (Johnson & Wayland, 1967), and therefore, we calculated CVC from mean cutaneous perfusion and mean arterial pressure. The pattern of changes at all five sites was the same in both the control and cooling experiment.

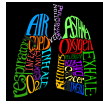
Systemic haemodynamics in the late recovery phase after exercise is characterized by an increase in systemic vascular conductance and a reduction in the mean arterial pressure (Halliwill, 2001; Lynn et al., 2007), while heart rate and cardiac output may be elevated or unchanged (Lynn et al., 2007). There is a reduced vascular responsiveness to sympathetic stimuli (Halliwill et al., 1996, 2003), and the cooling procedure did not induce a pressor response. The postexercise haemodynamic response is modulated by oestrogen and progesterone and is under the influence of the menstrual cycle. That could explain a lower reduction in DL_{CO} in women.

We conclude that the postexercise reduction in DL_{CO} in young well-trained subjects is present when thermal status is restored after exercise, and that any redistribution of blood volume by skin surface cooling is not large enough to affect DL_{CO} in the late recovery phase after exercise.

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ORIGINAL ARTICLE

Change in pulmonary diffusion capacity in a general population sample over 9 years

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Rationale: Data on the change in diffusion capacity of the lung for carbon monoxide (DL_{CO}) over time are limited. We aimed to examine change in DL_{CO} (Δ DL_{CO}) over a 9-year period and its predictors.

Methods: A Norwegian community sample comprising 1,152 subjects aged 18–73 years was examined in 1987 and 1988. Of the 1,109 subjects still alive, 830 (75%) were re-examined in 1996/97. DL_{CO} was measured with the single breath-holding technique. Covariables recorded at baseline included sex, age, height, weight, smoking status, pack years, occupational exposure, educational level, and spirometry. Generalized estimating equations analyses were performed to examine relations between Δ DL_{CO} and the covariables.

Results: At baseline, mean [standard deviation (SD)] DL_{CO} was 10.8 (2.4) and 7.8 (1.6) mmol·min⁻¹·kPa⁻¹ in men and women, respectively. Mean (SD) Δ DL_{CO} was -0.24 (1.31) mmol·min⁻¹·kPa⁻¹. Δ DL_{CO} was negatively related to baseline age, DL_{CO}, current smoking, and pack years, and positively related to forced expiratory volume in 1 second (FEV₁) and weight. Sex, occupational exposure, and educational level were not related to Δ DL_{CO}.

Conclusions: In a community sample, more rapid decline in DL_{CO} during 9 years of observation time was related to higher age, baseline current smoking, more pack years, larger weight, and lower FEV₁.

Keywords: diffusion capacity for carbon monoxide; longitudinal change; occupational exposure; socioeconomic status; smoking

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Diffusing capacity of the lung for carbon monoxide (DL_{CO}) is the most widely used non-invasive test of pulmonary gas transfer (1). The test has been used in both clinical and epidemiological settings and in surveys of occupational groups (2–8). Several cross-sectional community studies have presented predictors for DL_{CO} (9–17), and commonly used reference values are based on sex, age, and height. In some cross-sectional studies, smoking has been found to be associated with impaired DL_{CO}, while body mass and socioeconomic status (SES) have been shown to be related to DL_{CO} in some studies (14, 17). Only two community studies have been longitudinal in design, which is preferable to cross-sectional studies when studying change related to ageing (18, 19).

The two longitudinal studies were an 8-year follow-up study from Tucson, Arizona (18), including 543 subjects, and an 8-year follow-up study from Pisa, Italy, including 928 subjects (19). Both studies found that the decline in DL_{CO} during the follow-up period increased with increasing age, while no relationship to smoking was noted. The latter is somewhat surprising as smoking is the major cause of emphysema, which is associated with impaired DL_{CO} (20). A small cohort study of 84 subjects, followed for 22 years, has observed smoking to be a predictor for rapid decline of DL_{CO} (21, 22). The representativity of this cohort to the population at large is uncertain.

The purpose of this study was to explore predictors for the longitudinal change in DL_{CO} in a community sample examined twice 9 years apart. According to previous

findings in cross-sectional studies of this population sample (17, 23–26), we hypothesized that smoking habits, occupational airborne exposure, and SES were predictors of change in DL_{CO} .

Methods

Study population

Details of the sampling and characterization of the study population have been given elsewhere (27, 28). Briefly, a stratified sample ($n = 1,512$) from the general population in Hordaland, Norway, aged 18–73 years was invited to a clinical and respiratory physiological examination in 1987/88. Altogether 1,275 (84%) attended. DL_{CO} measurements were obtained from 1,152 (90%) of the 1,275 attendees.

All attendees from visit 1 were invited to a follow-up (visit 2) in 1996/97. From the 1,152 subjects with DL_{CO} measurements at visit 1, 881 (76%) attended visit 2. Of those lost to follow-up, 43 were dead, 81 no longer lived in the study area, 63 did not wish to participate further, and 23 could not attend because of serious illness. We were not able to establish contact with 61 of the visit 1 attendees. We obtained DL_{CO} measurements from 830 (94%) of the visit 2 attendees.

Questionnaires

At visit 1, data on smoking habits, educational level, and occupational airborne exposure were obtained through self-reported questionnaires (23, 29). Smoking habit was categorized into never smoking, ex-smoking, and current smoking. Pack years was calculated as average number of cigarettes smoked per day, divided by twenty and multiplied by total number of years of being a smoker. SES was assessed in terms of educational level which was categorized into primary school, secondary school, and higher education (17).

Occupational airborne exposure was based on the following data: self-reported past or present occupational exposure to dust or gas (24) and self-reported exposure to specific agents and work processes (asbestos, quartz, wood dust, welding, and soldering) (27).

Clinical examination and pulmonary function testing

Clinical examination included measurements of height and weight. Blood samples were analyzed for hemoglobin (Hb) concentration and fraction of carboxyhemoglobin (HbCO). Pulmonary function testing (PFT), including DL_{CO} , and forced spirometry were performed in accordance with current guidelines at the time of examination (1, 30–32).

PFT at both visit 1 and visit 2 was performed using a SensorMedics Gould 2100 automated system (SensorMedics BV, Bithoven, the Netherlands). The same instrument was used at both visits, with the same calibration

procedure and biological control throughout the observation period by regular measurements of the technicians operating the instrument. Details of the standardization of measurements, calibration processes, and the results of repeated measurements in the biological controls are given in the Supplementary file. At both visits, DL_{CO} , the alveolar volume (V_A), and the ratio of DL_{CO} to V_A (K_{CO}) were measured using the single breath-holding method, with a breath holding time of 10 seconds, a washout volume of 0.75 L, and a sample volume of 0.75 L. V_A was measured by helium dilution. The test gas was delivered and certified by Norsk Hydro A/S (Rjukan, Norway). The concentration of carbon monoxide was requested to be within 0.270 and 0.330% with an accuracy of 1%. The concentration of helium was requested to be within 9 and 11% with an accuracy of 1%. The mean of two measurements, with no more than 10% variability, is reported. The ATS/ERS guidelines require the DL_{CO} measurement to be performed after the subject had achieved an inspiratory vital capacity (IVC) of at least 85% of his or her forced vital capacity (FVC) (27). Only 531 subjects (64%) met this criterion on both visits, while 750 subjects (90%) achieved an IVC/FVC ratio of at least 0.7. Excluding the subjects with an IVC/FVC ratio of less than 0.85 did not alter the study results overtly as compared to including them in the analyses (Tables E1 and E2). Hence, the data are presented including all subjects with an IVC/FVC ratio > 0.7 . Predicted values for DL_{CO} were calculated using the formula estimated by Cotes et al. (1). It was decided not to use Norwegian predicted values, as they are based on the population sample also used in this study.

Spirometry was performed as an inhalation from functional residual capacity to total lung capacity, followed by a maximal forced expiration to residual volume. For forced expiratory volume in 1 second (FEV_1) and FVC, the highest value from three technically acceptable measurements, with variability between the two highest values within 300 mL, is reported. All subjects were shown how to perform the maneuvers before testing, using standardized instructions, for both forced spirometry and measurement of DL_{CO} . Subjects were seated and wearing a nose-clip during all efforts. Reference values calculated from healthy Norwegian subjects were used for FEV_1 (26).

Statistical methods

Descriptive statistics are presented using the mean and standard deviation (SD) for continuous variables and frequency and percentage for categorical variables. Comparisons of the study population and those lost to follow-up were performed using the independent samples t-test and the exact chi-squared test. Comparisons of means from baseline and follow-up were performed using paired samples t-test, testing for cohort effect was carried out using independent samples t-test, and modeling change in

DL_{CO} as a function of age was performed using curve estimation. Testing for normal distribution was performed using the Kolmogorov-Smirnov and the Shapiro-Wilk tests.

DL_{CO} at first and follow-up survey 9 years later was analyzed in a multiple linear regression model and estimated with generalized estimating equations (GEE) to account for correlation between the two measures of DL_{CO} in the same subject at the two surveys. In this model, time was given the values 0 and 9 (years), all other continuous explanatory variables were centered around their means, all categorical variables were represented by dummy variables, and all interactions between the explanatory variables (categorical and continuous) were included. From such a model, the estimated regression coefficients for the interactions give direct estimates of the average yearly change in DL_{CO} from the first to the last visit (Δ DL_{CO}) at the zero level for all explanatory variables (for continuous variables this is the mean value; for categorical variables it is the reference category), and for a value of 1 unit increase from 0 in each variable all others were fixed at 0. For the GEE estimation, an exchangeable correlation structure was assumed.

Models with adjustments for change in Hb and HbCO were also made. Finally, we decided a priori to test the following interactions: age versus sex, age versus smoking

habits, and sex versus smoking habits. A significance level of 5% was used for all analyses.

SPSS version 20 (IBM Corporation, New York, USA) was used for all analyses except for the GEE estimation for which Stata version 12 (StataCorp, College Station, Texas, USA) was applied.

Results

Study population description

The characteristics of those examined at baseline and at follow-up and those lost to follow-up are outlined in Table 1. Almost half of the sample was ever-smokers, and approximately one quarter of the subjects was current smokers. Those who were lost to follow-up were significantly older and had significantly lower lung function than those who remained in the study.

Analyses were performed to discover a cohort effect, if present, by comparing baseline FEV₁ and DL_{CO} values of those aged 40–44 years at baseline with the corresponding follow-up values of those aged 40–44 years at visit 2. Analyses were performed independently for men and women to adjust for difference in the ratio between the sexes in these sub-samples. There were no statistically significant differences in mean values of FEV₁ and DL_{CO}.

Table 1. Descriptive statistics for characteristics at baseline and follow-up of the stratified sample from the general population in Hordaland County, Norway, aged 18–73 years in 1987/88 with follow-up 9 years later

Variable	Baseline <i>n</i> = 1,152	Follow-up <i>n</i> = 830	Lost to follow-up <i>n</i> = 322
Sex (male), <i>n</i> (%)	590 (51.2)	436 (52.5)	154 (47.8)
Age (years), mean (SD)	41.6 (16.0)	49.8 (14.4)	44.4 (19.3)
Height (cm), mean (SD)	171.8 (9.3)	172.1 (9.4)	170.1 (9.3)
Weight (kg), mean (SD)	71.4 (12.8)	75.9 (13.9)	69.7 (12.1)
Smoking habits, <i>n</i> (%)			
Daily smokers	310 (26.9)	233 (24.7)	77 (23.9)
Ex-smokers	207 (18.0)	149 (21.8)	58 (18.0)
Never smokers	635 (55.1)	448 (53.5)	187 (58.1)
Pack years smoked, ^a mean (SD)	12.7 (11.1)	16.1 (12.3)	13.7 (14.1)
Occupational exposure, <i>n</i> (%)	337 (29.3)	259 (31.2)	78 (24.2)
Education level, <i>n</i> (%)			
Primary school	213 (18.5)	133 (16.0)	80 (24.8)
Secondary school	714 (62.0)	532 (64.1)	182 (56.5)
Higher education	225 (19.5)	165 (19.9)	60 (18.6)
FEV ₁ (L), mean (SD)	3.60 (1.02)	3.28 (0.96)	3.33 (1.12)
FEV ₁ percent predicted, mean (SD)	95 (14)	92 (15)	92 (16)
DL _{CO} (mmol·min ⁻¹ ·kPa ⁻¹), mean (SD)	9.37 (2.53)	9.35 (2.61)	8.81 (2.67)
DL _{CO} percent predicted, mean (SD)	94 (15)	98 (18)	91 (17)

SD, standard deviation; FEV₁, forced expiratory volume in 1 second; DL_{CO}, diffusing capacity of the lung for carbon monoxide.
^aNon-smokers excluded.

Baseline DL_{CO}

Mean DL_{CO} at baseline for the entire cohort ($n = 1,152$) was $9.37 \text{ mmol}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}$ (SD: 2.53). Using multiple linear regression, we found that female sex, higher age, current smoking, ex-smoking, and increased pack years were associated with lower DL_{CO}. Higher body height, larger weight, and higher FEV₁ were significantly associated with higher baseline DL_{CO}, as was higher education compared to secondary school. Occupational airborne exposure was not associated with baseline DL_{CO} regardless of whether the exposure characterization was based on self-reported dust or gas or self-reported exposure to specific airborne agents (Table 2, and Tables E3 and E4).

Change in DL_{CO}

Mean DL_{CO} at follow-up ($n = 830$) was $9.35 \text{ mmol}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}$ (SD: 2.61). Baseline DL_{CO} for the same 830 participants was $9.59 \text{ mmol}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}$ (SD: 2.44). Mean $\Delta\text{DL}_{\text{CO}}$ between baseline and follow-up for those who attended both visits was $-0.24 \text{ mmol}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}$ (95% CI: -0.33 to -0.15).

Mean change in DL_{CO} percent of predicted values for those subjects who attended both visits was 3.0% (95% CI: 2.3 to 4.1). Mean change in FEV₁ percent of predicted values for the same subjects was -3.0% (95% CI -3.9 to -2.7).

$\Delta\text{DL}_{\text{CO}}$ had a normal distribution, tested by both the Kolmogorov-Smirnov and the Shapiro-Wilk tests, with a large variation (Fig. 1). Approximately 40% had a decline of more than twice the average, while 5% had no change ($0 \pm 0.10 \text{ mmol}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}$), and 38% had an increase ($>0.10 \text{ mmol}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}$).

Univariate associations using GEE, adjusting only for baseline DL_{CO} and change in Hb concentration and HbCO, were found for age, height, baseline FEV₁, smoking habits, and pack years.

The multivariate analysis, including baseline DL_{CO}, sex, age, baseline height, baseline weight, baseline FEV₁, baseline smoking habits, pack years smoked before baseline, occupational exposure, and educational level, showed that higher baseline DL_{CO} and age were associated with a more rapid decline in DL_{CO}. Current smokers had a more rapid decline than never smokers, and increased pack years was associated with more rapid decline as well. Higher body height and weight, and higher FEV₁ were associated with a lower rate of decline in DL_{CO}. All the associations above persisted after adjusting for change in Hb and HbCO. Sex, occupational exposure to gas or dust, and level of education were not significantly associated with $\Delta\text{DL}_{\text{CO}}$ in the multivariate analyses (Table 3).

We found no interactions between age and sex, age and smoking habits, or sex and smoking habits on change in DL_{CO}.

Mean alveolar volume (V_A) was 6.49 L (SD: 1.30) at baseline and 6.29 L (SD: 1.38) at follow-up. There was a

significant reduction in V_A during the observation period. In a multivariate analysis, higher baseline V_A and female sex were significant predictors of a more rapid decline in V_A (Table E5).

Mean carbon monoxide diffusion coefficient (K_{CO}) at baseline was $1.48 \text{ mmol}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}\cdot\text{L}^{-1}$ (SD: 0.25) and $1.49 \text{ mmol}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}\cdot\text{L}^{-1}$ (SD: 0.32) at follow-up. When analyzing the values from only the participants who met the requirement of an IVC/FVC ratio of 0.85 or above, the corresponding means were $1.45 \text{ mmol}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}\cdot\text{L}^{-1}$ (SD: 0.24) and $1.46 \text{ mmol}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}\cdot\text{L}^{-1}$ (SD: 0.28), respectively. When analyzed in a multivariate model, we found that higher baseline K_{CO} , male sex, higher age, lower baseline body weight, current smoking, higher number of pack years smoked, and lower level of education were significant predictors of a more rapid decline in K_{CO} (Table E6).

Discussion

In this 9-year follow-up study of a general population sample, we observed that the rate of decline in gas diffusion capacity was highly variable. Mean change in DL_{CO} was $-0.025 \text{ mmol}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}\cdot\text{year}^{-1}$. Current smoking was the strongest predictor for decline in DL_{CO}. In addition, older age, higher cumulative smoking consumption in terms of pack years, lower level of FEV₁, lower body weight, and shorter body height were independent predictors of increased DL_{CO} loss. Sex, educational level, and occupational airborne exposure did not independently influence change in DL_{CO}.

This is the first community study to show that current smoking status and previous smoking consumption in terms of pack years predict loss of DL_{CO}. The study is also the first to examine the effect of educational level and occupational airborne exposure on change in gas diffusion capacity. Our study confirms the findings of others (18, 19) that the decline in DL_{CO} becomes more rapid with higher age.

The magnitude of the decline in DL_{CO} observed in our study is comparable to that found by Viegi et al. (19), while comparison to the decline found by Sherrill et al. (18) is more complicated because of differences in how the results are reported. Standard error of the mean of DL_{CO} seems to be comparable between all three studies.

Current smoking was related to a reduced baseline DL_{CO} and a larger subsequent decline in DL_{CO} in the multivariate analyses. Adjusting for HbCO did not change this association. Hence, current smoking has an effect on level and decline of DL_{CO} beyond that of previous exposure and that of HbCO. Smokers more often develop anemia that may impair gas diffusion (33). However, when change in Hb was added to the equation, the relationship between smoking and DL_{CO} persisted. The study was not designed to investigate mechanisms by which tobacco smoke could alter the rate of change in DL_{CO}.

Table 2. Descriptive statistics for baseline DL_{CO} in 1987/88 and average change per year during a 9-year follow-up, Δ DL_{CO}, for 830 subjects from Hordaland County, Norway, according to baseline characteristics

Characteristics at baseline	Baseline DL _{CO} (mmol·min ⁻¹ ·kPa ⁻¹), mean (SD)	Δ DL _{CO} (mmol·min ⁻¹ ·kPa ⁻¹ ·year ⁻¹), mean (SD)
Sex		
Male	10.85 (2.38)	-0.039 (0.161)
Female	7.83 (1.57)	-0.010 (0.114)
Age in years		
Up to 19	10.60 (2.39)	0.003 (0.158)
20–29	10.88 (2.49)	-0.021 (0.150)
30–39	10.00 (2.20)	0.001 (0.129)
40–49	9.45 (2.10)	-0.037 (0.163)
50–59	8.23 (2.01)	-0.032 (0.134)
60–69	7.54 (1.69)	-0.072 (0.103)
70–79	6.02 (1.46)	-0.050 (0.122)
Height in cm		
159 and below	6.55 (1.27)	-0.023 (0.118)
160–169	7.90 (1.61)	-0.018 (0.103)
170–179	9.93 (1.97)	-0.030 (0.142)
180–189	11.62 (2.31)	-0.034 (0.192)
190 and above	12.84 (2.16)	-0.005 (0.154)
Weight in kg		
< 49	6.08 (1.80)	0.001 (0.114)
50–59	7.76 (1.64)	-0.016 (0.111)
60–69	8.83 (2.24)	-0.026 (0.120)
70–79	10.06 (2.54)	-0.041 (0.156)
80–89	10.48 (2.41)	-0.001 (0.150)
90–99	10.61 (2.44)	-0.034 (0.207)
100	10.78 (2.89)	-0.049 (0.118)
Smoking habits		
Never smoker	9.62 (2.62)	-0.012 (0.144)
Ex-smoker	9.20 (2.31)	-0.037 (0.119)
Daily smoker	8.99 (2.43)	-0.044 (0.148)
Pack years smoked		
0	9.62 (2.62)	-0.012 (0.144)
1–20	9.23 (2.40)	-0.031 (0.136)
21–40	8.75 (2.19)	-0.080 (0.137)
> 40	6.79 (1.92)	-0.094 (0.125)
Occupational exposure		
No	9.08 (2.32)	-0.019 (0.138)
Yes	10.12 (2.53)	-0.029 (0.152)
Education level		
Primary school	8.15 (2.22)	-0.041 (0.131)
Secondary school	9.43 (2.44)	-0.023 (0.144)
Higher education	10.37 (2.62)	-0.020 (0.143)
FEV₁ quartiles		
2.89 L and below	6.87 (1.51)	-0.031 (0.109)
2.90–3.55 L	8.56 (1.27)	-0.030 (0.125)
3.56–4.36 L	9.95 (1.66)	-0.014 (0.145)
4.37 and above	12.20 (1.95)	-0.029 (0.174)

DL_{CO}, diffusing capacity of the lung for carbon monoxide; FEV₁, forced expiratory volume in 1 second; SD, standard deviation.

Cumulative smoking exposure in terms of pack years was also an independent predictor of future decline in DL_{CO} (Table 3). There may be several explanations for this finding. First, smoking exposure may cause airflow

limitation and air trapping that lead to impaired gas diffusion capacity. However, the effect of pack years on DL_{CO} decline persisted after taking baseline FEV₁ into account (Table 3). Second, we have recently shown in

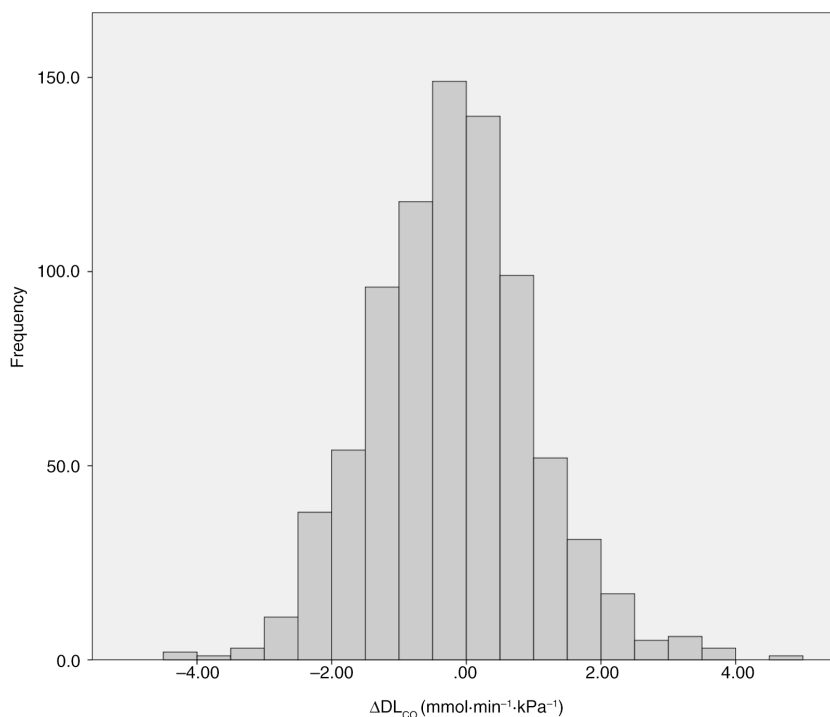


Fig. 1. The distribution of change in DL_{CO} during a 9-year follow-up from 1987/88 in 830 subjects from Hordaland County, Norway.

another data set that level of emphysema is related to DL_{CO} after adjusting for FEV_1 (34). Hence, increased smoking consumption may cause decline in DL_{CO} because of more emphysema.

Neither the Italian nor the American community study observed that current smoking or smoking consumption was related to decline in DL_{CO} (18, 19). The follow-up rate in the Italian study was lower than that in the current study, and smokers tend to drop out more often than non-smokers in longitudinal surveys (35). The American study comprised only about half the number of subjects of our study and they had no subjects above the age of 59 years at baseline (18).

In line with others (18, 19), we observed that the DL_{CO} decline becomes more rapid with increasing age. The best fit of the model was for age squared, adding further support to our finding that the decline accelerated with increasing age. In the multivariate analysis, this acceleration in the decline with increasing age was found to be independent of smoking, lung function, body height and weight, as well as occupational exposure and SES. Potential explanations might be age-related reduced alveolar ventilation, increased level of emphysema, increased

pulmonary blood pressure, and impaired cardiac function (36).

When comparing DL_{CO} with available European predicted values, we observed an increase in the percent predicted value while there was a decrease in the absolute value. These predicted values were based on a compilation of European cross-sectional studies, and the age coefficient may be overestimated because of a cohort effect and less precise characterization of the subjects with respect to symptoms, previous smoking, and occupational exposure. As for FEV_1 , the annual change in longitudinal studies is less than the estimated annual change from cross-sectional surveys.

The difference between cross-sectional and longitudinal estimates of annual change may also be influenced by regression to the mean. We included baseline DL_{CO} in the model which will partially account for that phenomenon.

We did not observe that occupational airborne exposure influenced level of DL_{CO} or decline of DL_{CO} in this general population sample. This may imply that there is no impact of occupational exposure on gas diffusion capacity in a community setting, or that we have not been able to show it. Regarding the latter possibility, the exposure

Table 3. Adjusted yearly change in DL_{CO} estimated by generalized estimating equations (GEE) of the stratified sample from the general population in Hordaland County, Norway, aged 18–73 years in 1987/88 with follow-up 9 years later

Characteristic at baseline	Estimate	<i>p</i>
DL _{CO} at baseline		
At DL _{CO} 9.6	−0.0293	
Per 1 unit increase	−0.0325	<0.0001
Age at baseline		
At age 45 years	−0.0293	
Per 10 years increase	−0.0243	<0.0001
Sex		
Men	−0.0293	
Women	−0.0162	0.410
Height at baseline		
At 170 cm	−0.0293	
Per 10 cm increase (at baseline)	0.0240	0.013
Weight at baseline		
At 70 kg	−0.0293	
Per 1 kg increase	0.0011	0.020
Smoking at baseline		0.001
Never	−0.0293	
Ex	−0.0238	0.700
Current	−0.0738	0.002
Pack years smoked before baseline		
At 6 pack years	−0.0293	
Per 10 pack years increase	−0.0196	0.003
Occupational exposure		
No	−0.0293	
Yes	0.0146	0.177
Educational level		0.310
Primary school	−0.0293	
Secondary school	−0.0443	0.270
Higher education	−0.0304	0.947
FEV ₁ at baseline		
At FEV ₁ 3.6 L	−0.0293	
Per 1 L increase	0.0235	0.013

DL_{CO}, diffusing capacity of the lung for carbon monoxide in mmol·min^{−1}·kpa^{−1}; FEV₁, forced expiratory volume in 1 second.

characterization applied in the present study has been used to show a relationship between lung function in terms of spirometry (27, 37), diagnosis of asthma and chronic obstructive pulmonary disease (27, 38), as well as the prevalence and incidence of respiratory symptoms (24, 38). The exposure data have a high specificity, but a lower sensitivity (29). Those stating exposure have in general been exposed to a higher degree than those falsely stating no exposure (29). Hence, we think that our study indicates that the level of occupational exposure in a general population sample is not high enough to cause impaired level of DL_{CO} and more rapid decline in DL_{CO}.

We have previously shown in cross-sectional analyses in this population that lower SES in terms of educational achievement is independently related to reduced level of DL_{CO} (17). However, we did not observe that SES predicted subsequent change in DL_{CO} after adjusting for the other covariates. As people tend to stay in the socioeconomic class into which they are born, the effect of SES on DL_{CO} may have been evident at an early stage in life after which the subsequent decline in DL_{CO} is independent of SES. However, it should be noted that low as compared to high SES was an independent predictor of rapid decline in K_{CO} (Table E6).

Strengths and limitations of the study

This study is based on a community survey with high response rates both at baseline and follow-up. The study sample is representative of the population at large with respect to sex, age, and smoking (25, 35). Except for the requirement of an IVC/FVC ratio above 0.85, the participants included in the analyses met the ATS-criteria for a satisfactory DL_{CO} test (28). The same equipment for measuring DL_{CO} was used at baseline and follow-up with the same technicians. The effect of smoking on change in DL_{CO} was adjusted for by change in HbCO, and finally validated questions on occupational exposure were used.

There are also some limitations to the study. First, we had only two points of observations, rendering the study susceptible to regression towards the mean. On the other hand, we adjusted for baseline level of DL_{CO}, which should at least partly take this bias into account. Second, we did not have data on menstrual cycle for female participants, and are therefore not able to adjust for the effects of the menstrual cycle on DL_{CO} (39–41).

In conclusion, we have observed that in the population at large both current smoking and cumulative smoking exposure, reduced FEV₁, and increasing age predict more rapid decline in gas diffusion capacity, while occupational exposure and SES do not. This knowledge may help physicians in their interpretation of DL_{CO} measurements.

Conflict of interest and funding

The authors have not received any funding or benefits from industry or elsewhere to conduct this study.

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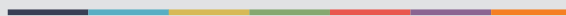
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