Assessment of Global Lung Function Initiative (GLI) reference equations for diffusing capacity in relation to respiratory burden in the Swedish CArdioPulmonary bioImage Study (SCAPIS)

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The LLN for D_{LCO} is above the GLI LLN in never-smoking, middle-aged Swedish adults. Individuals with D_{LCO} above the GLI LLN but below the SCAPIS LLN had an increased burden of respiratory disease, suggesting clinical implications for the present findings. https://bit.ly/39B455B

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ABSTRACT The Global Lung Function Initiative (GLI) has recently published international reference values for diffusing capacity of the lung for carbon monoxide (D_{LCO}). Lower limit of normal (LLN), *i.e.* the 5th percentile, usually defines impaired D_{LCO}. We examined if the GLI LLN for D_{LCO} differs from the LLN in a Swedish population of healthy, never-smoking individuals and how any such differences affect identification of subjects with respiratory burden.

Spirometry, D_{LCO}, chest high-resolution computed tomography (HRCT) and questionnaires were obtained from the first 15 040 participants, aged 50–64 years, of the Swedish CArdioPulmonary bioImage Study (SCAPIS). Both GLI reference values and the lambda-mu-sigma (LMS) method were used to define the LLN in asymptomatic never-smokers without respiratory disease (n=4903, of which 2329 were women).

Both the median and LLN for D_{LCO} from SCAPIS were above the median and LLN from the GLI ($p<0.05$). The prevalence of D_{LCO} <GLI LLN (and also <SCAPIS LLN) was 3.9%, while the prevalence of D_{LCO} >GLI LLN but <SCAPIS LLN was 5.7%. Subjects with D_{LCO} >GLI LLN but <SCAPIS LLN (n=860) had more emphysema (14.3% *versus* 4.5%, $p<0.001$), chronic airflow limitation (8.5% *versus* 3.9%, $p<0.001$) and chronic bronchitis (8.3% *versus* 4.4%, $p<0.01$) than subjects (n=13 600) with normal D_{LCO} (>GLI LLN and >SCAPIS LLN). No differences were found with regard to physician-diagnosed asthma.

The GLI LLN for D_{LCO} is lower than the estimated LLN in healthy, never-smoking, middle-aged Swedish adults. Individuals with D_{LCO} above the GLI LLN but below the SCAPIS LLN had, to a larger extent, an increased respiratory burden. This suggests clinical implications for choosing an adequate LLN for studied populations.

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Introduction

Diffusing capacity of the lung for carbon monoxide (D_{LCO}) is a valuable pulmonary function test (PFT) first described more than 100 years ago. It has been available for more than 60 years in the form currently used in clinics [1] and it adds important information to spirometry regarding the lungs' ability to transfer gas from inhaled air to blood in the pulmonary capillaries [2]. D_{LCO} is a valuable test in chronic obstructive pulmonary disease (COPD), with recent evidence indicating that impaired D_{LCO} relates to a higher symptom burden [3], decreased exercise performance [3, 4], increased exacerbation risk [3] and increased mortality [5]. In order to interpret the recorded values of a patient, adequate reference values are needed [6].

The Global Lung Function Initiative (GLI) collects values from lung function testing in healthy, never smoking individuals around the world, with the aim of publishing international reference values for all ages. The GLI has defined reference values using the lambda-mu-sigma (LMS) method, a mathematical approach originally applied for nonlinear growth charts. The reference values for spirometry were published in 2012 [7] and the reference values for D_{LCO} have been published recently [8].

The GLI represents an interesting alternative to national reference equations that are frequently based on small population samples. To our knowledge, the newly proposed GLI reference values for D_{LCO} have not been tested in large populations, except that in the GLI database, either in Sweden or in other countries. However, the reference values for spirometry have previously been evaluated in a Swedish population sample [9]. These GLI values were found to underestimate both forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) in adults from northern Sweden [9]. It is therefore appropriate to analyse whether the lower limit of normal (LLN) proposed by the GLI reference values for D_{LCO} is valid in Swedish subjects.

In clinical practice, corrections for haemoglobin (Hb) levels are recommended for D_{LCO} values [10]. However, it is not as clear if current Hb values should be accounted for when generating reference values.

The main aim of the present analysis was to test if GLI-predicted D_{LCO} values and the LLN for D_{LCO} were different from those defined in never smoking individuals without respiratory disease in the Swedish CArdioPulmonary bioImage Study (SCAPIS), using the same methodology as the GLI, and both with and without correction for Hb. A secondary aim was to examine respiratory burden (respiratory symptoms, physician-diagnosed COPD and emphysema) in relation to any discrepancies in LLN between the GLI and SCAPIS populations.

Methods

Study population

Participants from among the general population were randomly invited to participate in the Swedish CArdioPulmonary bioImage Study (SCAPIS) [11]. They attended six Swedish healthcare centres and underwent examinations including PFTs (spirometry and D_{LCO}) and chest high-resolution computed tomography (HRCT) scans (they also completed questionnaires). This is an interim analysis (including full information on D_{LCO}) from the first 15 040 participants, aged 50–64 years (7794 of whom were women) and this group is referred to as the "general population". We generated reference values from SCAPIS in a

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subset of subjects consisting of "never smokers" without "any respiratory symptom" and without "any respiratory disease." This group is referred to as the "reference population".

Smoking status retrieved from questionnaires was used to categorise subjects as "current smokers", "former smokers" or "never smokers". "Current smokers" were defined as those who answered that they were currently smoking, either on a regular basis or occasionally. "Former smokers" were defined as subjects who had smoked for at least 1 year, but not during the last year before the study. "Never smokers" were defined as subjects who stated that they had smoked less than 100 cigarettes in their lifetime.

"Any respiratory symptom" was defined as reporting fulfilment of any of the following criteria: 1) a cough in the absence of a cold; 2) coughing up phlegm or having phlegm in the chest in the absence of a cold; 3) having a wheeze or whistling; or 4) having dyspnoea (defined as a modified Medical Research Council (mMRC) score $\geqslant$2). "Any respiratory disease" was defined as having reported a diagnosis of asthma, COPD, chronic bronchitis, emphysema, tuberculosis (TB) or any other respiratory disease. "Chronic bronchitis" was defined as reporting expectoration of phlegm, even without simultaneous symptoms of upper respiratory tract infection, during a period of at least 3 months·year^{-1} for at least 2 years. Body mass index (BMI) was defined as measured weight (kg)/(height (m))^2.

Pulmonary function testing

Dynamic spirometry (including FEV_1, FVC and slow vital capacity (SVC), as well as D_{LCO}) was performed with the subject in a sitting position and wearing a nose clip, at least 15 min after inhalation of 400 µg of salbutamol. In all measurements, a Jaeger Master Screen PFT (Vyaire, Mettawa, IL, USA) was used. All procedures were performed in accordance with European Respiratory Society (ERS)/American Thoracic Society (ATS) standards [10, 12], with the exception that gas analyser linearity checks were not performed as required by the ERS/ATS for D_{LCO} measurement [10].

The following variables from diffusing capacity measurements for carbon monoxide were used: D_{LCO}, transfer coefficient of the lung for carbon monoxide (K_{CO}) and alveolar volume (V_A). Sensitivity analyses were conducted using D_{LCO} corrected for Hb (*e.g.*, equations 31 and 32 in [13]).

For males

$$D_{LCO}(\text{predicated for Hb}) = \frac{D_{LCO}(\text{predicted}) \cdot (1.7\text{Hb})}{(10.22 + \text{Hb})} \quad (1)$$

For females

$$D_{LCO}(\text{predicated for Hb}) = \frac{D_{LCO}(\text{predicted}) \cdot (1.7\text{Hb})}{(9.38 + \text{Hb})} \quad (2)$$

Emphysema assessment

All computed tomography (CT) scanning was performed using a Somatom Definition Flash Scanner (Siemens Healthcare, Forchheim, Germany) and the methodology has been described in detail [14]. If emphysema was present, the type (centrilobular, panlobular, paraseptal, bulla(e), or a combination thereof) was reported, as well as the grade (none, mild, moderate, or severe) and localisation (upper, middle and/or lower part of the right and/or left lung). To ensure consistent interpretation across radiologists, a consensus meeting was held before the start of the study. All imaging terminology was based on that of the Fleischner Society [15] and the radiologists were blinded to patient details when scoring CT. Emphysema was defined as CT findings of at least mild emphysema in any zone. We have recently validated the visual assessment of mild emphysema in the pilot study of SCAPIS [16]. At the time of analysis, data for emphysema assessment were available from five out of six of the participating centres.

Statistics

Following the instructions from GLI 2017, we applied the LMS methods to the reference population from SCAPIS (47.5% female). By setting D_{LCO} as the dependent variable and the splined log transformed age and log transformed height as the independent variables (choosing the Box–Cox–Cole Green power family and setting the "log" link function for the mean) we could estimate the models. The equations are presented in supplementary table S1. D_{LCO} was uncorrected for Hb at this stage.

After estimating the LMS models for the SCAPIS reference population for each sex separately, we predicted the LLN (or 5th percentile) of SCAPIS for the general population in the manner of GLI 2017. Thus, the LLN calculated from the SCAPIS reference population was compared with the GLI LLN, which was predicted using the GLI 2017 equations.

Also based on the equations from GLI 2017 (table 2 in [8]), the z-scores for D_{LCO}, K_{CO}, V_A and FEV_1/FVC were calculated for the general SCAPIS population. The median, 5th percentile and 95th percentile of the z-scores were then calculated accordingly. The outliers were defined as those with absolute z-scores larger than five [7]. The general population could then be clustered into three groups based on whether D_{LCO} was >GLI LLN and >SCAPIS LLN, >GLI LLN but <SCAPIS LLN, or <GLI LLN and <SCAPIS LLN.

Chi-squared tests were performed to evaluate if the prevalence of respiratory burden (any respiratory symptoms or any self-reported respiratory disease or emphysema) differed between those subjects with D_{LCO} >GLI LLN but <SCAPIS LLN and those with normal D_{LCO} according to both SCAPIS and GLI. Sensitivity analyses were conducted for D_{LCO} corrected for Hb and all analyses were performed using R version 3.5.3 [17]. Specifically, we estimated the LMS models using the generalised additive model for location, scale and shape package of R (GAMLSS). In case of multiple comparisons, p-values were adjusted for multiple tests in accordance with the Benjamini–Hochberg method [18].

Ethics

All participants gave written informed consent and SCAPIS has been approved as a multicentre study by the ethics committee at Umeå University (Dnr 2010-228-31M).

Results

Definitions of SCAPIS and LLN reference equations

The reference population of never smoking individuals without any respiratory symptoms or any respiratory disease was selected as indicted by the flowchart in figure 1 and consisted of 4903 participants, of which 2329 were female. LMS models were performed for males and females separately in order to generate SCAPIS reference equations.

Population characteristics

Table 1 displays the characteristics of males and females in the reference population and in the general population. D_{LCO} and K_{CO}, both expressed as z-scores, were higher in the reference population compared

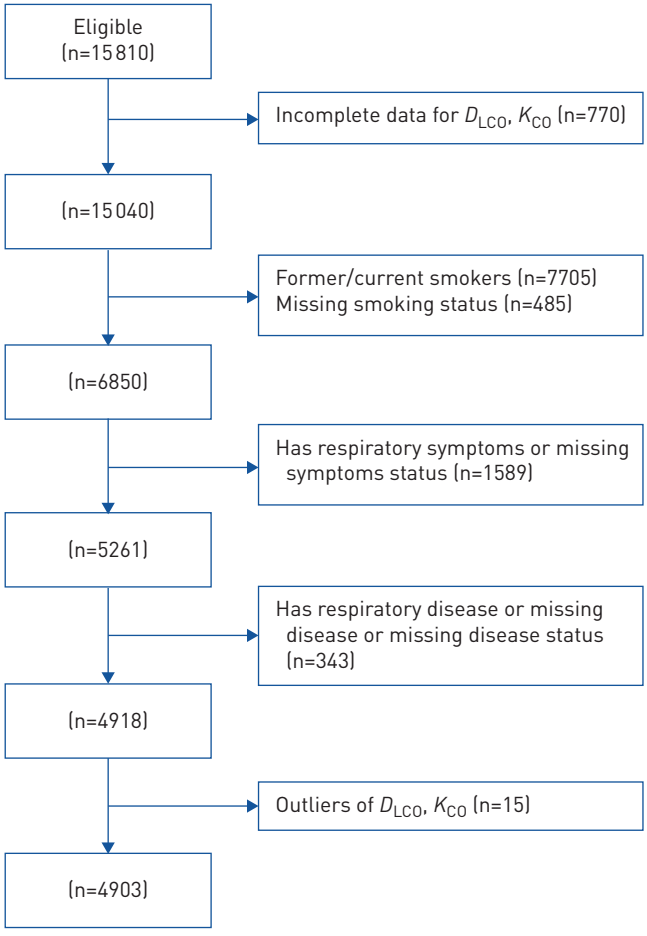

FIGURE 1 Flowchart describing the population studied for the Swedish CArdioPulmonary bioImage Study (SCAPIS) reference values. D_{LCO}: diffusing capacity of the lung for carbon monoxide; K_{CO}: transfer coefficient of the lung for carbon monoxide.

TABLE 1 Characteristics of the study participants in the general population, the reference population and the non-reference population, as stratified by sex

Characteristic	Males			Females		
	General population (n=7246)	Reference population (n=2574)	Non-reference population (n=4672)	General population (n=7794)	Reference population (n=2329)	Non-reference population (n=5465)
Age years	57.5±4.4	56.8±4.3	57.9±4.4	57.5±4.3	56.8±4.4	57.8±4.3
Height cm	179.3±7.0	180.2±6.8	178.8±7.1	165.5±6.5	165.9±6.4	165.3±6.6
BMI kg·m^{-2}	27.4±4.0	26.9±3.7	27.8±4.1	26.5±4.8	25.5±4.4	27.0±5.0
Hb g·dL^{-1}	14.8±0.98	14.8±0.93	14.8±1.01	13.4±0.93	13.3±0.89	13.4±0.95
FEV_1 GLI z-score	0.07±0.99	0.24±0.89	−0.03±1.03	0.19±0.99	0.36±0.89	0.12±1.03
FVC GLI z-score	0.06±0.91	0.14±0.87	0.02±0.93	0.22±0.88	0.27±0.85	0.20±0.89
FEV_1/FVC GLI z-score	0.00±0.97	0.16±0.84	−0.09±1.02	−0.12±0.99	0.07±0.75	−0.20±1.06
D_{LCO} GLI z-score	0.12±0.97	0.32±0.85	0.00±1.02	0.02±0.97	0.25±0.79	−0.08±1.02
K_{CO} GLI z-score	0.08±1.36	0.18±0.86	0.02±1.56	0.02±1.12	0.22±0.88	−0.07±1.19
Centre distribution %						
Gothenburg	30.2	31.1	29.7	30.5	29.1	31.1
Malmö	24.3	21.5	25.8	27.1	24.6	28.1
Linköping	18.5	18.1	18.6	17.7	17.3	17.9
Stockholm	14.5	16.6	13.4	12.7	15.5	11.5
Uppsala	9.9	9.9	10.0	9.8	11.0	9.3
Umeå	2.6	2.9	2.5	2.3	2.5	2.2
Smoking habits %						
Never smokers	47.7	100.0	18.9	43.5	100.0	19.4
Former smokers	34.4	0	53.4	39.5	0	56.3
Current smokers	14.2	0	22.1	14.2	0	20.2
Missing information	3.6	0	5.6	2.9	0	4.1
FEV_1/FVC <GLI LLN %	5.1	2.0	6.9	5.0	1.5	6.5
FEV_1/FVC <Brisman LLN %	9.8	5.1	12.5	8.1	3.0	10.3
FEV_1/FVC <0.7 %	11.1	6.0	13.9	8.0	3.0	10.1

Data are presented as n or mean±SD. BMI: body mass index; Hb: haemoglobin; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; GLI: Global Lung Function Initiative; D_{LCO}: diffusing capacity of the lung for carbon monoxide; K_{CO}: transfer coefficient of the lung for carbon monoxide; LLN: lower limit of normal.

with the general population. The LLN can be predicted for an individual of known sex for a given age and height. In figure 2, the GLI LLN and the SCAPIS LLN are compared for average height (180 cm for males and 165 cm for females) with age ranging from 50 to 65 years. There was a difference between the GLI LLN and the SCAPIS LLN, with lower values for the GLI LLN than for the SCAPIS LLN for both D_{LCO} and K_{CO}.

Sensitivity analyses

For sensitivity analyses, we estimated the models for D_{LCO} corrected for Hb, as introduced in the Methods section. The corresponding LLN for the corrected D_{LCO} in males with a height of 180 cm and females with a height of 165 cm are illustrated by the dotted lines in figure 3, which are close to the Hb-unadjusted SCAPIS LLN. The median (interquartile range (IQR)) difference between the LLN for Hb-unadjusted D_{LCO} and Hb-adjusted D_{LCO} was 0.06 mmol^{-1}·min^{-1}·kPa^{-1} (0.05–0.08 mmol^{-1}·min^{-1}·kPa^{-1}).

We also studied the effect of smaller samples (1000 men and 1000 women, or 500 men and 500 women) on defining the LLN for D_{LCO} (supplementary figure S1). The median (IQR) differences for the LLN for D_{LCO} when 1000 or 500 individuals were selected (instead of the whole reference population) were: 0.02 mmol^{-1}·min^{-1}·kPa^{-1} (−0.08 to 0.06 mmol^{-1}·min^{-1}·kPa^{-1}) and −0.07 mmol^{-1}·min^{-1}·kPa^{-1} (−0.13 to 0.08 mmol^{-1}·min^{-1}·kPa^{-1}), respectively.

Clinical importance of using different reference equations to define LLN

Table 2 shows the clinical importance of using different reference equations to define the LLN. We compared subjects with normal D_{LCO} (>GLI LLN and >SCAPIS LLN) and those with impaired D_{LCO} (>GLI LLN but <SCAPIS LLN). The SCAPIS median for D_{LCO} was higher than the GLI median and the median (IQR) difference was 0.51 mmol^{-1}·min^{-1}·kPa^{-1} (0.34–0.66 mmol^{-1}·min^{-1}·kPa^{-1}) for males and

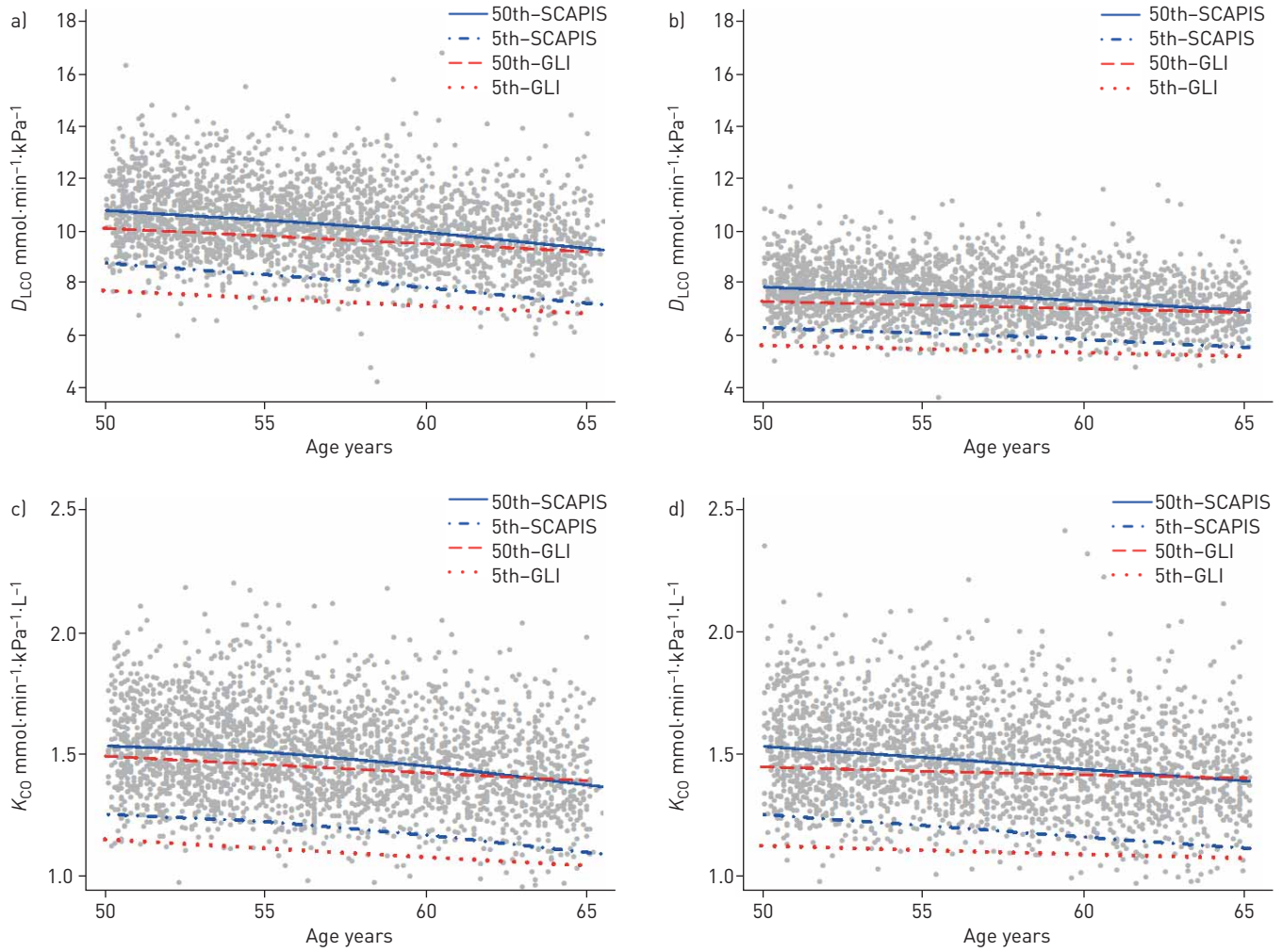

FIGURE 2 Comparison of median (50th percentile) and lower limit of normal (LLN) (5th percentile) values from the Swedish CArdioPulmonary bioImage Study (SCAPIS) cohort with the Global Lung Function Initiative (GLI) reference cohort for diffusing capacity of the lung for carbon monoxide (D_{LCO}) and transfer coefficient of the lung for carbon monoxide (K_{CO}). (a) and (c) LLN curves for males (height=180 cm); (b) and (d) LLN curves for females (height=165 cm).

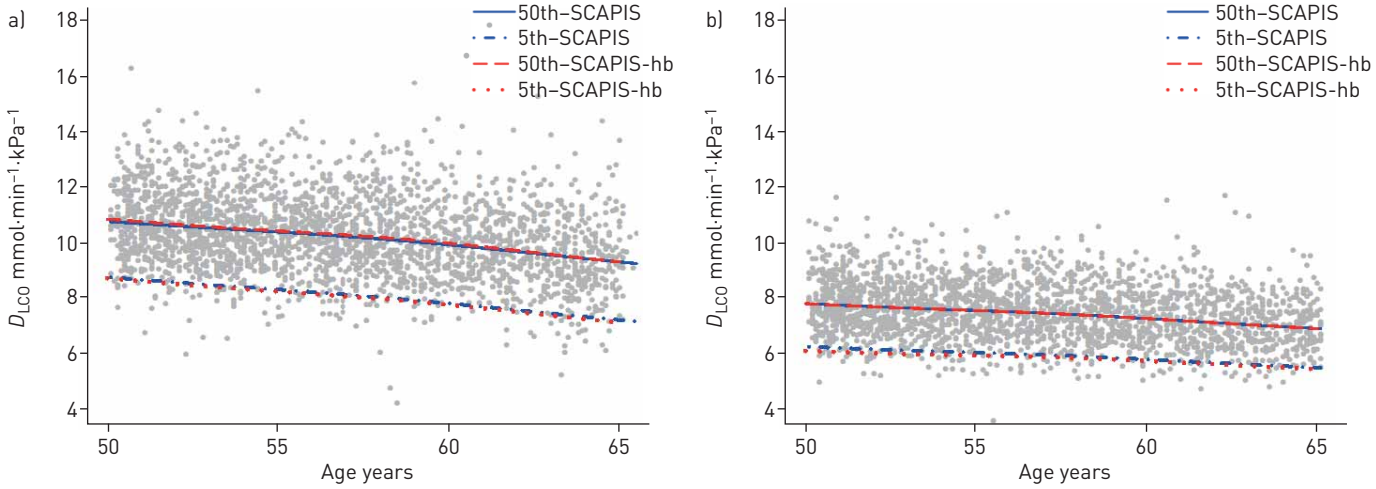

FIGURE 3 Comparison of median (50th percentile) and lower limit of normal (LLN) (5th percentile) values from the Swedish CArdioPulmonary bioImage Study (SCAPIS) cohort for diffusing capacity of the lung for carbon monoxide (D_{LCO}) both unadjusted for haemoglobin (Hb) (blue) and adjusted for Hb (red). (a) LLN curves for males (height=180 cm); (b) LLN curves for females (height=165 cm).

TABLE 2 Clinical characteristics of the general population from the Swedish CArdioPulmonary bioImage Study (SCAPIS) grouped according to diffusing capacity of the lung for carbon monoxide (D_{LCO}) in relation to the Global Lung Function Initiative (GLI) lower limit of normal (LLN) and the SCAPIS LLN (n=15 040)

Characteristic	D_{LCO}			p-value		
	>GLI LLN and >SCAPIS LLN (G1)	>GLI LLN but <SCAPIS LLN (G2)	<GLI LLN and <SCAPIS LLN (G3)	G1 *versus* G2	G1 *versus* G3	G2 *versus* G3
Subjects	13 600 (90.4)	860 (5.7)	580 (3.9)			
Age years	57.5±4.3	56.9±4.3	59.1±4.1			
Female %	51.6	53.6	54.7			
Smoking habits %						
Never smokers	47.7	30.2	18.8			
Former smokers	37.4	34.8	31.6			
Current smokers	11.6	31.9	48.1			
Missing information	3.3	3.1	1.6			
Cough %	17.3	27.9	39.2	<0.001	<0.001	<0.001
Chronic bronchitis %	4.4	8.3	13.9	<0.001	<0.001	<0.001
Sputum production %	10.3	18.3	26.9	<0.001	<0.001	<0.001
Wheeze %	6.6	12.8	23.7	<0.001	<0.001	<0.001
Dyspnoea# %	9.0	17.6	36.1	<0.001	<0.001	<0.001
Sick leave due to respiratory problems %	4.5	7.0	11.0	0.013	<0.001	0.015
Having reported asthma %	7.8	8.3	9.9	0.640	0.082	0.353
Having reported COPD %	0.9	3.1	11.4	<0.001	<0.001	<0.001
Emphysema¶ %	4.5	14.3	32.7	<0.001	<0.001	<0.001
FEV_1/FVC <SCAPIS LLN %	8.8	18.2	37.9	<0.001	<0.001	<0.001
FEV_1/FVC <GLI LLN %	3.9	8.5	26.9	<0.001	<0.001	<0.001

Data are presented as n, n (%) or mean±SD. COPD: chronic obstructive pulmonary disease; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity. #: modified Medical Research Council (mMRC) score ⩾2; ¶: based on high-resolution computed tomography (HRCT) findings.

0.34 $mmol^{-1}\cdot min^{-1}\cdot kPa^{-1}$ (0.19–0.48 $mmol^{-1}\cdot min^{-1}\cdot kPa^{-1}$) for females (supplementary figure S2). The SCAPIS LLN for D_{LCO} was higher than the GLI LLN and the median (IQR) difference was 0.81 $mmol^{-1}\cdot min^{-1}\cdot kPa^{-1}$ (0.63–0.94 $mmol^{-1}\cdot min^{-1}\cdot kPa^{-1}$) for males and 0.53 $mmol^{-1}\cdot min^{-1}\cdot kPa^{-1}$ (0.43–0.63 $mmol^{-1}\cdot min^{-1}\cdot kPa^{-1}$) for females (supplementary figure S3). The prevalence of D_{LCO} <GLI LLN was 3.9% and the prevalence of D_{LCO} <SCAPIS LLN was 9.6% for the general population.

Subjects with D_{LCO} >GLI LLN but <SCAPIS LLN (n=860) had a three-fold higher ($p<0.01$) prevalence of emphysema than subjects with normal D_{LCO} (>GLI LLN and >SCAPIS LLN, n=13 600). Similar differences with two to three times higher prevalence were found regarding chronic airflow limitation (defined as FEV_1/FVC <GLI LLN), chronic bronchitis and physician-diagnosed COPD ($p<0.01$ in all cases).

Subjects with D_{LCO} <GLI LLN and <SCAPIS LLN (n=580) had further increased prevalence of emphysema, chronic airflow limitation, chronic bronchitis and physician-diagnosed COPD in comparison to the group with D_{LCO} >GLI LLN but <SCAPIS LLN, as well as in comparison to the group with normal D_{LCO} according to both SCAPIS and GLI (table 2) ($p<0.001$ in all cases).

The prevalence of K_{CO} <GLI LLN was 4.3% while the prevalence of K_{CO} <SCAPIS LLN was 9.8% for the general population. Similar results were found for K_{CO}, with two to three times higher prevalence rates of chronic airflow limitation, chronic bronchitis, emphysema and physician-diagnosed COPD in the group with K_{CO} >GLI LLN but <SCAPIS LLN compared to the group with normal K_{CO} (table 3).

Subjects with K_{CO} <GLI LLN and <SCAPIS LLN (n=649) had further increased prevalence of emphysema, chronic airflow limitation, chronic bronchitis and physician-diagnosed COPD compared with both the group with K_{CO} >GLI LLN but <SCAPIS LLN and the group with normal K_{CO} according to both SCAPIS and GLI (table 3) ($p<0.001$ in all cases).

Discussion

Our main finding was that the LLN for D_{LCO}, as suggested by the GLI, was lower than the LLN in never smoking, middle-aged Swedish women and men without respiratory disease and/or respiratory symptoms. Moreover, subjects classified as having normal D_{LCO} by the GLI but not by the LLN in our population

TABLE 3 Clinical characteristics of the general population from the Swedish CArdioPulmonary bioImage Study (SCAPIS) grouped according to transfer coefficient of the lung for carbon monoxide (K_{CO}) in relation to Global Lung Function Initiative (GLI) lower limit of normal (LLN) and SCAPIS LLN (n=15 040)

Characteristic	K_{CO}			p-value		
	>GLI LLN and >SCAPIS LLN (G1)	>GLI LLN but <SCAPIS LLN (G2)	<GLI LLN and <SCAPIS LLN (G3)	G1 *versus* G2	G1 *versus* G3	G2 *versus* G3
Subjects	13 573 (90.2)	818 (5.4)	649 (4.3)			
Age years	57.5±4.3	56.8±4.3	59.0±4.2			
Female %	51.5	52.9	56.9			
Smoking habits %						
Never smokers	47.9	31.1	14.0			
Former smokers	37.1	36.7	35.3			
Current smokers	11.6	30.2	48.8			
Missing information	3.4	2.1	1.8			
Cough %	17.5	27.3	34.0	<0.001	<0.001	0.009
Chronic bronchitis %	4.4	8.3	13.2	<0.001	<0.001	0.006
Sputum production %	10.4	16.7	24.9	<0.001	<0.001	<0.001
Wheeze %	6.8	11.5	18.2	<0.001	<0.001	0.001
Dyspnoea[#] %	9.5	13.6	27.4	<0.001	<0.001	<0.001
Sick leave due to respiratory problems %	4.6	6.8	8.3	0.006	<0.001	0.359
Having reported asthma %	7.8	7.9	9.5	0.955	0.016	0.359
Having reported COPD %	1.0	2.0	10.3	0.024	<0.001	<0.001
Emphysema[¶] %	4.3	13.9	35.9	<0.001	<0.001	<0.001
FEV_1/FVC <SCAPIS LLN %	8.1	22.1	44.4	<0.001	<0.001	<0.001
FEV_1/FVC <GLI LLN %	3.6	10.4	29.6	<0.001	<0.001	<0.001

Data are presented as n, n (%) or mean±SD. COPD: chronic obstructive pulmonary disease; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity. [#]: modified Medical Research Council (mMRC) score ⩾2; [¶]: based on high-resolution computed tomography (HRCT) findings.

sample had a two- to three-fold increase in chronic airflow obstruction, chronic bronchitis, emphysema and self-reported physician-diagnosed COPD. Similar findings regarding underestimation by the GLI and higher disease burden among subjects with normal K_{CO} by the GLI but not based on data from our population were also made for K_{CO}. Finally, we confirmed that correction for Hb does not appear to be necessary when defining the LLN for D_{LCO} in large population-based samples.

This is the first study analysing the suitability of the GLI D_{LCO} reference equations in an external population. However, there are several studies that have explored the suitability of the GLI reference equations for spirometry in Europe, including research from Sweden [9, 19], Norway [20] and Germany [21]. The GLI reference values for spirometry were considered to be suitable in elderly German women and in the Norwegian population [20, 21]. However, an underestimation of both FEV_1 and FVC has been reported by the Swedish OLIN studies [9]. The poorer fit for D_{LCO} values might be due to the fact that the reference populations used to generate the GLI equations did not include North Europeans, as opposed to the GLI spirometry reference values. However, this is not confirmed by the present data as it appears that GLI spirometry also underestimates values in the present population sample. Another reason might be that the number of subjects in the GLI database for the age range tested by SCAPIS is relatively small compared with the present population sample, but this does not appear to be supported by the analyses performed (when smaller sample sizes were used to define the LLN). In addition, SCAPIS had the advantage of employing a single type of device to measure D_{LCO}, while the GLI reference values were based on studies using four different devices. Although no significant differences could be identified with regard to device usage, this might have introduced a larger scattering in the reference values. As such, it is reasonable to believe that this effect could only partly explain the differences in the LLN. However, we noted that both the mean and the LLN of D_{LCO} are underestimated by the GLI, suggesting that there is an underestimation of D_{LCO} values for never smoking, middle-aged Swedish adults without respiratory symptoms. This might to some extent be explained by larger lung volumes in Swedes, as they are taller compared with Southern Europeans [22]. Finally, we could demonstrate that the individuals categorised as having normal D_{LCO} by the GLI, but abnormal D_{LCO} when compared with the SCAPIS data, are characterised by a higher burden of respiratory symptoms, emphysema and chronic airflow limitation than subjects with normal D_{LCO}. This supports the interpretation that the observed differences between the GLI and our population sample are clinically important. The group with abnormal D_{LCO} according to both

SCAPIS and the GLI had a further increase in respiratory burden, which is likely explained by a more severe impairment of diffusing capacity in this group.

Lower Hb levels lead to reduction in diffusing capacity and some laboratories therefore choose to measure Hb in clinical routine and correct the values for actual Hb. The current technical guidelines do not discuss the clinical importance of this [13]. Our findings are in line with those of STANOJEVIC *et al.* [8], who reported no significant effect of including corrected values for Hb on generating reference equations for D_{LCO}.

The main strength of our study is the large number of subjects included, making it one of the largest single studies with measurements of D_{LCO}. Another advantage of the study is that it includes subjects from six different Swedish regions and the results are thus considered generalisable to the Swedish population for the studied ages. The main limitation is the narrow age range (50–65 years). However, this is a relevant interval, as many subjects in this age range might present with breathlessness/respiratory symptoms and diffusing capacity is one of the measures included when extensive pulmonary function testing is indicated. Selection bias can be argued, as approximately one out of every two invited subjects chose not to participate. However, as we have focused on healthy individuals to generate reference values, non-participation probably had only a limited effect on the results. The diffusing capacity measurements are performed after bronchodilation, but this should not impact on diffusing capacity values, as highlighted by the recent guidelines [13] and studies in COPD [23]. Furthermore, the LLN has been defined in healthy individuals and, therefore, the potential effect of bronchodilation on D_{LCO} should be very small. We have based our definition of LLN in SCAPIS on never smoking subjects without respiratory disease or respiratory symptoms. SCAPIS is a population-based study and is expected to include mainly healthy subjects and subjects with only mild respiratory disease. When selecting the reference population for calculation of reference values and the LLN we tried, in so far as it was possible, to use the same criteria as those used in the GLI and included only never smokers without any respiratory symptoms or disease, as assessed based on the self-completed questionnaires. Finally, a limitation of the present study is that we focused solely on the findings related to D_{LCO}, while the clinical algorithms usually account for spirometry findings as well [6]. However, this was beyond the scope of our study.

In conclusion, the GLI LLN for D_{LCO} and for K_{CO} are lower than the estimated LLN in never smoking, middle-aged Swedish adults. Individuals with D_{LCO} >GLI LLN but <SCAPIS LLN had a higher prevalence of chronic airflow obstruction, chronic bronchitis and emphysema, suggesting an increased burden of respiratory disease. Similar findings were also reported with regard to K_{CO}. As our study analysed differences between reference values for D_{LCO} in a Swedish sample with a narrow age range, the findings may not be applicable in other countries and they should be replicated for other age ranges. However, we believe that our findings emphasise the clinical importance of adequate reference values and the importance of evaluating the GLI-based reference values in specific populations.

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