CO DIFFUSING CAPACITY IN THE HUMAN LUNG DEPENDENT ON ALVEOLAR VOLUME

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CO DIFFUSING CAPACITY IN THE HUMAN LUNG DEPENDENT ON ALVEOLAR VOLUME

CO DIFFUSIECAPACITEIT VAN DE LONGEN ALS FUNCTIE VAN HET ALVEOLAIRE VOLUME

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR

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Dedicated to
Anneke, my children
and my father
To the memory of my mother

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CHAPTER 1

INTRODUCTION

General aspects of gasexchange.

A main function of the lungs is to establish gas exchange between body tissues and the surrounding air. O_2 is taken up and CO_2 is eliminated.

This process of gas exchange can be subdivided into three stages:

1. *Ventilation,* which is the mechanism by which the alveolar gas is intermittently refreshed with ambient air. As a result O_2 concentration in the alveolar gas is kept high and CO_2 concentration low.
2. *Alveolar-capillary diffusion,* which is the passage of gases across the blood-gas barrier by passive diffusion.
3. *Perfusion,* which involves the distribution of blood in the lungs and the removal from the lungs by the blood circulation process.

The studies presented in this thesis are concerned with the characteristics of the alveolar to capillary diffusion, the second stage in the classification above. An aim was to develop, adapt and evaluate methods to study the characteristics of this diffusion process in patients.

A diffusion process in one medium by which molecules are transferred from a place with a high concentration to a place with a low concentration is governed by Fick's law:

$$\frac{\delta n}{\delta t} = -AK \frac{\delta C}{\delta d} \qquad (1.1)$$

where:
- n = number of mols
- t = time in s
- A = surface area in m^2
- K = diffusion coefficient in $m^2.s^{-1}$
- C = concentration in $mol.m^{-3}$
- d = distance in m

In the lungs diffusion occurs between a gas and a liquid phase. The concentration in a liquid is a function of the solubility of the gas in the liquid and the pressure of the gas, since the quantity of dissolved gas is proportional to the pressure (Henry's law):

$$C = \alpha P \tag{1.2}$$

where: α = Bunsens solubility coefficient in $mol.m^{-3}.kPa^{-1}$

P = pressure of the gas in kPa

Substitution of eq. 1.2 in eq. 1.1 gives:

$$\frac{\delta n}{\delta t} = -AK\alpha \frac{\delta P}{\delta d} \tag{1.3}$$

Because we do not know the pressure gradient at each distance in the gas-blood barrier the differential quotient $\delta P/\delta d$ has been replaced by the total pressure difference divided by the total diffusion distance d. Then, we rewrite eq. 1.3 to:

$$\frac{\delta n}{\delta t} = -AK\alpha \frac{(P_1-P_2)}{d} \tag{1.4}$$

where: P_1 = the gas tension at one side of the gas-blood barrier

P_2 = the gas tension at the other side.

In the lung the O_2 transport across the gas-blood barrier per unit of time, V_{O_2}', is:

$$V_{O_2}' = -\frac{AK\alpha}{d} (P_{AO_2}-P_{cO_2}) = -D_{LO_2} (P_{AO_2}-P_{cO_2}) \tag{1.5}$$

where: P_{AO_2} = alveolar P_{O_2} in kPa

P_{cO_2} = capillary P_{O_2} in kPa

D_{LO_2} = diffusing capacity for O_2

The diffusion coefficient K depends on the mobility of gas molucules, and therefore on the viscosity of the medium where diffusion occurs and on the size of the gas molecules. According to Graham's law the diffusion coefficient K for each gas at a specific temperature and in a specific medium is proportional to $1/\sqrt{}$(mol.mass). According to Forster [1] Graham's law approximates reality for respiratory gases dissolved in water. This means

that V_{O_2}' is proportional with the pressure difference across the alveolar-capillary membrane with a proportionality constant D_{LO_2}. D_{LO_2} is proportional with the surface area A and the reciprocal of the barrier thickness d. A proper gas exchange occurs at a large alveolar surface area and a thin gas-blood barrier. This large surface area is achieved by a large number of alveoli. A rough estimate of the number of alveoli has been made at about 300.10^6 [2, 3] and between 200 and 600.10^6 [4]. According to Weibel [5] and Weibel and Gomez [3] surface area is about 80 m^2 at 75% of the total lung capacity (TLC) and according to Gehr et al. [6] alveolar surface area is 143 m^2. In a normal male volunteer with a height of 1.89 m and a reference TLC of 8 liters 75% of TLC corresponds with a volume of 6 liters. If his lungs are modelled as two identical spheres of 3 liters each with a radius R (Volume = $4/3\pi R^3$) the corresponding surface area ($4\pi R^2$) is only 0.2 m^2. If the volume is subdivided in an increasing number of equal alveoli the surface area is increasing fast until at the final number of 300.10^6 alveoli the total surface area is about 107 m^2 (Fig. 1.1).

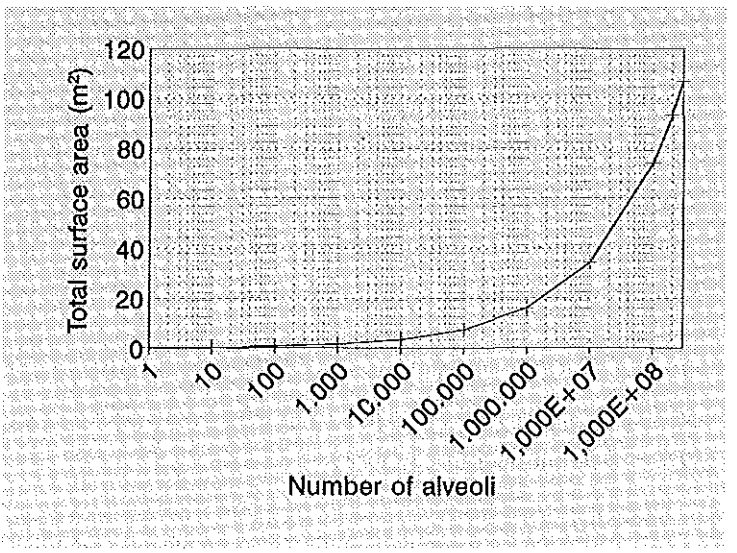

Fig. 1.1 *Lung surface area as function of number of alveoli for a lung volume of 6 liter.*

Around the alveoli there is a capillary network which contains 60 to 80 ml blood in contact with alveolar air [7]. This capillary network has such a density that nearly the

complete alveolar surface area is covered with capillaries. Mean barrier thickness is about 0.5 μm [8, 9], but inside the capillaries O_2 molecules have to diffuse for a mean distance of 2 μm through the plasma to the erythrocyte. This distance contributes to the "membrane" thickness.

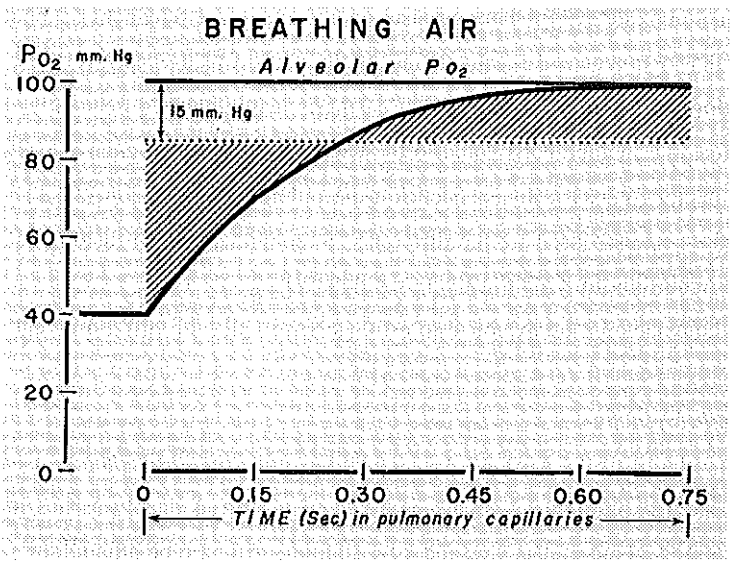

Fig. 1.2. Rate of oxygen transfer in the pulmonary capillaries (From [10], with permission).

To determine D_{LO_2} we need to measure P_{AO_2} and a mean value for P_{cO_2} during the blood passage in the capillary bed. P_{cO_2} of the blood is increasing and therefore, the difference in O_2 tension across the gas-blood barrier is decreasing. In Fig. 1.2 this increase in capillary P_{O_2} is plotted against the capillary passage time of the blood [10]. In normal subjects capillary P_{O_2} equals P_{AO_2} in about half of this capillary passage time, causing in the other part of the passage time a difference in O_2 tension of zero. If pressure equilibration occurs diffusion is not a limiting factor and V_{O_2}' will only depend on the perfusion rate. When mixed venous and end-capillary P_{O_2} are measured, the calculation of the D_{LO_2} cannot simply be based on the mean value of mixed venous and end-capillary P_{O_2}, because of the non-linear increase in P_{cO_2}. After calculating the change in capillary P_{O_2} according to Bohr's integration procedure, mean capillary P_{O_2} can be determined graphically by drawing the horizontal dotted line (Fig. 1.2) so that the shaded areas are

equal [10].

However, end-capillary P_{O_2} is difficult to measure due to venous admixture before the blood reaches the arterial system and mixed venous and arterial blood sampling is invasive. Therefore, Bohr [11] and Krogh [12] suggested to study the diffusing capacity with carbon monoxide (CO). This gas has an affinity for Hb which is about 230 times larger than that of oxygen. The calculation of the CO diffusing capacity D_{LCO} is based on the assumption that the CO tension in the plasma is negligible. Consequently, the pressure gradient across the membrane is equal to the CO tension in the alveolar gas (P_{ACO}) and the diffusing capacity for CO is independent on the pulmonary perfusion rate. The difference in D_{LO_2} and D_{LCO} is caused by the difference in solubility and diffusion coefficient K of both gases. According to Krogh [12] $D_{LO_2} = 1.23\ D_{LCO}$.

Several methods have been developed to estimate D_{LCO}, all with different conditions.

Single Breath Method

To determine the diffusing capacity in patients the *single breath method* is usually applied. After a maximal expiration the subject is asked to inspire as deeply as possible a volume of air containing about 0.3% CO and 5% Helium. After a breathholding period of 10 seconds the subject expires and an alveolar gas sample is collected. Alveolar fractions of CO and He are usually obtained from an alveolar gas sample of 750 ml after discarding 750 ml for washout of airways and apparatus deadspace. This technique was first described by Krogh [12]. It is based on the condition, that after inspiring a gas mixture containing CO, the CO fraction decreases exponentially with time during breathholding as CO diffuses into the blood. If the alveolar CO fraction, F_{ACO}, is known at the beginning and end of a time interval, it is possible to calculate the exponential decay constant k_{CO} of the relationship according to:

$$F_{ACO,\,t_1} = F_{ACO,\,t_0} \cdot e^{-k_{CO}\,(t_1 - t_0)} \tag{1.6}$$

where: t_0 = beginning time in s

t_1 = end time in s

$F_{ACO,\,t_1}$ = F_{ACO} at time t_1

$F_{ACO,\,t_0}$ = F_{ACO} at time t_0

During the first application by Krogh a subject inspired air with a small amount of CO as deeply as possible and expired immediately a small portion to determine the initial CO concentration. A second sample was collected at the end of a breathholding period to measure the final alveolar CO concentration. The period of breathholding was estimated from a spirogram, from which also the inspired volume was measured. The actual alveolar volume was estimated by adding the residual volume RV to the inspired volume. RV was separately determined by a multiple breath dilution technique.

Forster et al. [13] modified the technique by adding the inert gas He to the inspired gas mixture. They measured the He fraction both in the inspired gas and after 10 seconds of breathholding in the expired gas. Assuming He is insoluble in blood and tissue, they calculated alveolar volume V_A from the He dilution and the inspired volume V_I. In a mass balance the total volume of He in V_A is equal to the inspired amount of He, according to:

$$V_A\, F_{AHe} = F_{IHe}\, (V_I - V_D) \tag{1.7}$$

where:
- V_A = alveolar volume in liters BTPS
- F_{IHe} = He fraction in the inspiration gas
- F_{AHe} = alveolar He fraction at time t_1
- V_I = inspired volume in liters BTPS
- V_D = total dead space in liters BTPS

Usually V_I is equal to the vital capacity (VC), the maximum volume which can be inspired after a maximum expiration. If VC is inspired, maximum alveolar volume $V_{A,max}$ is calculated according to:

$$V_{A,\max} = \frac{F_{IHe}}{F_{AHe}}\, (VC - V_D) \tag{1.8}$$

Foster et al. [13] assumed that He and CO are diluted in a comparable way, which is still

generally accepted. Then, the initial fraction of CO can be calculated from the measured inspired CO fraction and the degree to which He is diluted by RV, according to:

$$\frac{F_{AHe}}{F_{IHe}} = \frac{F_{ACO_0}}{F_{ICO}} \tag{1.9}$$

where: F_{ICO} = F_{CO} of the inspired gas mixture

F_{ACO_0} = alveolar CO fraction at zero time

A further improvement was made by Jones and Meade [14], who demonstrated that the effective breathholding time was not equal to the time the subjects hold their breath at TLC. The effective breathholding time lasted from the time that 0.3 part of the vital capacity VC was inspired until the time that half of the alveolar sample was collected.
In equations 1.6 and 1.10 k_{CO} (s^{-1}) represents the ratio $D_{LCO}(P_B-P_{H2Osat})/K_{STPD}V_{A,max}$, where D_{LCO} is in $\mu mol.s^{-1}.kPa^{-1}$, P_B is the barometric pressure and P_{H2Osat} the saturated water vapour pressure in kPa at body temperature (usually 37 °C), $V_{A,max}$ is the alveolar volume at TLC level in liters BTPS, and K_{STPD} is the conversion factor for the conversion from liters BTPS to μmol.

$$\ln\left(\frac{F_{ACO,\,t_0}}{F_{ACO,\,t_1}}\right) = k_{CO}\,(t_1-t_0) = \frac{D_{LCO}\,(P_B-P_{H_2Osat})}{K_{STPD}\,V_{A,max}}\,(t_1-t_0) \tag{1.10}$$

Rearrangement of equation 1.10 gives:

$$D_{LCO} = V_{A,max}\,\frac{1}{(t_1-t_0)}\,\frac{K_{STPD}}{(P_B-P_{H_2Osat})}\,\ln\left(\frac{F_{ACO,\,t_0}}{F_{ACO,\,t_1}}\right) \tag{1.11}$$

Not every patient will be able to perform the single breath procedure to determine D_{LCO}, for several reasons. Either the patient cannot hold his or her breath at TLC for 10 seconds

or cannot deliver a VC of 1.5 l (0.75 l for deadspace washout and 0.75 l alveolar gas sample) for a proper analysis. For that reason multiple breath methods have been developed.

Multiple Breath Methods

In the *steady state technique* subjects breathe during a certain time from a gas container with a gas mixture with a low CO concentration. During the test period mixed expired CO is monitored until a steady state is reached. The diffusing capacity under steady state conditions is estimated from:

$$D_{LCO} = \frac{V_{CO}'}{P_{ACO}} \tag{1.12}$$

where V_{CO}' is the CO uptake, which is calculated from inspired and expired amount of CO and P_{ACO} is the alveolar CO tension. P_{ACO} fluctuates throughout the respiratory cycle and cannot be determined directly. Two methods have been described to estimate the mean P_{ACO}. Filley et al. [15] stated that mean P_{ACO} can be obtained by partitioning the concentration in the expired gas into components due to the alveolar and dead space ventilation. Assuming the dead space for CO_2 and CO are similar, P_{ACO} can be calculated according to:

$$P_{ACO} = P_{ICO} - \left(\frac{(P_{ICO} - P_{ECO})\ P_{ACO_2}}{P_{ECO_2}} \right) \tag{1.13}$$

where:

P_{ICO} = CO tension in the inspired gas

P_{ECO} = mixed expired CO tension

P_{ACO_2} = alveolar CO_2 tension

P_{ECO_2} = mixed expired CO_2 tension

Bates et al. [16] assumed end-tidal CO pressure, $P_{ET_{CO}}$, to be equal to mean P_{ACO}.

The results of this method will be influenced by the breathing pattern of the subject. The CO load is relatively large compared to other methods, because the patient is inspiring a constant and relatively large CO concentration for several minutes, until a steady state is reached. In the calculation of the CO diffusing capacity CO tension in the lung capillary bed is assumed to be zero. However, during such a procedure CO tension in mixed venous and lung capillary blood will gradually increase, causing a high CO tension (CO back tension) in the blood, which invalidate the computation.

A second method is the *rebreathing technique* introduced by Krühoffer [7]. The subjects hyperventilate for about 30 s from a bag containing an air mixture with a low CO concentration, characterized by a large tidal volume and a rate of about 30 breaths per minute. The gas in the lungs is well mixed with that in the rebreathing bag. An inert gas is added to measure lung volume and total volume of the system, i.e. volume of lungs and rebreathing bag. From the initial and final CO concentrations the D_{LCO} is calculated in a comparable way as in the single breath method. The results of this method are dependent on breathing pattern too. An advantage above the steady state method is that the CO load is smaller, because the inspiratory CO concentration decreases during the measurement.

The D_{LCO} values obtained with the various methods are not the same. A main reason is that with the single breath method D_{LCO} is estimated at TLC, whereas with the steady state method D_{LCO} is estimated at a changing lung volume between FRC and the sum of FRC and tidal volume. On average FRC and half tidal volume is taken. With the rebreathing method lung volume is on average equal to the sum of end-expired volume and half of the tidal volume, where end expiratory volume will be smaller and tidal volume larger than the corresponding volumes during the steady state method.

Outline of this thesis

As mentioned above, D_{LCO} is proportional to the area A of the blood gas barrier and the reciprocal of the barrier thickness. A voluntary decrease in lung volume will cause a difference in surface area A, but probably not a change in barrier thickness [3, 5, 9].

Patients with a restrictive lung disease have a smaller TLC than reference TLC. Severely ill patients are often not able to perform a single breath procedure at TLC. Therefore, we aimed to study the effect of alveolar volume on the D_{LCO} and D_{LCO}/V_A, estimated with the

single breath method. D_{LCO}/V_A has been described by Krogh [12] as a permeability constant K_{CO}, but D_{LCO}/V_A appeared to be dependent on V_A. The European Community for Coal and Steel report [17] warns: "The association between D_{LCO}/V_A and lung volume can lead to difficulty in interpretation, particularly during childhood and adolescence, in non-Caucasians and in patients in whom the total lung capacity is reduced". Based on our studies in chapters 2 and 3 we clarify and solve these problems. Because in children and adolescents anatomical changes in volume due to growth could interfere, we also studied the response of the diffusion variables to changes in alveolar volume in adults (chapter 2) and children (chapter 3) separately. Furthermore, we described the diagnostic consequences in patients who developed a restrictive lung disease due to chemotherapy (chapter 4).

In severely ill patients and small children the single breath technique can not always be applied. Patients, who are too ill to perform a single breath test, also will have problems with the usual rebreathing procedure during hyperventilation. Therefore, we developed a rebreathing technique in which patients breathe spontaneously at rest ventilation as an alternative method. We tried to explain the different results of single breath and rebreathing technique by differences in lung volume and we compared the results of both techniques in patients with as well as without ventilation distribution unequality. This study is described in chapter 5.

Diffusion of CO will occur only where alveoli are in contact with functioning capillaries filled with erythrocytes. D_{LCO} is independent of pulmonary perfusion, but D_{LCO} depends on the effective capillary blood volume.

In healthy volunteers the lungs appear to be unequally perfused in the sitting position due to hydrostatic pressure differences. In the supine position the lungs are more equally perfused, because the hydrostatic pressure differences are smaller. The effects of a change in body position from sitting to supine on the effective capillary blood volume (Q_c), the membrane conductance (D_m) and D_{LCO}/V_A vs V_A relationships are also presented in Chapter 6.

Roughton and Forster [8] described a model in which D_{LCO} was separated in a diffusing capacity of the alveolar-capillary membrane D_m and the capacity of the blood present in the alveolar capillaries to bind the gas molecules chemically (θQ_c).

$$\frac{1}{D_{LCO}} = \frac{1}{D_m} + \frac{1}{\theta Q_c\,[Hb]} \qquad (1.14)$$

where: Q_c = effective capillary blood volume in ml.

$[Hb]$ = hemoglobin concentration as fraction of normal.

θ = constant for the rate of uptake of CO by the erythrocytes in one ml normal blood in $\mu mol.s^{-1}.kPa^{-1}.ml^{-1}$.

D_{LCO} varies with the O_2 tension, because the reaction rate θ for the reaction between CO and Hb is dependent on this O_2 tension. The estimation of D_m and Q_c is based on D_{LCO} measurements at two different O_2 levels. They found $1/D_m$ and $1/Q_c$ graphically from the intercept with the ordinate and the slope of the linear relationship between $1/D_{LCO}$ and $1/\theta$ respectively (Fig. 1.3).

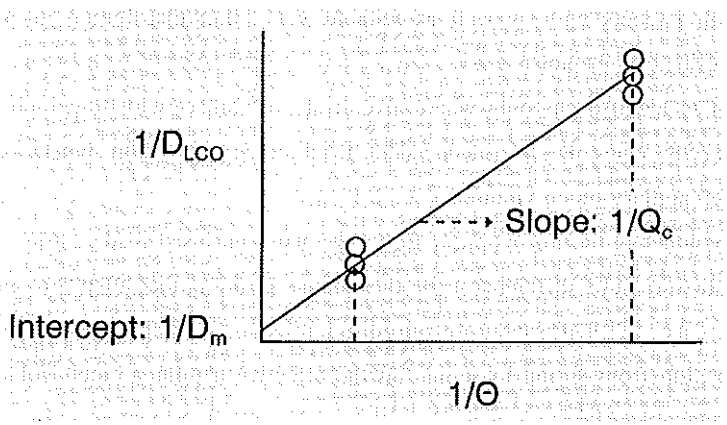

Fig. 1.3. Graphical method to separate D_{LCO} in D_m and Q_c.

The accuracy of the estimation of D_m is limited, because $1/D_m$ is about zero. To improve this accuracy and to study the relationships D_m vs V_A and Q_c vs V_A, we estimated the relationship between D_{LCO}/V_A and V_A at two different oxygen tensions and analysed the relationship between D_m and V_A and between Q_c and V_A [9].

REFERENCES

1. Forster RE. Exchange of gases between alveolar air and pulmonary capillary blood: pulmonary diffusing capacity. *Physiol. Rev.* 1957; 37: 391-452.
2. Dunnill MS. Postnatal growth of the lung. *Thorax* 1962; 17: 329-333.
3. Weibel ER and Gomez DM. Architecture of the human lung. *Science* 1962; 137: 577-585.
4. Angus GE and Thurlbeck WM. Number of alveoli in the human lung. *J. Appl. Physiol.* 1972; 32: 483-485.
5. Weibel ER. Morphometry of the human lung. Springer, Berlin-Göttingen-Heidelberg, 151pp.
6. Gehr P, Bachofen M and Weibel ER. The normal lung: Ultrastructure and morphometric estimation of diffusion capacity. *Respir. Physiol.* 1978; 32: 121-140.
7. Krühoffer P. Lung diffusion coefficient for carbon monoxide in normal human subjects by means of $C^{14}O$. *Acta Physiol. Scand.* 1954; 32: 106-123.
8. Roughton FJ and Forster RE. Relative importance of diffusion and chemical reaction rates in determining the rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volumes of blood in the lung capillaries. *J. Appl. Physiol.* 1957; 11: 290-302.
9. Stam H, Versprille A and Bogaard JM. The components of the carbon monoxide diffusing capacity in man dependent on alveolar volume. *Bull. Eur. Physiopathol. Respir.* 1983; 19: 17-22.
10. Comroe JH, Forster RE, Dubois AB, Briscoe WA and Carlsen E. The Lung. Clinical physiology and pulmonary function tests. Second Edition 1973. Year Book Medical Publishers, Inc. Chicago, U.S.A.
11. Bohr C. Über die spezifische Tätigkeit der Lungen bei der respiratorischen Gasaufnahme. *Skand. Arch. Physiol.* 1909; 22: 221.
12. Krogh M. The diffusion of gases through the lungs of man. *J. Physiol.* 1915; 49: 271-300.

13. Forster RE, Fowler WS, Bates DV and Van Lingen B. The absorption of carbon monoxide by the lungs during breath-holding. *J. Clin. Invest.* 1954 b; 33: 1135-1145.

14. Jones RS and Meade F. A theoretical and experimental analysis of anomalies on the estimation of pulmonary diffusing capacity by the single breath method. *Quart. J. Exp. Physiol.* 1961; 46: 131-143.

15. Filley CF, McIntosh DJ, and Weight GW. Carbon monoxide uptake and pulmonary diffusing capacity in normal subjects at rest and during exercise. J. Clin. Invest. 1954; 33: 530-539.

16. Bates DV, Boucot NG and Dormer AF. Pulmonary diffusing capacity in normal subjects. *J. Physiol. Lond.* 1955; 129: 237-252.

17. Cotes JE, Chinn DJ, Quanjer PhH, Roca J and Yernault JC. Standardization of the measurement of transfer factor (diffusing capacity). Report working party standardization of lung function tests European Community for Steel and Coal. *Eur. Respir. J.* 1993; 6 Suppl. 16: 41-52.

CHAPTER 2

DIFFUSING CAPACITY DEPENDENT ON LUNG VOLUME AND AGE IN NORMAL SUBJECTS

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The diffusing capacity of carbon monoxide (D_{LCO}) and its value normalized to alveolar volume (D_{LCO}/V_A) are usually estimated at total lung capacity (TLC) [for references see 1].
In normal subjects D_{LCO} decreases and D_{LCO}/V_A increases when V_A is decreased [1-10]. Consequently, D_{LCO} is lower and D_{LCO}/V_A is higher at lower lung volumes, compared with reference values estimated at reference TLC.
In patients with a restrictive disease, due to intra or extra parenchymal diseases, diffusing capacity is determined at a lung volume lower than their disease-free TLC. In order to compare the diffusion indices of such a patient with the reference values at the same lower lung volume, we aimed to determine reference values at lung volumes lower than TLC in non-smoking normal subjects of both gender.
Mangado et al. [11] described a conversion of D_{LCO} for reduced V_A based on a relationship between the membrane conductance D_m and V_A according to $D_m = kV_A^{2/3}$, whereas capillary blood volume (Q_c) was assumed to be constant. In a previous study we found this D_m vs V_A relationship appropriate in only 64% of a group of normal volunteers in sitting position, whereas Q_c varied with V_A according to a second order polynomial [1]. In the majority of subjects older than 40 years of age the maximum Q_c was at TLC or close to TLC, whereas, in younger volunteers, a plateau in Q_c was found between TLC and 60% of TLC. Thus, Mangado's assumption of constant Q_c was acceptable in the younger group, but not in the older subjects.
We determined reference values of D_{LCO} and D_{LCO}/V_A at TLC and at lower lung volumes in the sitting position, related these values to age for both sexes, and derived two mathematical methods for calculation of the reference values and their standard deviations.

SUBJECTS AND METHODS

Subjects

In 55 healthy non-smokers reference values of D_{LCO} and D_{LCO}/V_A at TLC and at lung volumes below TLC were determined after informed consent. The subjects were recruited from Rotterdam and its suburbs, an industrial area in the Netherlands. All were healthy Caucasians with no history of chronic pulmonary or cardiac disease or previous thoracic surgery, and no history of acute respiratory symptoms in the three weeks prior to the investigation.

We selected normal subjects within a weight range of about 20 % above and below ideal body weight. To estimate ideal body weight we used the modified Metropolitan Life Insurance Company charts [12]. No subjects had contact with harmful chemicals, which may influence lung function. The group consisted of 28 males and 27 females. All had normal lung volumes in % of the reference values of the European Community for Coal and Steel (ECCS) [13], (mean ± SD): TLC=105 ± 10 %; VC=113 ± 14 % and FEV_1=103 ± 12 %. Their ages ranged from 20 to 85 years. In a subgroup of 16 male volunteers, 20-69 years old, we tested whether D_{LCO} and D_{LCO}/V_A were dependent on the normal variability in the Hb concentration.

Procedure

In a series of single breath maneuvers D_{LCO} and D_{LCO}/V_A were determined in the sitting position at various levels of alveolar volume. We followed the single breath procedure as recommended by the ECCS [13]. The subjects expired to residual volume (RV) and holding their breath after inspiration of volumes ranging from 1.5 l up to vital capacity (VC) in random order. The start of breathholding was taken when 30% of the volume had been inspired, and the end when half of the expired sample had been collected [14]. Overall breath holding time slightly exceeded 10 s. Inspirations and expirations were performed rapidly. Alveolar fractions of CO and He were obtained from expired gas after discarding 800 ml for washout of airways and apparatus dead space. The size of the alveolar sample was 800 ml. Maneuvers were performed with a "Masterlab Transfer" manufactured by Jaeger (Würzburg, Germany). The interval between consecutive measurements was 5 min. To minimize CO back tension, we restricted the number of consecutive measurements to six. Back tension was ignored in these non-smokers, because it was less than 1% of the alveolar CO tension at the start of breath holding. We used a heat conductivity type He analyzer, which is sensitive to CO_2. Therefore, we absorbed CO_2 prior to both He and CO analysis. The remaining gas concentrations were corrected for an absorbed amount of 5% CO_2 [15]. D_{LCO} and D_{LCO}/V_A were expressed in $\mu mol.s^{-1}.kPa^{-1}$ and $\mu mol.s^{-1}.kPa^{-1}.l^{-1}$ respectively and the correction for the normal variability in Hb concentration was performed according to the procedure described by Cotes and recommended by the ECCS [13, 16]. Reference values of Hb concentration in men and women are 9.2 ± 0.5 (SD) $mmol.l^{-1}$ (n=120) and 8.3 ± 0.5 (SD) $mmol.l^{-1}$ (n=120) respectively, as determined in a group of volunteers from the same

demographic background in the Laboratory for Clinical Chemistry in our hospital.

Data analysis and Statistics

We analysed our results according to two methods.

Firstly, a "*Random coefficients linear (RCL) model*" for all subjects was fitted assuming linear D_{LCO}/V_A vs V_A relationships within individuals. This model is a direct generalisation of the model described by Feldman [17]. The parameters were estimated by the restricted maximum likelihood method using module 5V of the BMDP package [18]. This method uses the least squares slope and intercept to refine its previous estimates of variance components, recalculates the weights, re-estimates the slope and intercept, and so forth. Secondly, we determined a conversion of the conventional reference values at TLC based on the parameters of all linear regression equations through the individual data, obtained by the least squares method, which we will call the *conversion method*. Differences between two groups of data were regarded as significant at P-value < 0.05.

RESULTS

Reference values at TLC

D_{LCO} and D_{LCO}/V_A values were determined at TLC level as a function of age (A, in years) in men and women respectively (n=55).

Males between 20 and 85 years:

$D_{LCO} = 251 - 1.4A$ r=-0.83 RSD = 15 (2.1)

Females between 20 and 77 years:

$D_{LCO} = 177 - 0.8A$ r=-0.56 RSD = 19 (2.2)

Males and females between 20 and 85 years:

$D_{LCO}/V_A = 29.1 - 0.10A$ r=-0.54 RSD = 2.5 (2.3)

where RSD is the residual standard deviation.

The relationships of D_{LCO}/V_A vs A were not significantly different between the male and female subjects; the P-value varied between 0.23 and 0.97 for the measured values of both sexes in five ten-year age groups.

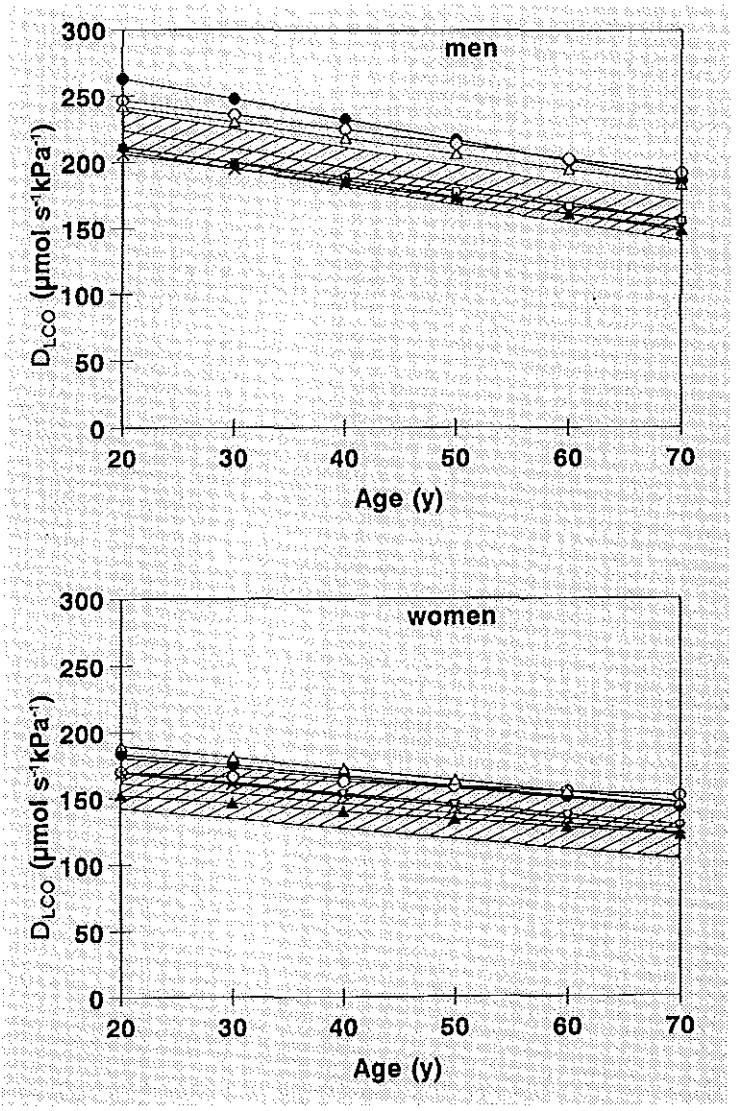

Fig. 2.1 *Comparison of reference values for D_{LCO} from our study with reference values published in the literature in men and women respectively. For the regression equations in which height is a parameter a mean height of 180 cm and 170 cm is used for males and females respectively [13, 16, 19-22].*

x	*Cotes*	*[16]*
△	*Crapo et al.*	*[19]*
□	*ECCS, Quanjer*	*[13]*
●	*Knudson et al.*	*[20]*
▲	*Miller et al.*	*[21]*
○	*Paoletti et al.*	*[22]*
	This paper	± *1 RSD*

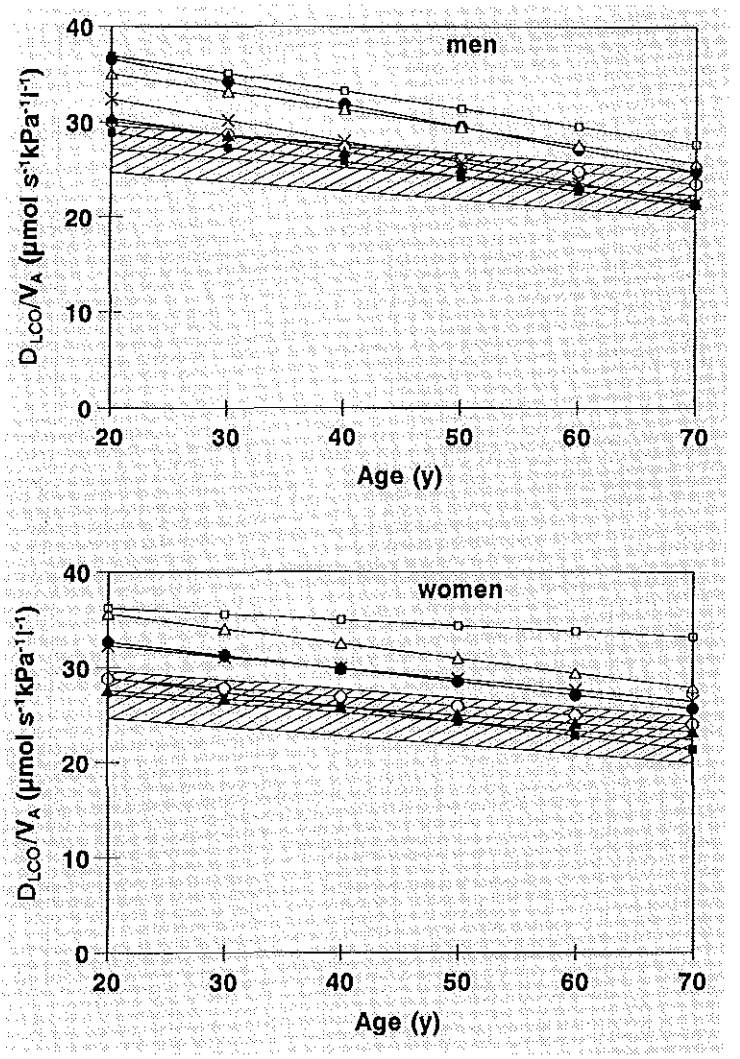

Fig. 2.2 *Comparison of reference values for D_{LCO}/V_A from our study with reference values published in the literature in men and women respectively. For the regression equations in which height is a parameter a mean height of 180 cm and 170 cm is used for males and females respectively [20-22].*

x	*Cotes*	*[16]*
△	*Crapo et al.*	*[19]*
□	*ECCS, Quanjer*	*[13]*
●	*Knudson et al.*	*[20]*
■	*Love et al.*	*[23]*
▲	*Miller et al.*	*[21]*
○	*Paoletti et al.*	*[22]*
	This paper	*± 1 RSD*

In Fig. 2.1 the regression lines of D_{LCO} vs A for both men and women are given as well as those of Cotes [16], Crapo [19], ECCS [13], Knudson [20], Miller [21] and Paoletti [22]. In Fig. 2.2 the same is done for the regression lines of D_{LCO}/V_A vs A. Moreover, the references proposed by Love and Seaton [23] are added.

Hb correction

In a subgroup of 16 male subjects Hb concentration was on average 9.5 (SD=0.6) mmol.l^{-1}. The regression equations $D_{LCO}/V_A = 29.6 - 0.11$ A (r=-0.56) and $D_{LCO}/V_A = 30.5 - 0.12$ A (r=-0.61) for the Hb corrected and uncorrected measurements respectively, are not significantly different. Hb correction did not decrease the standard deviation.
The correction for the normal variability in the Hb concentration resulted in a mean change of 1.0 $\pm$ 2.0(SD) % of the uncorrected values of D_{LCO} and D_{LCO}/V_A respectively.

Reference values at TLC and V_A levels below TLC with the RCL model

In all volunteers D_{LCO}/V_A increased linearly with decreasing V_A. Three typical examples of males of 20, 58 and 85 years of age are presented in Fig. 2.3. D_{LCO} decreased with decreasing V_A.
The parameters a and b of the relationship $D_{LCO}/V_A = a - bV_A$ varied substantially between volunteers. In both parameters a decreasing trend with increasing age was present. Therefore, we postulated the following RCL model:

$$D_{LCO}/V_A = c + d\,A - (e + f\,A)V_A$$

In this model the intercept ($c + d$ A) and the slope ($e + f$ A) depend in a linear fashion on age. The parameters c and e were assumed to be random, i.e. each individual is allowed to have his or her own intercept and slope, whereas d and f were considered fixed, i.e. systematic dependence of intercept and slope on age. This model was fitted to the data of all subjects with a coefficient related to sex. As this sex related coefficient was significantly different from zero (P-value $<$ 0.001), we performed the analysis for men and women separately.

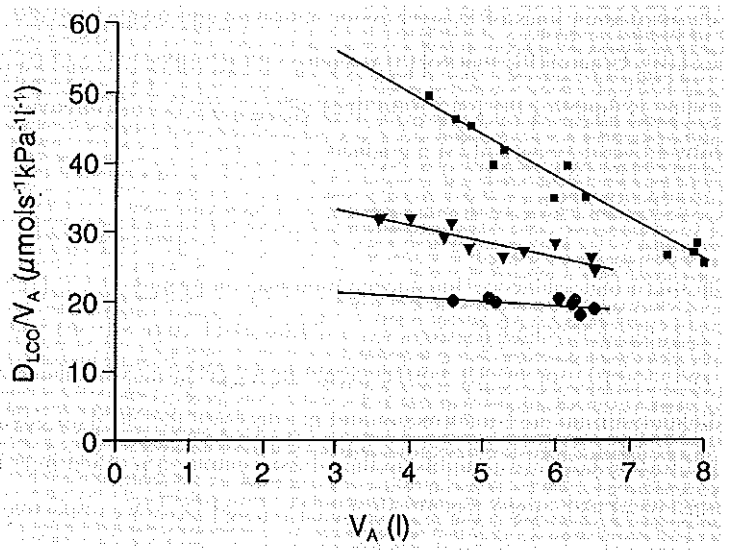

Fig. 2.3. *D_{LCO}/V_A vs. V_A relationship in 3 male subjects from Table 2.1.*
- ■ *Subject 5, 20 yr. old*
- ▼ *Subject 26, 58 yr. old*
- ● *Subject 25, 85 yr. old*

The resulting regression equations were as follows:

Males: $D_{LCO}/V_A = 76.5 - 0.62\ A - (6.0 - 0.07\ A)V_A$ (2.4)

with RSD $= \sqrt{(\ 0.55V_A^2 - 8.69V_A + 37.82)}$

Females: $D_{LCO}/V_A = 67.7 - 0.52\ A - (6.9 - 0.08\ A)V_A$ (2.5)

with RSD $= \sqrt{(\ 0.59V_A^2 - 6.66V_A + 27.83)}$

where V_A is in liters and A in years.

The regression coefficients of age (*d* and *f*) turned out to be significantly different from zero (P-value < 0.001), showing that both intercept and slope decrease with increasing age. In Fig. 2.4 we have illustrated the dependence of D_{LCO}/V_A on A and V_A by using equation 2.4.

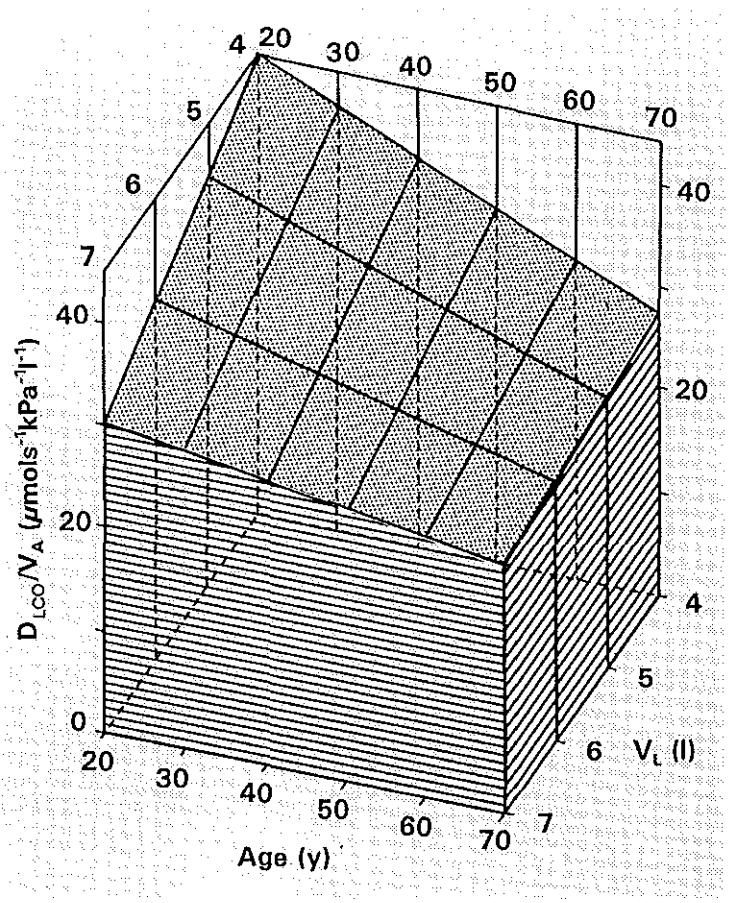

Fig. 2.4. Three dimensional representation of reference values of D_{LCO}/V_A for males, obtained from the RCL-method, as a function of age in years and lung volume (V_L) in liters.

To compare the predictions of D_{LCO}/V_A at TLC from eqs. 2.4 and 2.5 with the measured values at TLC we plotted the differences between predicted and measured values as a percentage of the measured values of all 55 individuals in Tables 2.1 and 2.2 (difference 3.9 ± 10 %, mean ± SD) as a function of TLC (Fig. 2.5).

The predicted values are significantly higher than the measured values (P-value=0.006), the slope of the regression line through the differences between predicted and measured values was not significantly different from zero, P-value=0.08.

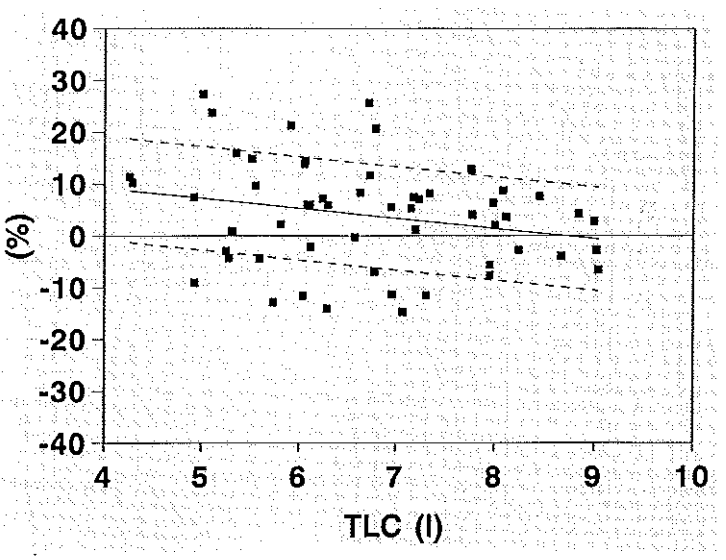

Fig. 2.5. *Differences between RCL reference values and measured D_{LCO}/V_A values at total lung capacity (TLC) level as percentage of measured D_{LCO}/V_A values at TLC are plotted as a function of TLC for subjects in Tables 2.1. and 2.2. Solid horizontal line, zero line, which implies no difference between prediction and estimate; solid line with negative slope, regression line of differences vs. TLC; dashed lines, ± 1 RSD.*

Reference values at V_A levels below TLC with the Conversion method

The conversion method is based on the slopes of all D_{LCO}/V_A vs V_A relationships, where V_A is expressed as a fraction of the predicted TLC (Tables 2.1 and 2.2). This conversion is illustrated in Fig. 2.6.

D_{LCO}/V_A decreases linearly with increasing V_A with a slope *b*, which decreases with age according to:

Males: $b = -0.50\ A + 46.1$ r=-0.78 RSD = 6 (2.6)

Females: $b = -0.41\ A + 38.0$ r=-0.74 RSD = 6 (2.7)

Differences in *b* were only significant in the over-50 age groups.

Table 2.1 Anthropomorphic data and regression equations for D_{LCO}/V_A ($\mu mol.s^{-1}.kPa^{-1}.l^{-1}$) as a function of V_A (fraction of reference TLC (ECCS [13]) of the male normal subjects.

Subj. No.	Age (yr)	Height (cm)	Weight (kg)	TLC (l)	TLC_{ref} (l)	n	$D_{LCO}/V_A = a - bV_A$		
							b	a	r
1	43	183	83	8.06	7.54	12	21.61	45.19	-0.92
2	41	189	82	8.67	8.02	14	19.50	45.33	-0.94
3	24	178	78	7.17	7.14	12	20.03	47.63	-0.94
4	24	180	63	7.30	7.30	11	35.95	66.78	-0.97
5	20	192	85	8.23	8.26	12	47.59	73.11	-0.98
6	56	175	62	6.94	6.90	11	31.38	56.92	-0.96
7	48	181	86	7.76	7.38	12	19.29	44.99	-0.98
8	23	190	75	9.01	8.10	12	23.78	48.90	-0.96
9	38	178	75	7.99	7.14	12	30.26	57.79	-0.94
10	52	178	80	7.22	7.14	6	31.41	56.60	-0.98
11	55	170	87	6.11	6.50	12	14.27	41.60	-0.80
12	54	193	92	8.44	8.34	6	14.10	36.00	-0.96
13	41	186	91	8.83	7.78	12	27.80	51.56	-0.93
14	58	177	84	8.10	7.06	6	13.91	39.56	-0.90
15	32	173	78	6.62	6.74	12	34.13	62.19	-0.98
16	69	172	82	6.06	6.66	12	10.53	31.91	-0.64
17	23	187	70	7.94	7.86	14	42.43	69.84	-0.95
18	49	175	80	7.14	6.90	12	24.37	49.98	-0.93
19	29	180	73	7.94	7.30	12	33.22	60.79	-0.97
20	34	183	84	7.75	7.54	11	23.80	48.30	-0.97
21	52	187	92	9.03	7.86	12	17.75	42.62	-0.92
22	56	180	90	6.78	7.30	12	21.30	42.83	-0.93
23	59	183	92	8.99	7.54	12	11.10	35.63	-0.95
24	31	188	80	7.97	7.94	12	34.11	58.13	-0.95
25	85	181	81	6.72	7.38	8	4.58	23.39	-0.54
26	58	173	70	6.73	6.74	12	15.06	39.99	-0.91
27	56	182	88	6.29	7.46	5	18.79	42.92	-0.94
28	51	180	80	7.33	7.30	12	18.03	42.88	-0.96

a and b are constants, n is the number of observations and r is the correlation coefficient.

Table 2.2 Anthropomorphic data and regression equations for D_{LCO}/V_A ($\mu mol.s^{-1}.kPa^{-1}.l^{-1}$) as a function of V_A (fraction of reference TLC (ECCS [13]) of the female normal subjects.

Subj. No.	Age (yr)	Height (cm)	Weight (kg)	TLC (l)	TLC_{ref} (l)	n	$D_{LCO}/V_A = a - bV_A$		
							b	a	r
29	36	178	62	7.07	5.96	12	20.67	48.27	-0.92
30	28	176	80	4.91	5.83	12	41.86	62.85	-0.96
31	44	164	48	5.58	5.03	12	26.45	54.13	-0.94
32	71	172	63	5.73	5.56	6	13.25	41.35	-0.62
33	30	180	75	4.92	6.09	12	37.19	63.42	-0.74
34	26	178	65	6.77	5.96	12	29.50	55.48	-0.95
35	56	157	56	5.27	4.57	12	9.87	38.49	-0.80
36	59	166	53	5.54	5.17	12	17.17	41.23	-0.92
37	58	161	68	5.10	4.84	6	12.47	34.31	-0.99
38	27	178	60	6.04	5.96	12	30.21	57.91	-0.94
39	47	171	63	6.57	5.50	12	11.50	37.27	-0.92
40	31	171	56	6.08	5.50	6	22.47	47.97	-0.98
41	21	178	70	5.80	5.96	12	26.75	51.57	-0.89
42	37	168	61	6.05	5.30	12	19.38	43.12	-0.95
43	69	171	78	5.01	5.50	12	5.85	26.03	-0.51
44	60	166	74	5.35	5.17	12	7.37	29.71	-0.86
45	42	159	54	5.50	4.70	11	14.89	40.42	-0.94
46	41	172	63	6.10	5.56	12	13.84	37.85	-0.81
47	69	157	66	5.24	4.57	8	8.14	34.65	-0.87
48	76	159	59	4.25	4.70	8	19.44	39.77	-0.94
49	43	167	57	5.90	5.23	11	21.21	45.13	-0.89
50	24	158	52	4.28	4.64	12	29.92	57.56	-0.94
51	60	176	71	6.23	5.83	6	9.92	32.01	-0.91
52	57	176	64	7.19	5.83	12	19.79	44.25	-0.94
53	22	172	63	6.28	5.56	11	26.89	58.24	-0.98
54	53	168	70	6.95	5.30	12	13.67	42.24	-0.94
55	50	168	66	5.30	5.30	11	11.28	37.89	-0.92

a and b are constants, n is the number of observations and r is the correlation coefficient.

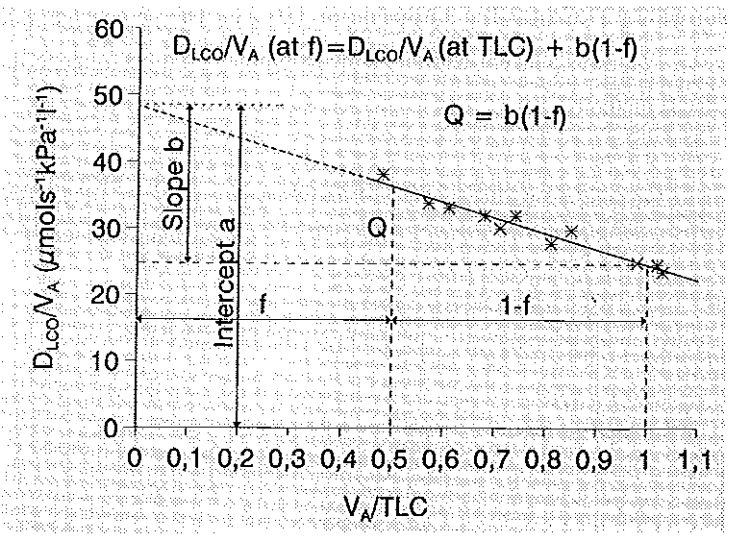

Fig. 2.6. Linear relationship between D_{LCO}/V_A and V_A/TLC (TLC from ECCS reference value [13]) in subject 20. Conversion factor Q=b(1-f), where f is V_A fraction of reference TLC at which breath-holding procedure was carried out and b is slope of linear relationship between D_{LCO}/V_A and V_A found from eq. 2.6.

The reference value of D_{LCO}/V_A at a lower V_A level than TLC will be equal to the sum of the reference value of D_{LCO}/V_A at TLC and a conversion factor b(1-f) according to:

$$D_{LCO}/V_A(\text{at f}) = D_{LCO}/V_A(\text{at TLC}) + b(1\text{-f}) \qquad (2.8)$$

where f is V_A as a fraction of reference TLC. The D_{LCO}/V_A values at TLC were obtained from equation 2.3.

The residual standard deviation after volume conversion will be:

$$RSD = \sqrt{(RSD(D_{LCO}/V_A \text{ at TLC})^2 + RSD(b)^2)} \qquad (2.9)$$

With equations (2.3), (2.8) and (2.6) or (2.7) a three-dimensional diagram could be made corresponding to that of Fig. 2.4.

To assess whether the conversion method is satisfactory for determining reference values of D_{LCO}/V_A at V_A levels below TLC we compared predicted values of the conversion method with those of the RCL method at three different volumes and ages. We not only converted the D_{LCO}/V_A references at TLC of eq. 2.3, but also those of Miller et al. [21], Paoletti et al. [22] and Cotes [16] (Table 2.3). D_{LCO}/V_A obtained from the conversion method was expressed as a percentage of the D_{LCO}/V_A from the RCL method. These calculations were carried

out for a man with a TLC of 7 l and a height of 180 cm. The percentage values indicate that the D_{LCO}/V_A values calculated with the conversion method are similar to those calculated with the RCL method.

Table 2.3 *D_{LCO}/V_A reference values at lower lung volumes are calculated with the conversion method from D_{LCO}/V_A reference values at TLC (eq. 2.3, Miller et al. [21], Paoletti et al. [22] and Cotes [16]).*

Age	Fraction	D_{LCO}/V_A (Conversion method) as a % of D_{LCO}/V_A (RCL)			
(y)	of TLC	Eq.3	Miller	Paoletti	Cotes
20	1.	85	95	94	101
	0.8	90	98	97	103
	0.6	93	100	99	104
45	1.	86	91	94	94
	0.8	90	94	97	97
	0.6	93	97	99	99
70	1.	87	86	93	85
	0.8	90	89	96	88
	0.6	93	92	98	91

D_{LCO}/V_A obtained with the conversion method is expressed as a percentage of the D_{LCO}/V_A from the RCL method (%RCL) at different ages and alveolar volume fractions for male normal subjects with a TLC=7 l and a height of 180 cm.

DISCUSSION

Potential errors

In a previous study [10] we reported that the diffusion variables estimated at various alveolar volumes were not influenced by unequal ventilation. This is in agreement with the fin-

dings of other studies where nonsequential emptying of the lungs is described [14, 24]. Lebecque reported a positive effect of a preceding sigh on D_{LCO}/V_A [25]. Cotton et al. [4] reported that D_{LCO}/V_A was larger after a deep breath than after tidal breathing at all lung volumes. After a deep breath, D_{LCO}/V_A increased by about 5% at 100% of inspiratory capacity (IC) and by about 17% at 25% of IC. We found a maximum increase of 5% after an inflation-deflation maneuver. In 5 min, 62% of this increase disappeared [10]. Cotton et al. [4] carried out the single breath maneuvers immediately after a deep breath or after 10 min. of tidal breathing. They defined tidal breathing as no breaths exceeding 50% of IC for 10 min. In the present study we instructed the volunteers to sit quietly at rest and not to talk or sigh between the experiments and we verified the absence of sighs visually. If the volunteer was nevertheless sighing, we waited at least 5 min before the next diffusion measurement was started. Therefore, we regard the occurrence of sighs as having a negligible influence upon our data.

We also checked whether our data could have been affected by high intrapulmonary pressures during the breath holding periods. At a mouth pressure of 2.5 kPa above atmospheric pressure, D_{LCO}/V_A was decreased by not more than 4% at TLC [10]. We concluded that our results obtained over a wide range of V_A were not significantly influenced by possible differences in alveolar pressure.

Hb correction

A correction for normal variability in Hb concentration did not change the spread of D_{LCO} and D_{LCO}/V_A values. In our volunteers, Hb concentration was normally distributed, which resulted in an equal amount of positive and negative corrections of the diffusing capacity. Therefore, the regression equations of D_{LCO} and D_{LCO}/V_A vs age were not significantly changed. Also the standard deviations were not changed, which can be explained by the lower variation coefficient in the Hb concentration of about 5 %, with respect to the variation coefficients of the diffusion indexes, which are more than 10 %. We concluded that our reference values for D_{LCO} and D_{LCO}/V_A, as well as those in literature obtained from normal volunteers whose Hb concentrations were not determined, can be reliably used for assessing patients' diffusion indexes. This does not mean that a hemoglobin correction is not necessary in patients. By comparing the diffusing capacity both with and without a hemoglobin correction, a real disturbance in diffusing capacity can be distinguished from the effect caused

by anemia or polycythemia.

Reference values at TLC

The reference values for both D_{LCO} and D_{LCO}/V_A correlate well with age between 20 and 85 years in men as well as in women.

Our references of D_{LCO} at TLC in women (eq. 2.2) are comparable to those of Cotes [16], Crapo et al. [19], Knudson et al. [20], Miller et al. [21], Paoletti et al. [22] and ECCS [13]. In male subjects our references (eq. 2.1) are comparable to those of Cotes, Miller et al. and ECCS. The references of the other authors exceed our references by more than one residual standard deviation.

No gender difference was found in the D_{LCO}/V_A, which is in agreement with the results of Burrows et al. [26] and Bradley et al. [27]. The reference values for D_{LCO}/V_A (eq. 2.3) correspond best with those published by Miller [21], Paoletti [22] and Love and Seaton [23]. The reference values published by Cotes [16] are significantly higher in the female subjects, but in males they are only significantly higher at younger ages. The ECCS [13], Crapo [19] and Knudson [20] published significantly higher D_{LCO}/V_A reference values. Love and Seaton [23] noticed that the ECCS reference values for D_{LCO}/V_A are at a much higher level than the reference values of Cotes [16]. Better results were obtained when they divided the ECCS reference values for D_{LCO} by the reference values of TLC. These values are less than 1 RSD different from our reference values.

Reference values at V_A levels below TLC

The dependence of D_{LCO} and D_{LCO}/V_A on V_A is extensively reported in the literature [1-5, 7-10]. Besides a linear relationship of D_{LCO}/V_A vs V_A, also hyperbolic and biphasic relationships were reported [2, 7, 8]. In a previous study [10] we observed that in only 21% of the subjects a hyperbolic or biphasic fit provided a negligibly small improvement of the correlation coefficient with respect to the linear fit. Therefore, further calculations were based on the linear relations, which we continued in this study, because in all subjects D_{LCO}/V_A decreased with V_A and yielded a linear relationship as the best mathematical description (Tables 2.1 and 2.2).

As mentioned in the introduction, we considered the method of Mangado et al. [11] to be unsatisfactory for calculating a reference of D_{LCO} and D_{LCO}/V_A at lower lung volume from

the corresponding references at TLC. A single correction factor as proposed by Mangado et al. cannot be sufficiently accurate, because our results showed that the D_{LCO}/V_A vs V_A relationship also depends on age (Figs. 2.3 and 2.4).

The ageing effect on the D_{LCO}/V_A vs V_A relationship could not be attributed to a difference in height between older and younger volunteers, because the height of male and female subjects between 20 and 30 years was not significantly different from that of the subjects aged over 60 (P-value=0.15 and 0.12 respectively).

We think the decrease in slope of the D_{LCO}/V_A vs V_A relationship with increasing age should be attributed to other mechanisms. Previously [1] we observed that the effective capillary blood volume (Q_c) vs V_A relationship can be described by a second order polynomial. In the younger subjects we found a more or less flat maximum between TLC and 60 % of TLC. Therefore, Q_c will be approximately stable in this alveolar volume range in the younger subjects. Furthermore, we found physiological indications for isotropic volume change with constant barrier thickness [1], implying an increase in diffusion area relative to alveolar volume when volume decreases. This is in agreement with morphometric data of Weibel et al. [28]. Therefore, we concluded that in the younger subjects this positive effect of decreasing V_A on D_{LCO}/V_A coincides with an approximately constant effect of Q_c on diffusion. In the elderly the maximum of the Q_c vs V_A relationship has been shifted to TLC. In these subjects an increased membrane conductance per liter alveolar volume (D_m/V_A) at lower alveolar volumes might be partly compensated by a smaller capillary blood volume, leading to a smaller increase in D_{LCO}/V_A with decreasing V_A.

In 1983 we mentioned the controversy surrounding the difference in results between our study [1] and those of Hamer [29] and Werner et al. [30], who described a minimum instead of a maximum in the Q_c vs V_A relationship somewhere between FRC and TLC. We had no fundamental explanation for this discrepancy, nor do we have now. In a later study with other subjects, we again found a maximum in the Q_c vs V_A relationship [10]. In rats, Forrest [31] also described a maximum. Factors which favour a maximum are the much greater number of volunteers (in total n=76) and intra-individual observations (6-12) in comparison with the results of the authors mentioned above (n=9 and 3 respectively). Moreover, our result was based on two continuous mathematical functions in air and high oxygen respectively, derived from all observations in each volunteer. The other authors, however, based their calculations on single observations in air and high oxygen respectively, which were not

always performed at exactly the same lung volume.

RCL method

In the relationship based on the RCL method, the factor (c + d A) represents the decrease in the intercept and the factor (e + f A) represents the decrease in slope with age, because d and f are negative (eqs. 2.4 and 2.5).

The use of the equations 2.4 and 2.5 imply a calculation of reference values of D_{LCO}/V_A at all lung volumes including TLC, which is a new approach for determining reference values. With equation 2.3, reference values can only be determined at TLC. The predicted values at TLC obtained with the RCL method (eqs. 2.4 and 2.5) showed an overestimation of 3.9% (Fig. 2.5). The predictability is also illustrated in Table 2.3, where D_{LCO}/V_A references at TLC (= 7 l) are compared to D_{LCO}/V_A references at TLC in larger populations as presented by Miller et al. [21], Paoletti et al. [22] and Cotes [16]. In the majority of predictions the RCL method predicts larger values at TLC. A maximum difference of 18% was found for TLC values lower than 7 l.

According to the reference equations of the RCL method, D_{LCO}/V_A is the same for all individuals of the same age and sex if lung volume is the same, implying the same diffusion conditions at the same lung volume, even though this volume is at TLC in one individual or below TLC in another. This is illustrated in Fig. 2.4. Because height is not a significant parameter, the RCL method implies the principle that individuals with a small TLC have the same geometric relationship between lung volume and diffusion membrane as individuals with a large TLC, but after expiration to the same smaller volume. The RCL method predicts a smaller D_{LCO}/V_A at larger TLC. This is in agreement with the predictions of D_{LCO}/V_A by Miller et al. [21] and Paoletti et al. [22], which are negatively related to height and, therefore, also to TLC, because TLC is positively related to height [13]. The predictions by our equation 2.3 and by Cotes [16] are basically different from the RCL method, because both predict the same D_{LCO}/V_A values if age and gender are the same, independent of height and TLC. Nevertheless, the comparison of D_{LCO}/V_A at TLC levels did not reveal that one method is better than the other.

Because D_{LCO}/V_A is linearly related to V_A, according to $D_{LCO}/V_A = a - bV_A$, the relationship between D_{LCO} and V_A is a second order polynomial according to: $D_{LCO} = aV_A - bV_A^2$. A statistical operation for a second order polynomial, comparable with the RCL method for a

linear relationship needs complicated, time-consuming computer analyses. To avoid such a procedure we derived D_{LCO} references at volumes lower than TLC from the corresponding reference values for D_{LCO}/V_A by multiplying them with the lower lung volume.

Conversion method

We derived the conversion method for calculating reference values at V_A levels below TLC to enable the use of reference values at TLC obtained from other populations, assuming similar effects of ageing (eqs. 2.6 and 2.7) on the parameter *b* of eq. 2.8. The conversion method implies that D_{LCO}/V_A is not linearly related to the absolute lung volume as in the RCL method, but to the fractional decrease in lung volume. As a consequence, for individuals of the same age and gender the conversion method predicts a different D_{LCO}/V_A at the same lung volume below TLC, if their TLC is different, e.g. 6 and 8 l respectively. According to equation 2.3 both have the same D_{LCO}/V_A (=x) at TLC. At half TLC (3 and 4 l respectively) $D_{LCO}/V_A = x + 1/2b$. However, at 3 l the individual with the largest TLC has a predicted $D_{LCO}/V_A = x + 5/8b$, which is larger than the value of the individual with the smaller TLC.

Comparison of reference values

To assess whether the conversion method is satisfactory we compared its predictions at two volumes below TLC and at three different ages with the corresponding predictions based on the RCL method (Table 2.3). This conversion was applied to the D_{LCO}/V_A reference values at TLC, obtained from eq. 2.3 and the regression equations of Miller et al. [21], Paoletti et al. [22] and Cotes [16] respectively. The differences between the predictions of D_{LCO}/V_A by the conversion and the RCL method for V_A levels below TLC are smaller than the differences between the RCL method and the predictions at TLC from equation 2.3, Miller et al., Paoletti et al. and Cotes. A preference cannot be made based on the numerical data, probably due to the fact that differences are within the variation of the data. Nevertheless, we have a preference to the RCL method for two reasons.

1. According to the RCL method, gas transfer changes with lung volume in accordance with morphometric changes. During the first two [32-34] or eight [35] years of life alveoli increase to their final number. Subsequently, an increase in lung volume occurs by an increase in linear dimensions of the alveolar septa at constant thickness

[34]. Since lung volume is directly correlated to body height [13], it might be assumed that the growth of the lungs lasts as long as the body is growing, or the chest volume is increasing [36]. During this type of growth, D_{LCO}/V_A will change in the same way as during voluntary changes of lung volume in a full grown individual, where we found physiological evidence for an isotropic volume change of alveoli at constant barrier thickness [1].

2. The smaller standard deviation of the RCL predictions (eqs. 2.4 and 2.5), when compared with that of the conversion method (eq. 2.9), implies a more sensitive testing of a patient's value.

Applications

In our pulmonary function laboratory we routinely compare D_{LCO} and D_{LCO}/V_A in a patient suffering from a restrictive lung disease with reference values both at the patient's reference TLC and at the lung volume equal to the patient's actual TLC. Comparing D_{LCO}/V_A of a patient with a decreased TLC with the reference D_{LCO}/V_A at the same lung volume implies the assumption that the effect of decreasing lung volume by disease has the same effect on D_{LCO}/V_A as the voluntary reduction in lung volume in healthy volunteers. We have no evidence to support this assumption. However, we also lack of evidence that the comparison with D_{LCO}/V_A at normal TLC is correct. If TLC is smaller due to a restrictive disease D_{LCO}/V_A is normally compared to reference values at an equal lower lung volume, we intend to conclude that the lower D_{LCO} is due to the decrease in lung volume. If in such a case D_{LCO}/V_A is compared to a reference value at normal TLC, D_{LCO}/V_A seems abnormally large, often close to or more than 2 SD above predicted. We believe that this abnormally large D_{LCO}/V_A is compared to the wrong reference value.

If in a patient suffering from a restrictive lung disease D_{LCO}/V_A is lower than the reference D_{LCO}/V_A at the same lung volume, the question arises whether we have a disorder at alveolar-capillary membrane level, a decreased capillary blood volume or a changed D_{LCO}/V_A vs V_A relationship. In such patients, D_{LCO}/V_A at the "symptom limited" TLC can be normal when compared with the D_{LCO}/V_A reference at reference TLC. This does not necessarily mean a normal diffusion at alveolar-capillary level. In this case we suspect that the patient is suffering from some underlying disease.

Conclusion

To determine reference values of D_{LCO}/V_A at alveolar volumes lower than TLC, we recommend the use of either our RCL method (eqs. 2.4 and 2.5) or our conversion method (eqs. 2.6, 2.7 and 2.8), together with a D_{LCO}/V_A reference equation at TLC. A disadvantage of the conversion method is its larger standard deviation, caused by the standard deviations in *b* (from eqs. 2.6 or 2.7) and the reference value at TLC. References for D_{LCO} at each lung volume can be calculated from the corresponding D_{LCO}/V_A references.

Acknowledgement.

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REFERENCES

1. Stam H, Versprille A, and Bogaard JM. The components of the carbon monoxide diffusing capacity in man dependent on alveolar volume. *Bull. Eur. Physiopathol. Respir.* 1983; 19: 17-22.
2. Billiet L. De bepaling van de Pulmonaire Diffusie Capaciteit. *(Thesis) Leuven: University of Leuven*, 1965.
3. Cadigan JB, Marks A, Ellicott MF, Jones RH, and Gaensler EH. An analysis of factors affecting the measurement of pulmonary diffusing capacity by the single breath method. *J. Clin. Invest.* 1961; 40: 1495-1514.
4. Cotton DJ, Taher F, Mink JT, and Graham BL. Effect of volume history on changes in D_{LCO}^{SB}-3EQ with lung volume in normal subjects. *J. Appl. Physiol.* 1992; 73(2): 434-439.
5. Gurtner GH, and Fowler WS. Interrelationships of factors affecting pulmonary diffusing capacity. *J. Appl. Physiol.* 1971; 30: 619-624.
6. Lipscomb DJ, Patel K, and Hughes JMB. Interpretation of increases in the transfer coefficient for carbon monoxide (TL_{CO}/V_A or K_{CO}). *Thorax* 1978; 33: 728-733.
7. Marks A, Cugell DW, Cadigan JB, and Gaensler EA. Clinical determination of the diffusing capacity of the lungs. *Am. J. Med.* 1957; 22: 51-73.
8. McGrath MW, and Thomson ML. The effect of age body size and lung volume change on alveolar-capillary permeability and diffusing capacity in man. *J. Physiol. Lond.* 1959; 146: 572-582.
9. Rosenberg E, and Young RC. Potential value of diffusing capacity per liter of lung volume (D_{LCO}/V_A) for early detection of alveolar capillary defects. *Lung* 1979; 157: 23-29.
10. Stam H, Kreuzer FJA, and Versprille A. Effect of lung volume and positional changes on pulmonary diffusing capacity and its components. *J. Appl. Physiol.* 1991; 71: 1477-1488.
11. Mangado NG, Inglés MJA, Peces-Barba G, González MA, and Navarro FL. A simple method of correcting diffusing capacity for alveolar volume reduction in restrictive lung diseases. *Respiration* 1987; 52: 163-170.

12. Blackburn GL, Benotti PN, Bistrian BR, Bothe A, Maini BS, Schlamm HT, and Smith MF. Nutritional assessment and treatment of hospital malnutrition. *Infusionstherapie* 1979; 6: 238-250.
13. Quanjer PhH. Standardized lung function testing. *Bull. Eur. Physiopathol. Respir.* 1983; 19, *Suppl.*: 5.
14. Jones RS, and Meade F. A theoretical and experimental analysis of anomalies in the estimation of pulmonary diffusing capacity by the single breath method. *Q. J. Exp. Physiol.* 1961; 46: 131-143.
15. Ferris BG. Epidemiology standardization project. *Am. Rev. Respir. Dis.* 1978; 118 (suppl.): 62-72.
16. Cotes JE. *Lung Function: Assessment and Application in Medicine* (4th ed.). Oxford, UK: Blackwell, 1979.
17. Feldman HA. Families of lines: random effects in linear regression analysis. *J. Appl. Physiol.* 1988; 64: 1721-1732.
18. Dixon WJ, Brown MB, Engelman L, Hill MA, and Jennrich RJ. BMDP Statistical Software Manual Vol. 2, University of California Press, Berkeley, 1988.
19. Crapo RO, and Morris AH. Standardized single breath normal values for carbon monoxide diffusing capacity. *Am. Rev. Respir. Dis.* 1981; 123: 185.
20. Knudson RJ, Kaltenborn WT, Knudson DE, and Burrows B. The single-breath carbon monoxide diffusing capacity. Reference equations derived from a healthy nonsmoking population and effects of hematocrit. *Am. Rev. Respir. Dis.* 1987; 135: 805-811.
21. Miller A, Thornton JC, Warshaw R, Anderson H, Teirstein AS, and Selikoff IJ. Single breath diffusing capacity in a representative sample of the population of Michigan, a large industrial state. *Am. Rev. Respir. Dis.* 1983; 127: 270-277.
22. Paoletti P, Viegi G, Pistelli G, Di Pede F, Fazzi P, Polato R, Saetta M, Zambon R, Carli G, Giuntini C, Lebowitz MD, and Knudson RJ. Reference equations for the single-breath diffusing capacity. A cross-sectional analysis and effect of body size and age. *Am. Rev. Respir. Dis.* 1985; 132: 806-813.
23. Love RG, and Seaton A. About the ECCS summary equations (letter to the editor). *Eur. Respir. J.* 1990; 3: 489.

24. Newth CJL, Cotton DJ, and Nadel JA. Pulmonary diffusing capacity measured at multiple intervals during a single exhalation in man. *J. Appl. Physiol.* 1977; 43: 617-625.

25. Lebecque P, Mwepu A, Veriter C, Rodenstein D, Nemery B, and Frans A. Hysteresis of the alveolar capillary membrane in normal subjects. *J. Appl. Physiol.* 1986; 60: 1442-1445.

26. Burrows B, Kasik JE, Niden AH, and Barclay WR. Clinical usefulness of the single-breath pulmonary diffusing capacity test. *Am. Rev. Respir. Dis.* 1961; 84: 789-806.

27. Bradley J, Bye C, Hayden SP, and Hughes DTD. Normal values for transfer factor and transfer coefficients in healthy males and females. *Respiration* 1979; 38: 221-226.

28. Weibel ER, Untersee P, Gill J, and Zulauf M. Morphometric estimation of pulmonary diffusing capacity. VI. Effect of varying positive pressure inflation of airspaces. *Resp. Physiol.* 1973; 18: 285-308.

29. Hamer NAJ. Variations in the components of the diffusing capacity as the lung expands. *Clin. Sci.* 1963; 24: 275-285.

30. Werner FM, and Beneken Kolmer H. The CO single breath transfer factor of the lung. Generally acceptable normal values. *Pflügers Arch.* 1982; 393: 269-274.

31. Forrest JB. Lung tissue plasticity: Morphometric analysis of anisotropic strain in liquid filled lungs. *Resp. Physiol.* 1976; 27: 223-239.

32. Davies G, and Reid L. Growth of the alveoli and pulmonary arteries in childhood. *Thorax* 1970; 25: 669-681.

33. Thurlbeck WM. Postnatal human lung growth. *Thorax* 1982; 37: 564-571.

34. Zeltner TB, Caduff JH, Gehr P, Pfenninger J, and Burri PH. The postnatal development and growth of the human lung. I. Morphometry. *Resp. Physiol.* 1987; 67: 247-267.

35. Dunnill MS. Postnatal growth of the lung. *Thorax* 1962; 17: 329-333.

36. Zeltner TB, and Burri PH. The postnatal development and growth of the human lung. II. Morphology. *Resp. Physiol.* 1987; 67: 269-282.

CHAPTER 3

PULMONARY DIFFUSING CAPACITY AT REDUCED ALVEOLAR VOLUMES IN CHILDREN

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The carbon monoxide diffusing capacity of the lungs (D_{LCO}) and the diffusing capacity normalized to alveolar volume (D_{LCO}/V_A) are usually estimated at total lung capacity (TLC) [1]. In normal subjects aged 20 and 85 years D_{LCO} increases as alveolar volume (V_A) increased, whereas D_{LCO}/V_A decreases, yielding a linear relationship with V_A, characterized by a negative slope [2, 3]. The regression line of D_{LCO}/V_A vs V_A shifts downwards and becomes less steep with increased age. In adults with restrictive lung disease, D_{LCO} and D_{LCO}/V_A should probably be compared to reference values obtained at the same lung volume as the symptom limited total lung capacity (TLC) [4]. No information on the effect of a change in lung volume on the diffusion variables is available for normal children. We, therefore, studied the relationships of D_{LCO} and D_{LCO}/V_A to V_A, and determined the relevant regression parameters of these relationships in a group of normal subjects below 20 years of age.

SUBJECTS AND METHODS

Subjects

We studied 103 healthy school children of European descent with ages ranging from 6 to 18 years. They had no signs of an acute respiratory disease, no history of chronic pulmonary or cardiac disease according to the selection criteria of Taussig et al. [5], no history of pneumonia or thoracic surgery, and no other disease which might influence the respiratory system or their general state of health, either directly or indirectly. Furthermore, there was no history of an upper respiratory tract infection during the three weeks prior to the investigation. The group consisted of 48 male and 55 female nonsmokers. Their heights and weights (Table 3.1) relative to their ages closely approximated the means observed for healthy Dutch children, indicating that we investigated a representative sample of normal Dutch children [6]. All had normal lung volumes in % of the reference values of Zapletal [7], (mean ± SD), ♂: TLC = 93 ± 10 %; vital capacity: VC = 98 ± 8 %; forced expired volume in 1 second: FEV_1 = 98 ± 10 %; D_{LCO} at TLC = 101 ± 11 % and ♀: TLC = 93 ± 9 %; VC = 101 ± 9 %; FEV_1 = 103 ± 10 %; D_{LCO} at TLC = 94 ± 10 %.

Procedure

The spirometric and diffusion variables were determined in an upright seated posture; volume changes were obtained by integration of the flow signal from a pneumotachograph and pressure transducer. Functional residual capacity (FRC) was determined with a closed circuit helium dilution technique [8]. We estimated single breath D_{LCO} and D_{LCO}/V_A at six different alveolar volumes. The single breath procedure was performed as recommended by the ECCS [1]. The subjects expired to residual volume (RV) and then inhaled volumes ranging from 1.5 l up to vital capacity (VC) in random order. Breath holding time started when 30% of the volume was inspired, and ended when half of the expired sample was collected [9]. Overall, breath holding time slightly exceeded 10 s. Inspirations and expirations were performed rapidly.

Alveolar fractions of CO and He were obtained from expired gas after discarding 750 ml for washout of airways and apparatus dead space. The size of the alveolar sample was 500 ml. Measurements were performed with "Masterlab Transfer" equipment (Jaeger, Würzburg, Germany). The interval between consecutive measurements was 5 min. To minimize the effect of CO back tension, we restricted the number of measurements to six a day. We neglected in our calculations the effects of CO back tension as it was less than 1% of the alveolar CO tension at the start of breath holding. We used a heat conductivity type He analyzer, which is also sensitive to CO_2. CO_2 was therefore absorbed prior to the gas analysis. The remaining gas concentrations were corrected for an absorbed volume corresponding to 5% CO_2 [10].

D_{LCO} and D_{LCO}/V_A were not corrected for normal variability in Hb concentration, because it appeared not to be necessary in healthy volunteers as noted in a former study [4].

Data analysis and Statistics

We analysed our results according to a 'Random coefficients model' [4], in which a linear D_{LCO}/V_A vs V_A relationship within all individuals was assumed. This model is a direct generalization of the model described by Feldman [11]. The parameters were estimated by the 'restricted maximum likelihood' method using module 5V of the BMDP package [12]. This method uses the least squares slope and intercept to refine its previous estimates of variance components, recalculates the weights, re-estimates the slope and intercept, and so forth.

Differences between two groups of data or differences from zero were regarded as signifi-

cant at P-value < 0.05.

RESULTS

D_{LCO} and D_{LCO}/V_A at TLC

Cotes et al. [13] found D_{LCO} as well as D_{LCO}/V_A, estimated at TLC, to be a power function of height. We found similar relationships:

Boys:	D_{LCO}	$= 39.9\ H^{2.45}$	$r = 0.93$	RSD of $\ln D_{LCO} = 0.096$	(3.1)
	D_{LCO}/V_A	$= 40.1\ H^{-0.60}$	$r = -0.50$	RSD of $\ln(D_{LCO}/V_A) = 0.107$	(3.2)
Girls:	D_{LCO}	$= 41.3\ H^{2.23}$	$r = 0.90$	RSD of $\ln D_{LCO} = 0.108$	(3.3)
	D_{LCO}/V_A	$= 43.9\ H^{-0.84}$	$r = -0.59$	RSD of $\ln(D_{LCO}/V_A) = 0.112$	(3.4)

D_{LCO} is in $\mu mol.s^{-1}.kPa^{-1}$, D_{LCO}/V_A in $\mu mol.s^{-1}.kPa^{-1}.l^{-1}$, and height H in m.

Our regression equations in boys were compared with those of Cotes et al. [13], Baran et al. [14], Bucci et al. [15] and Nasr et al. [16], and are graphically presented in Fig. 3.1. The D_{LCO} vs height relationships of Bucci et al. and Nasr et al. and the D_{LCO}/V_A vs height relationship of Cotes et al. were within 1 RSD of our regression equations. We found similar results for the girls we tested.

Furthermore, D_{LCO} and D_{LCO}/V_A appeared to be linearly related to TLC in boys and girls (Figs. 3.2 and 3.3). Since age and height were not significant predictors, both variables were adequately represented by TLC.

Boys:	D_{LCO}	$= 29.5 + 22.9$ TLC	$r = 0.94$	RSD=11	(3.5)
	D_{LCO}/V_A	$= 37.4 - 1.56$ TLC	$r = 0.54$	RSD=3.4	(3.6)
Girls:	D_{LCO}	$= 35.8 + 20.2$ TLC	$r = 0.88$	RSD=11	(3.7)
	D_{LCO}/V_A	$= 41.1 - 2.78$ TLC	$r = 0.69$	RSD=3.0	(3.8)

where TLC is in liters (l).

In our group of boys TLC (l) depended on height H (m) according to $TLC = 1.01H^{3.1}$ with RSD of $\ln TLC = 0.090$ and in girls $TLC = 0.99H^{3.0}$ with RSD of $\ln TLC = 0.087$. Comparison of our prediction equations for TLC in boys and girls with the reference equations published in the literature gave similar results [13, 17-23]. The TLC values for boys, in 10 cm increments between 120 and 180 cm in height, were not significantly different from those of girls (P-values between 0.14 and 0.88). The heights of boys and girls were not significantly

different between 6 and 12 years of age (P-values between 0.07 and 0.83).

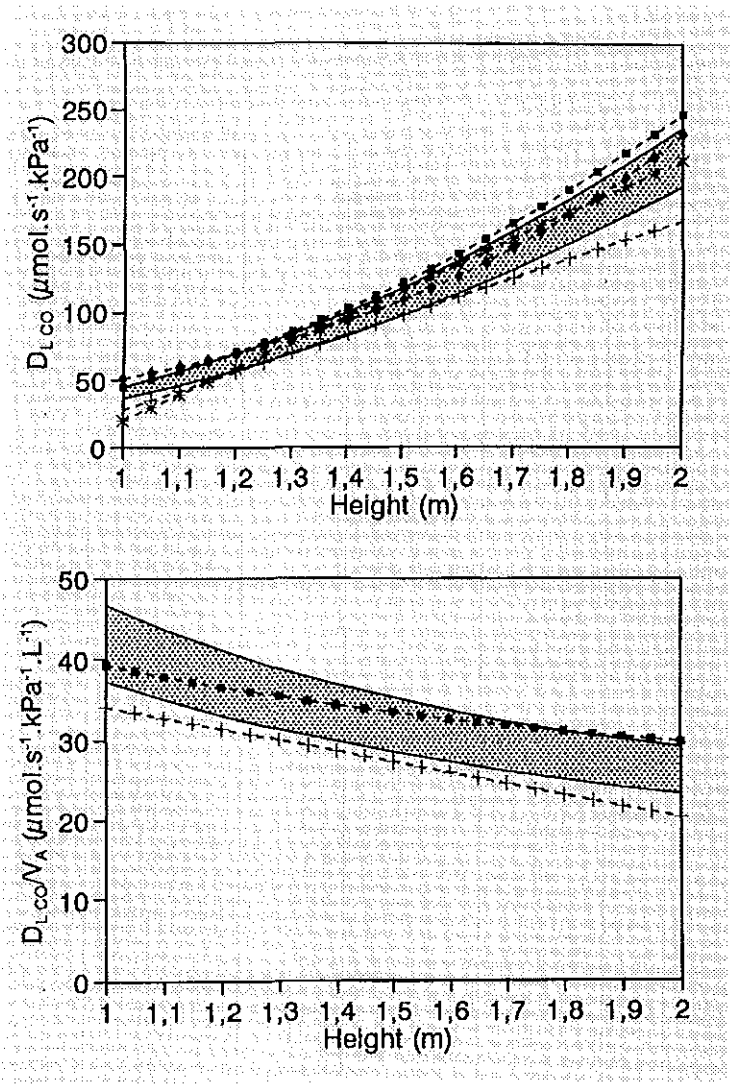

Fig. 3.1. *Comparison of reference values for D_{LCO} and D_{LCO}/V_A at TLC in µmol.s^{-1}.kPa^{-1} and µmol.s^{-1}.kPa^{-1}.l^{-1} respectively from the boys of our study with reference values published in literature as function of height (m).*

- ▪ *Cotes et al. [13]*
- \+ *Baran et al. [14]*
- ♦ *Bucci et al. [15]*
- * *Nasr et al. [16]*

Shaded area, this paper ± 1 RSD
Similar results were found in the girls.

Table 3.1. Anthropometric data and regression equations for $D_{L,CO}/V_A$ as function of V_A of the boys and girls. We performed a separation in age groups of three years.

Age group	sex m/f	n	Height (m) mean ± SD	Weight (kg) mean ± SD	TLC (l) mean ± SD	$D_{LCO}/V_A = a - bV_A$	
						b mean±SD	a mean±SD
6-8	m	4	1.31 ± 0.04	27 ± 3	2.28 ± 0.4	11.8 ± 6.4	59.6 ± 13.0
6-8	f	6	1.26 ± 0.09	27 ± 7	2.04 ± 0.5	10.3 ± 2.1	54.4 ± 2.8
9-11	m	23	1.49 ± 0.07	38 ± 7	3.37 ± 0.5	8.3 ± 3.3	59.1 ± 9.3
9-11	f	24	1.47 ± 0.09	39 ± 9	3.14 ± 0.5	8.7 ± 2.9	58.5 ± 8.9
12-14	m	10	1.62 ± 0.13	48 ± 11	4.58 ± 1.2	5.3 ± 2.0	53.0 ± 8.6
12-14	f	14	1.65 ± 0.08	50 ± 7	4.59 ± 0.7	7.2 ± 1.9	58.8 ± 8.0
15-17	m	11	1.81 ± 0.09	63 ± 8	6.49 ± 1.0	5.2 ± 1.3	60.2 ± 8.8
15-17	f	11	1.65 ± 0.06	56 ± 9	4.79 ± 0.8	6.6 ± 1.9	58.1 ± 7.9

D_{LCO}/V_A, diffusing capacity of CO per alveolar volume ($\mu mol.s^{-1}.kPa^{-1}.l^{-1}$); TLC, total lung capacity (l); a ($\mu mol.s^{-1}.kPa^{-1}.l^{-1}$) and b ($\mu mol.s^{-1}.kPa^{-1}.l^{-2}$) are constants; n, no of volunteers.

However, between 12 and 14 years of age the girls (P-value=0.03) and above 14 years of age the boys were significantly taller than the opposite gender (P-value=0.01), implying

significantly larger TLC values (P-value=0.0003) and D_{LCO} values at TLC (P-value=0.000003) at a given age in boys above 14 years of age. D_{LCO}/V_A at TLC was not significantly different between boys and girls in all age ranges (P-values between 0.72 and 0.84).

D_{LCO} and D_{LCO}/V_A dependent on V_A

In all 103 normal volunteers D_{LCO}/V_A decreased linearly with V_A. D_{LCO} increased as V_A increased. In Table 3.1 the mean values of the anthropometric data and the slopes (column "b" in Table 3.1) and intercepts (column "a" in Table 3.1) of the D_{LCO}/V_A vs V_A relationships are given for age groups of three years each.

The parameters a and b of the relationship $D_{LCO}/V_A = a - bV_A$ varied substantially between normal subjects. Parameter a was independent of height and age, but the parameter b was linearly related to height (H), according to: $b = c - d\text{H}$.

We assumed the following model:

$$D_{LCO}/V_A = a - (c - d\text{H})V_A \qquad (3.9)$$

in which parameters a and c were regarded as varying randomly, i.e. each individual was allowed to have his or her own values, and d was considered to be constant, i.e., a systematic dependence of the slope on height. Significant gender difference (P-value=0.015) was found when a gender-related component was introduced in this model. We therefore calculated regression equations for boys and girls separately. When an age-related factor was added to this model, it resulted in a parameter, which was not significantly different from zero (P-value=0.08 and 0.06 for boys and girls respectively).

Using the RCL method [4] the resulting regression equations for D_{LCO}/V_A were:

Boys: $$D_{LCO}/V_A = 58.9 - (23.1 - 9.98\text{H})V_A \qquad (3.10)$$
with RSD $= \sqrt{(2.01V_A^2 - 16.4V_A + 46.6)}$

Girls: $$D_{LCO}/V_A = 57.6 - (22.5 - 9.52\text{H})V_A \qquad (3.11)$$
with RSD $= \sqrt{(1.81V_A^2 - 13.8V_A + 36.7)}$

To demonstrate that height is a significant factor in the slope value of both reference equations (3.10 and 3.11), we compared their predictions with all individually determined slopes. The differences were not significant at P-value=0.83 and 0.60 for boys and girls respectively (paired t-test). According to the model of equation 3.9, D_{LCO} reference values can be

calculated according to:

$$D_{LCO} = aV_A - cV_A^2 + dHV_A^2 \quad (3.12)$$

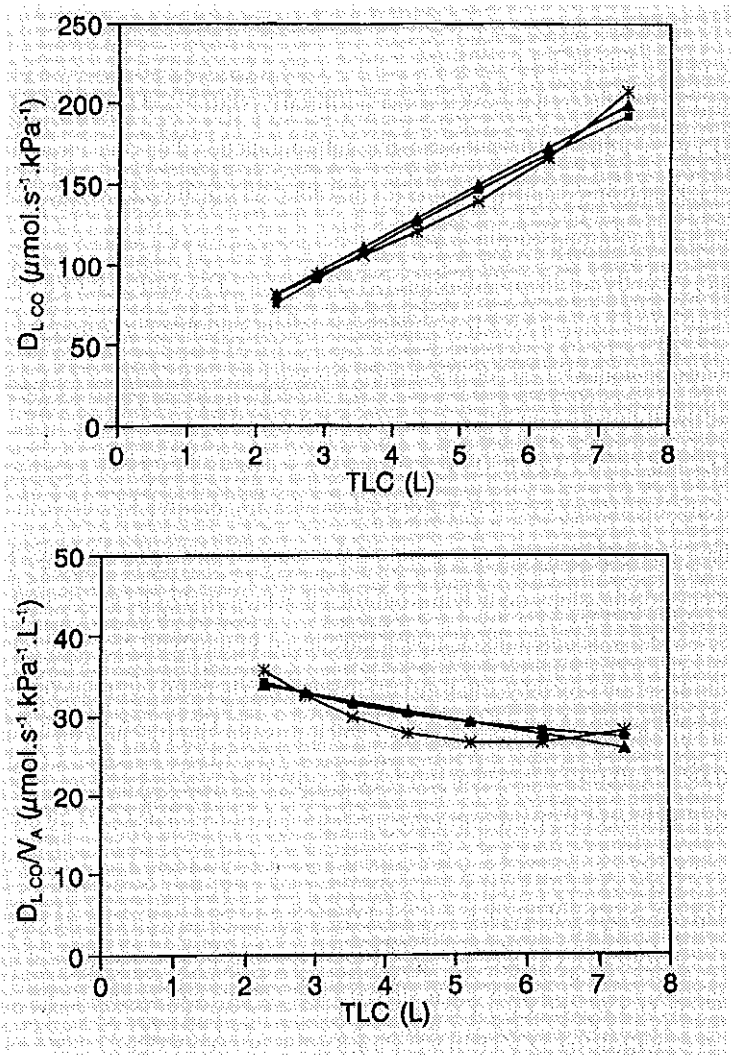

Fig. 3.2. Comparison of our reference equations for D_{LCO} and D_{LCO}/V_A at TLC with those at all alveolar volume levels in the boys.

- ■ *equations 3.1 and 3.2*
- ▲ *equations 3.5 and 3.6*
- ✱ *equations 3.12 and 3.10*

D_{LCO} can then be calculated for boys and girls by using the constants of equations 3.10 and 3.11 respectively. At reference TLC we compared the various models in boys and girls, for D_{LCO} (♂: eqs. 3.1, 3.5 and 3.12 and ♀: eqs. 3.3, 3.7 and 3.12) and D_{LCO}/V_A (♂: eqs. 3.2, 3.6 and 3.10 and ♀: eqs. 3.4, 3.8 and 3.11) (Figs. 3.2 and 3.3). The differences were

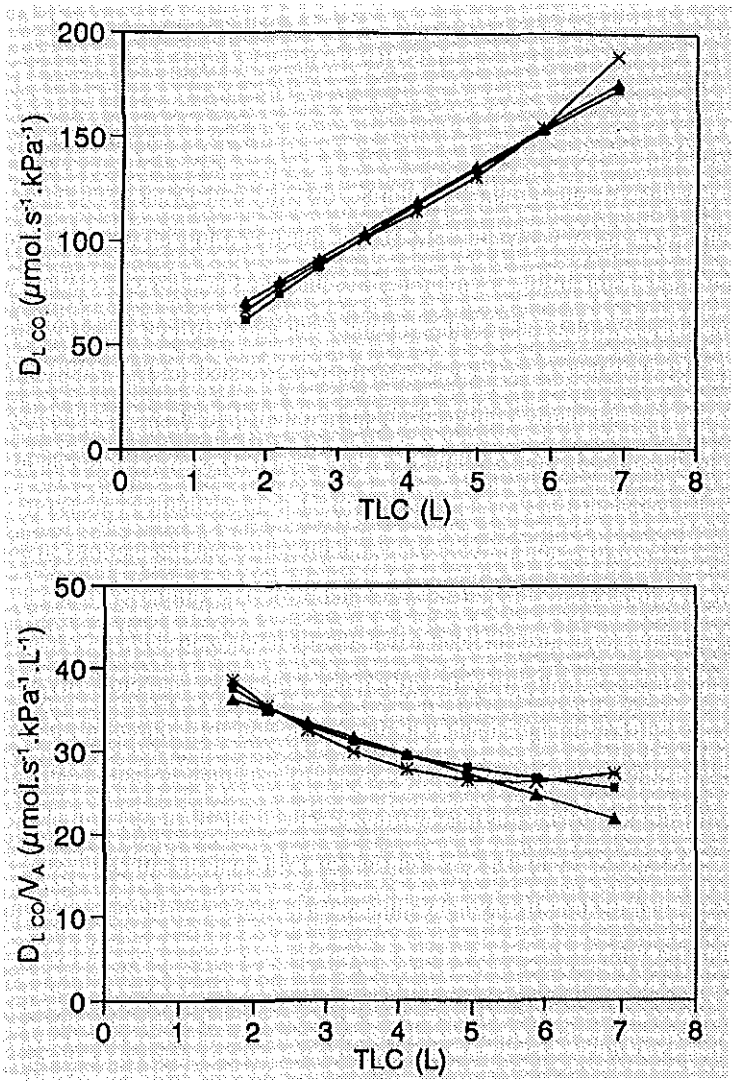

Fig. 3.3 *Comparison of our reference equations for D_{LCO} and D_{LCO}/V_A at TLC with those at all alveolar volume levels in the girls.*

- ■ *equations 3.3 and 3.4*
- ▲ *equations 3.7 and 3.8*
- * *equations 3.12 and 3.11*

not significant (paired t-test ♂: P-value=0.79 and 0.43; ♀: P-value=0.87 and 0.73 for D_{LCO} and D_{LCO}/V_A respectively). For the calculation of the reference value of TLC we used the equations mentioned above.

DISCUSSION

D_{LCO} and D_{LCO}/V_A at TLC

The reference values of our group of children for D_{LCO} and D_{LCO}/V_A at TLC level are in agreement with other reference values [13-16], as illustrated in Fig. 3.1. Age did not appear to be a significant factor in these children. Presumably, the poor correlation with age for both D_{LCO} and D_{LCO}/V_A depends on the variability in age at which the growth spurt starts. Nasr et al. [16] found that D_{LCO}/V_A was negatively correlated to TLC and not significantly correlated to height. Similarly, Bucci et al. [15] found that D_{LCO} was better correlated to TLC than to height. In our volunteers D_{LCO} and D_{LCO}/V_A were significantly (P-value$<$0.001) dependent on TLC (eqs. 3.5-3.8). The logarithmic regression equations on height (eqs. 3.1-3.4) do not provide substantially different predictions from the linear equations on TLC (eqs. 3.5-3.8 and Figs. 3.2 and 3.3). The similarity of the TLC vs height relationships of boys and girls indicates that the difference in TLC between both groups above 14 years of age is caused by the difference in height. The similarity in D_{LCO}/V_A at TLC in boys and girls is in agreement with the conclusions of Cotes et al. [13], namely that the proportion of the alveolar wall occupied by alveolar capillaries is the same in both sexes and that the difference in D_{LCO} in this age range reflects the difference in size of the lungs. Furthermore, the decrease in D_{LCO}/V_A during growth indicates that the increase in lung volume exceeds that of lung tissue and capillary surface area, resulting in less diffusing area relative to lung volume.

D_{LCO} and D_{LCO}/V_A dependent on V_A

In our former studies [2, 3] we observed in healthy adult volunteers that D_{LCO}/V_A vs V_A yields a linear relationship, characterized by a negative slope. The regression line shifts downwards and the slope becomes less steep with increasing age [4]. D_{LCO} increases with increasing V_A. Since below 20 years of age the anatomical change in volume due to growth

could interfere with the D_{LCO} and D_{LCO}/V_A vs V_A relationships, we did not extrapolate the results of our former study to ages below 20 years. In children and adolescents the D_{LCO}/V_A vs V_A relationships appeared to be independent of age. Table 3.1 and equations 3.10 and 3.11 illustrate that the slopes of the D_{LCO}/V_A vs V_A relationships appeared to be related to height. In the Appendix we present an explanation for the decrease in slope if height is increasing.

The predictions of the diffusion indexes at TLC from equations 3.10, 3.11 and 3.12 were similar to the predictions based on equations 3.1-3.8, which were obtained from measurements at TLC only. We conclude that the equations 3.10-3.12 can be reliably used to predict the diffusion indexes at all levels of alveolar volume.

Clinical application

These relationships imply that comparison of D_{LCO} and D_{LCO}/V_A, determined at a lower lung volume than TLC, with reference values obtained at reference TLC, will lead to a relatively low D_{LCO} and high D_{LCO}/V_A. In patients with restrictive lung disease due to intra or extra parenchymal diseases, diffusing capacity is determined at a decreased TLC. In such patients a decreased D_{LCO}, compared with the reference value at normal TLC, will comprise the decrease due to the lower lung volume. However, comparison of D_{LCO}/V_A with the reference value at normal TLC will lead to an underestimation of the change in D_{LCO}/V_A. We prefer to compare D_{LCO}/V_A of such patients at their actual decreased TLC, because it will reveal better whether a diffusion disorder, either due to a disorder of the membrane or to a decreased lung capillary blood volume, is present. We are aware of the fact that the comparison of the D_{LCO} and D_{LCO}/V_A in a patient with a decreased TLC with the reference values for the diffusion indexes at the same lung volume assumes that the effect of decreasing lung volume by disease has the same effect on D_{LCO} and D_{LCO}/V_A as a voluntary reduction in lung volume in healthy volunteers. In absence of any evidence that the comparison of D_{LCO} and D_{LCO}/V_A with reference values at reference TLC is correct, we compare D_{LCO} with reference values at reference TLC and D_{LCO}/V_A with reference values at a volume equal to the symptom limited TLC.

To illustrate this reasoning an example of an 11 year old girl with inflammatory interstitial lung disease of unknown etiology, which showed gradual worsening despite agressive anti-

inflammatory treatment, is presented in Fig. 3.4. D_{LCO}/V_A at the actual TLC of 3.15 l is 70% of reference D_{LCO}/V_A at reference TLC. However, compared to reference

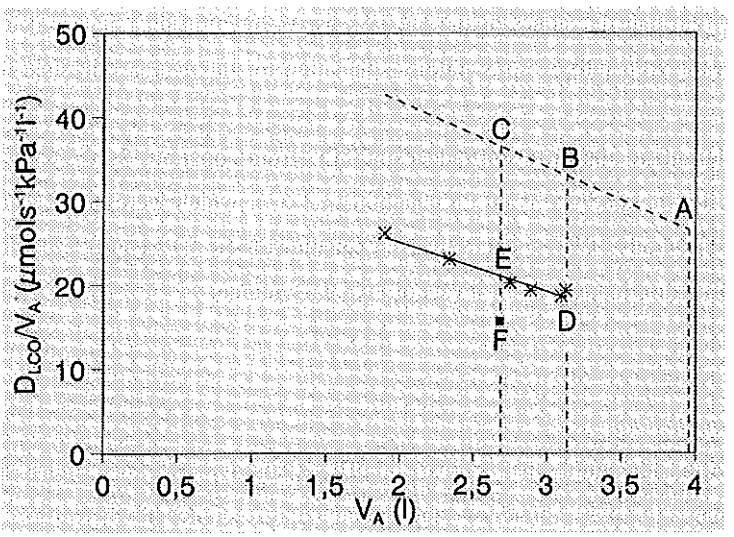

Fig. 3.4. *D_{LCO}/V_A vs V_A in an 11 year old female patient with a height of 1.54 m, a restrictive lung function and interstitial inflammatory changes. The dashed line represents the predicted values, based on eq. 3.11.*

- *A:* *D_{LCO}/V_A reference value at reference TLC (3.95 l).*
- *D:* *Actual D_{LCO}/V_A value at her actual TLC (3.15 l).*
- **:* *D_{LCO}/V_A estimates at various volumes. The solid line indicates her actual D_{LCO}/V_A vs. V_A relationship.*
- *B:* *reference D_{LCO}/V_A at her TLC of 3.15 l.*
- *F:* *D_{LCO}/V_A at TLC (=2.68 l) half a year later.*
- *C:* *D_{LCO}/V_A reference at her TLC of 2.68 l.*
- *E:* *D_{LCO}/V_A at the same volume half a year earlier to evaluate the further decrease.*

D_{LCO}/V_A at the actual TLC, the patient's D_{LCO}/V_A is 56% of predicted. This indicates a large decrease in diffusing capacity per liter lung volume. Furthermore, a change in lung volume in this patient elicited an almost parallel change in D_{LCO}/V_A with V_A as observed in normal individuals. Half a year later both D_{LCO}/V_A and TLC were decreased even more. Again the change in D_{LCO}/V_A (to 43%) is largest if compared to reference D_{LCO}/V_A at the same volume as the decreased TLC.

APPENDIX

The height dependence of equations 3.10-3.12

Based on the analysis of Roughton and Forster [24] we separated in previous studies [2, 3] the diffusing capacity in its components, the membrane conductance D_m and by analogy, θQ_c the diffusing capacity of the total mass of erythrocytes in the capillary bed:

$$\frac{1}{D_{LCO}} = \frac{1}{D_m} + \frac{1}{\theta Q_c} \tag{3.13}$$

where Q_c is the volume of the capillary bed in ml and θ the rate at which 1 ml whole blood will take up CO and $D_m/\theta Q_c \approx 1$ [25]. In normal adults the Q_c vs V_A relationship could be described by a second order polynomial with a maximum at an alveolar volume between 50 to 100% of TLC. In young adult volunteers the parabolic relationship had a flat appearance, implying an approximately constant Q_c around FRC.

Based on the diffusion equation of Fick we assumed a proportionality of D_m and the effective diffusion area A and an inverse proportionality between D_m and the membrane thickness δ according to: $D_m = k_1 A/\delta$.

The membrane conductance varied with V_A according to $D_m = kV_A^x$, where k was a proportionality constant and x was a constant reflecting the type of membrane expansion. Combination of both equations results in:

$$D_m = k_1 A/\delta = kV_A^x \tag{3.14}$$

In 64% of the adult volunteers x was varying around 2/3, which could be explained by an isotropic expansion of alveoli and a constant membrane thickness. In the remaining group of volunteers x was larger than 2/3, indicating isotropic expansion probably in combination with alveolar recruitment or a decrease in membrane thickness. The isotropic expansion with constant barrier thickness and the exponent 2/3 were also reported by Weibel et al. based on morphometric studies [26, 27].

When diffusing capacity is measured at lower alveolar volume V_A than maximum alveolar volume V_{Amax} which is a fraction y of V_{Amax} ($y = V_A/V_{Amax}$), the membrane conductance D_m can be derived from eq. 3.14:

At TLC: $D_m = kV_{Amax}^x$.

At lower V_A: $D_m' = kV_A^x$.

Thus: $D_m' = k(V_{Amax}.V_A/V_{Amax})^x = D_m.y^x$

$$D_m/D_m' = y^{-x} \quad (3.15)$$

Assuming that $D_m/\theta Q_c = 1$ [25] and $Q_c \approx$ constant [2, 3], we can derive from eqs 3.13 and 3.15:

$(1/D_{LCO})/(1/D_{LCO}') = D_{LCO}'/D_{LCO} = (1/D_m + 1/\theta Q_c)/(1/D_m' + 1/\theta Q_c) = 2y^x/(1+y^x)$

$$D_{LCO}' = (2y^x/(1+y^x))D_{LCO} \quad (3.16)$$

where D_{LCO} is measured at V_{Amax} and D_{LCO}' is calculated at a fraction y of V_{Amax}.

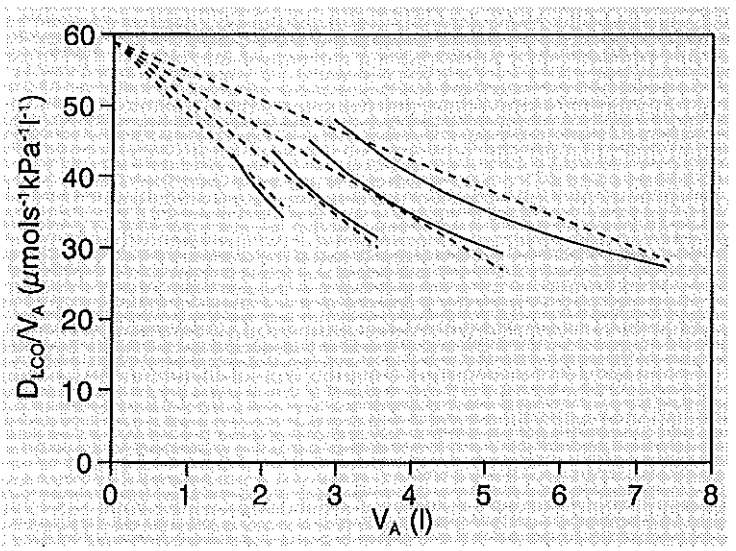

Fig. 3.5 *D_{LCO}/V_A vs V_A relationships according to eq. 3.18 for boys with heights of 1.3, 1.5, 1.7 and 1.9 m respectively. TLC was calculated from TLC = $1.01H^{3.1}$ and D_{LCO}/V_A at TLC was calculated with eq. 3.2 (solid lines). The dashed lines represent the predictions of eq. 3.10 for the four examples.*

From eqs 3.15 and 3.16 we derive:

$(D_{LCO}/V_A)'/(D_{LCO}/V_A) = D_{LCO}'/(D_{LCO}V_A/V_{Amax}) = D_{LCO}'/(D_{LCO}.y) = 2y^x/((1+y^x)y) = 2/(y^{1-x}+y)$

$$(D_{LCO}/V_A)' = (2/(y^{1-x}+y))D_{LCO}/V_A \quad (3.17)$$

where D_{LCO}/V_A is measured at V_{Amax} and $(D_{LCO}/V_A)'$ is calculated at a fraction y of V_{Amax}.

According to the model x=2/3 is:

$$(D_{LCO}/V_A)' = (2/(y^{1/3}+y))D_{LCO}/V_A \qquad (3.18)$$

In fig.3.5 the solid lines represent the calculated relationships between D_{LCO}/V_A and V_A of boys with heights of 1.3, 1.5, 1.7 and 1.9 m respectively according to equation 3.18. TLC is calculated from the reference equation $TLC=1.01H^{3.01}$ and reference D_{LCO}/V_A at TLC is calculated from equation 3.2. These relationships are not linear, but are curved upwards at lower V_A levels. This progressive increase at lower V_A levels explains the steeper slopes of the D_{LCO}/V_A vs V_A relationships when TLC is smaller. When deriving equations 3.10 and 3.11 we assumed for reasons of simplicity in each individual subject a linear D_{LCO}/V_A vs V_A relationship between 50 and 100% of maximum alveolar volume V_{Amax}. In our opinion this was an acceptable assumption, because in 73% of the boys and in 61% of the girls the correlation coefficient was larger then 0.9.

The dashed lines with the same intercept on the y-axis and height dependent slopes illustrate equation 3.10 at these heights. These lines are more or less in parallel to the solid lines of eq. 3.18. In individuals with smaller height (and thus smaller TLC) the prediction of the slope of eq. 3.10 and the calculated change in D_{LCO}/V_A from eq. 3.18 correspond acceptably. Equation 3.18 is derived assuming Q_c is constant when V_A decreases. However, in our previous studies [2, 3] we illustrated in young adults that the Q_c vs V_A relationship had a maximum between 50 and 100% of

V_{Amax}. A maximum in this relationship will reduce the curvature of the D_{LCO}/V_A vs V_A relationship, causing smaller differences in solid and dashed lines.

REFERENCES

1. Quanjer PhH. Standardized lung function testing. *Bull. Eur. Physiopathol. Respir.* 1983; 19: Suppl. 5.
2. Stam H, Versprille A, Bogaard JM. The components of the carbon monoxide diffusing capacity in man dependent on alveolar volume. *Bull. Eur. Physiopathol. Respir.* 1983; 19: 17-22.
3. Stam H, Kreuzer FJA, Versprille A. Effect of lung volume and positional changes on pulmonary diffusing capacity and its components. *J. Appl. Physiol.* 1991; 71: 1477-1488.
4. Stam H, Hrachovina V, Stijnen T, Versprille A. Diffusing capacity dependent on lung volume and age in normal subjects. *J. Appl. Physiol.* 1994; 76(6): 2356-2363.
5. Taussig LM, Cherniack V, Wood R, Farrell P, Mellins RB. Standardization of lung function testing in children. Proceedings and recommendations of the GAP Conference Committee, Cystic Fibrosis Foundation. *J. Pediatr.* 1980; 97: 668-676.
6. Verweij GCG. Health Interview Surveys. Height and weight of children and adolescents up to 21 years, 1981-1992. [Dutch edition] Report Netherlands Central Bureau of Statistics; 1994.
7. Zapletal A, Samánek M, Paul T. Lung function in children and adolescents. Methods, Reference values. Basel: Karger, 1987.
8. Cotes JE. Lung Function: Assessment and Application in Medicine (4th ed.). Oxford, UK: Blackwell, 1979.
9. Jones RS, Meade F. A theoretical and experimental analysis of anomalies in the estimation of pulmonary diffusing capacity by the single breath method. *Q. J. Exp. Physiol.* 1961; 46: 131-143.
10. Ferris BG. Epidemiology standardization project. *Am. Rev. Respir. Dis.* 1978; 118 (suppl.): 62-72.
11. Feldman HA. Families of lines: random effects in linear regression analysis. *J. Appl. Physiol.* 1988; 64: 1721-1732.
12. Dixon WJ, Brown MB, Engelman L, Hill MA, Jennrich RJ. BMDP Statistical Software Manual Vol. 2. Berkeley, USA: University of California Press, 1988.

13. Cotes JE, Dabbs JM, Hall AM, Axford AT, Laurence K. Lung volumes, ventilatory capacity and transfer factor in healthy British boy and girl twins. *Thorax* 1973; 28: 709-715.
14. Baran D, Englert M. Les valeurs normales du facteur de transfert pulmonaire d'oxyde de carbone et de la "perméabilité" alveolo-capillaire chez l'enfant. *Bull. Eur. Physiopath. Resp.* 1968; 4: 659-672.
15. Bucci G, Cook CP, Barrie H. Studies of respiratory physiology in children, Part V. Total lung diffusion, diffusing capacity of pulmonary membrane and pulmonary capillary blood volume in normal subjects from 7 to 40 years of age. *J. Pediatr.* 1961; 58: 820-828.
16. Nasr SZ, Amato P, Wilmott RW. Predicted values for lung diffusing capacity in healthy children. *Pediatr. Pulmonol.* 1991; 10: 267-272.
17. Cook CD, Hamann JF. Relation of lung volumes to height in healthy persons between ages of 5 and 38 years. *J. Pediatr.* 1961; 59: 710-714.
18. Engström I, Karlberg P, Swarts CL. Respiratory studies in children. IX. Relationships between mechanical properties of lungs, lung volumes and ventilatory capacity in children 7-15 years of age. *Acta Paediatr.* 1962; 51: 68-80.
19. Geubelle F, Brény H. Volumes pulmonaires de filles et de garçons sains, agés de 5 à 16 ans. *Le Poumon et le Coeur* 1969; 25: 1051-1064.
20. Guerini C, Pistelli G, Paci A, Taddeucci G, Dalle Luche A. Pulmonary volumes in children. I. Normal values in males of 6 to 15 years old. *Bull. Eur. Physiopath. Resp.* 1970; 6: 701-719.
21. Helliesen PJ, Cook CD, Friedlander L, Agathon S. Studies of respiratory physiology in children. I. Mechanics of respiration and lung volumes in 85 normal children 5 to 17 years of age. *Pediatrics* 1958; 22: 80-93.
22. Pistelli G, Paci A, Dalle Luche A, Giuntini C. Pulmonary volumes in children. II. Normal values in female children 6 to 15 years old. *Bull. Eur. Physiopathol. Respir.* 1978; 14: 513-523.
23. Thiemann HH. Normwerte der Lungenfunktionsdiagnostik im Kindersalter. *Z. Erkrank. Atm-Org.* 1976; 145: 249-257.

24. Roughton FJW, and Forster RE. Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. *J. Appl. Physiol.* 1957; 11: 290-302.

25. Mangado NG, Avilés Inglés MJ, Peces-Barba G, González MA, and Navarro FL. A simple method of correcting diffusing capacity for alveolar volume reduction in restrictive lung diseases. *Respiration* 1987; 52: 163-170.

26. Weibel ER, and Gomez DM. Architecture of the human lung. *Science* 1962; 137: 577-585.

27. Weibel ER, Untersee P, Gil J, and Zulauf M. Morphometric estimation of pulmonary diffusion capacity. VI. Effect of varying positive pressure inflation of air spaces. *Resp. Physiol.* 1973; 18: 285-308.

CHAPTER 4

EVALUATION OF DIFFUSING CAPACITY IN PATIENTS WITH A RESTRICTIVE LUNG DISEASE

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This chapter has been submitted for publication.

The CO diffusing capacity of the lung, D_{LCO} and D_{LCO} per liter alveolar volume, D_{LCO}/V_A, are usually estimated at total lung capacity, TLC [1] In normal volunteers D_{LCO} decreases and D_{LCO}/V_A increases if V_A is decreased [2-12]. We hypothesized that a volume restriction due to a disease has a similar effect on the diffusion indexes as a voluntary volume reduction in normal volunteers, implying an increase in D_{LCO}/V_A at the decreased TLC. As a consequence a decreased D_{LCO}/V_A in such patients should be compared with a reference D_{LCO}/V_A at the disease limited TLC. To test this hypothesis, we aimed to study the volume dependence of the diffusion indexes in a group of patients, who developed a diffusion disorder in combination with a volume restriction in a short period of time. Such rapidly developing restrictive lung disorder may occur in patients receiving bleomycin in a chemotherapeutic regimen. An important side effect of bleomycin is lung damage, characterized by pneumonitis or diffuse interstitial pulmonary fibrosis [13, 14] with a decrease in TLC, D_{LCO}, or both [15]. D_{LCO} appeared to be the best indicator of early lung damage [16]. This index enables an early discontinuation of bleomycin treatment at a stage where lung toxicity is still reversible [17]. In a group of patients receiving bleomycin we estimated D_{LCO} and D_{LCO}/V_A at different lung volumes before, during and after treatment and compared these results with the diffusion indexes of healthy controls.

SUBJECTS AND METHODS

Subjects

In 13 adult patients suffering from germ cell tumors D_{LCO} and D_{LCO}/V_A were measured. The spirometric data before the chemotherapeutic treatment, expressed in percent of the reference values of the European Community for Coal and Steel (ECCS) [1], (mean $\pm$ SD) were: total lung capacity, TLC = 101 $\pm$ 8 %; vital capacity, VC = 99 $\pm$ 12 % and forced expired volume in one second as a fraction of VC, FEV_1/VC = 98 $\pm$ 10 %. Mean D_{LCO} and D_{LCO}/V_A at TLC, corrected to a normal Hb concentration, were 87 $\pm$ 14 % and 94 $\pm$ 15 % of reference [12] respectively. Their ages ranged from 20 to 35 years.

Reference values of Hb concentration are 9.2 $\pm$ 0.5 and 8.2 $\pm$ 0.5(SD) $mmol.l^{-1}$ in men and women respectively, as determined in a group of 120 volunteers with the same demographic background in the Laboratory for Clinical Chemistry in our hospital.

Procedure

In a series of 12 single breath maneuvers D_{LCO} and D_{LCO}/V_A were determined in sitting position at various alveolar volume levels as described previously [12]. The single breath procedures were performed with a "Masterlab Transfer" (Jaeger, Würzburg, Germany). The interval between consecutive measurements was at least 5 min and the number of measurements was restricted to six a day, to minimize the influence of CO back tension. Back tension was neglected, because it was less than 1% of the alveolar CO tension at the start of breath holding. Between the measurements we observed the ventilation visually and inserted a minimal period of 5 minutes between each observed sigh and the next measurement [4, 11, 12, 18]. The "Masterlab Transfer" used a heat conductivity type He analyzer, which is sensitive to CO_2. Therefore, CO_2 is absorbed prior to both He and CO analysis. The expiratory gas concentrations were corrected for an absorbed volume corresponding with 5% CO_2 [19]. D_{LCO} and D_{LCO}/V_A were expressed in $\mu mol.s^{-1}.kPa^{-1}$ and $\mu mol.s^{-1}.kPa^{-1}.l^{-1}$ respectively.

Effects of variation in Hb concentration during the period of chemotherapy were eliminated by correction according to the procedure described by Cotes [20] and advised by the ECCS [1]. To compare the diffusion results before, during and after the treatment with bleomycin we corrected all D_{LCO} and D_{LCO}/V_A values to a patient's Hb concentration before the treatment.

Protocol of the lung function study

Spirometry, performed with a water-sealed spirometer, and the D_{LCO}/V_A vs V_A relationships were determined before, after 2 and 4 chemotherapeutic treatments, and ½ and 1 year after the last treatment.

Chemotherapy regime

Patients were treated with combination chemotherapy, consisting of cisplatin 20 $mg.m^{-2}$ days 1-5, etoposide 100 $mg.m^{-2}$ days 1-5 and bleomycin 30 mg i.v. push on day 2, 9 and 16. Courses were repeated every 3 weeks. The maximum total dose of bleomycin was 360 mg.

RESULTS

In all patients the Hb concentration was significantly decreased with respect to the initial Hb concentration due to the chemotherapy (Tables 4.1 and 4.2; paired t-test P-value< 0.00001 after two and four treatments respectively). After two treatments with bleomycin the mean decrease in Hb concentration was 11% and after four treatments 19%.
We separated the patients in two groups, one in which TLC decreased more than 10% of the pretreatment TLC (Table 4.1.) and another in which TLC was less than 10% different during the chemotherapy from baseline TLC (Table 4.2.).

D_{LCO}/V_A vs V_A relationships

A typical example of the relationships between the diffusion variables and V_A before and after 4 treatments with bleomycin (solid lines) and after a period of ½ and 1 year recovery respectively (dotted lines), is given in Fig. 4.1 for a patient in which both D_{LCO}/V_A, D_{LCO} and TLC are decreased due to chemotherapy. The dashed lines in this figure represent the volume dependent reference values according to the RCL method [12]. The D_{LCO}/V_A vs V_A relationship before treatment is close to this reference line. The D_{LCO}/V_A vs V_A relationship after 4 treatments nearly runs parallel to this reference equation. The D_{LCO}/V_A vs V_A relationship was increased after a half year of recovery, but did not improve further in the next half year. If we regard the initial value of D_{LCO}/V_A at the initial TLC to be 100%, we observed a decrease in D_{LCO}/V_A at the symptom limited TLC after 4 treatments with bleomycin of 25% of the pretreatment value at TLC. When we compared D_{LCO}/V_A after 4 treatments with the pretreatment D_{LCO}/V_A at the same volume level as the symptom limited TLC we observed a decrease by 36%. An example of a patient, in which we observed a decrease in the diffusion variables due to 4 treatments with bleomycin without a change in TLC is given in Fig. 4.2. This patient already had a decreased diffusing capacity before the treatment with bleomycin. However, the relationships of D_{LCO} vs V_A and D_{LCO}/V_A vs V_A before treatment were in parallel to his corresponding reference lines. The relationship after the chemotherapy, remained in parallel to the volume dependent reference equations [12]. Half and one year after the treatment the relationships were partly recovered and remained in parallel to the initial relationships.

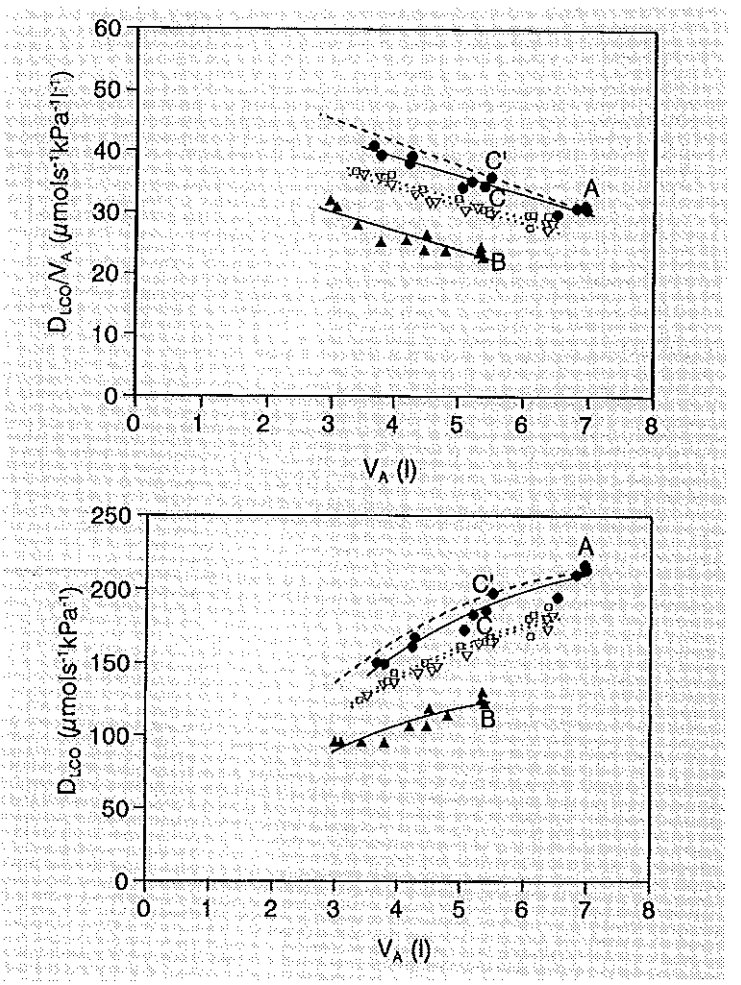

Fig. 4.1. *An individual example of D_{LCO}/V_A vs V_A and D_{LCO} vs V_A relationships respectively before and after four courses with bleomycin containing chemotherapy (solid lines) and after a half and one year recovery (dotted lines) in a patient, who developed a diffusion disorder as well as a volume restriction. The dashed lines represent the volume dependent reference values [12].*

- ● *: Before chemotherapy.*
- ▲ *: After four courses with bleomycin containing chemotherapy.*
- ▫ *: Half a year after the last treatment.*
- ▽ *: One year after the last treatment.*
- *A* *: D_{LCO}/V_A and D_{LCO} values at initial TLC.*
- *B* *: D_{LCO}/V_A and D_{LCO} values at the disease limited TLC after four courses with bleomycin containing chemotherapy.*
- *C* *: D_{LCO}/V_A and D_{LCO} values before chemotherapy at an alveolar volume equal to the TLC after chemotherapy.*
- *C'* *: D_{LCO}/V_A and D_{LCO} reference values at an alveolar volume equal to the TLC after chemotherapy.*

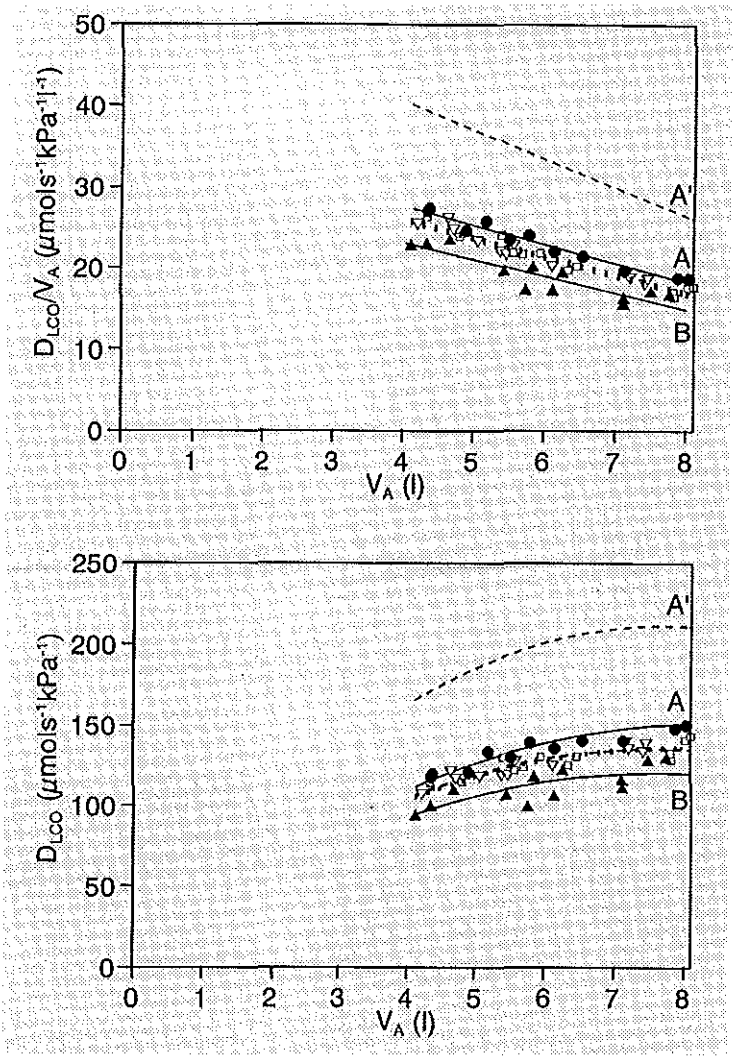

Fig. 4.2. *An individual example of D_{LCO}/V_A vs V_A and D_{LCO} vs V_A relationships respectively before and after four courses with bleomycin containing chemotherapy (solid lines) and after a half and one year recovery (dotted lines) in a patient, who developed a diffusion disorder without a volume restriction. The dashed lines represent the volume dependent reference values [12].*

- ● : *Before chemotherapy.*
- ▲ : *After four courses with bleomycin containing chemotherapy.*
- □ : *Half a year after the last treatment.*
- ▽ : *One year after the last treatment.*
- *A* : *D_{LCO}/V_A and D_{LCO} values at initial TLC.*
- *B* : *D_{LCO}/V_A and D_{LCO} values at TLC after four courses with bleomycin containing chemotherapy.*
- *A'* : *D_{LCO}/V_A and D_{LCO} reference values.*

Table 4.1. Change in Hb concentration, TLC and D_{LCO}/V_A vs V_A relationship dependent on the stage of the chemotherapy in the patients, who developed a restrictive ventilatory defect.

Nr.	After bleomycine treatment and recovery	Hb (mmol.l^{-1})	TLC (l)	$D_{LCO}/V_A = a - bV_A$			D_{LCO}/V_A μmol.s^{-1}.kPa^{-1}.l^{-1} (%Pretreatment)	
				b	a	r	At actual TLC	At equal lung volume *
1	pre	9.6	8.00	3.91	60.21	-0.98	28.9(100)	34.0(100)
	2t	8.7	7.77	4.44	60.36	-0.96	25.9(90)	30.6(90)
	4t	8.0	6.71	4.30	53.83	-0.98	25.0(87)	25.0(74)
	½y	9.7	7.13	4.11	53.07	-0.97	23.8(82)	25.5(75)
	1y	9.3	7.35	4.26	56.27	-0.98	25.0(87)	27.7(81)
2	pre	9.8	7.56	2.31	40.23	-0.99	22.8(100)	24.6(100)
	2t	8.2	7.91	2.76	41.37	-0.99	19.5(86)	22.7(92)
	4t	5.6	6.78	2.30	34.62	-0.99	19.0(83)	19.0(77)
3	pre	9.5	7.13	3.02	51.16	-0.97	29.6(100)	33.9(100)
	2t	7.9	7.21	3.04	45.10	-0.96	23.2(78)	27.7(82)
	4t	6.2	5.72	3.09	39.28	-0.89	21.6(73)	21.6(64)
	½y	8.8	6.54	2.90	46.77	-0.97	27.8(94)	30.2(89)
	1y	9.2	6.61	2.82	45.83	-0.98	27.2(92)	29.7(88)
4	pre	9.3	9.43	1.24	31.02	-0.93	19.3(100)	21.0(100)
	2t	7.6	9.39	1.39	31.61	-0.95	18.6(96)	20.3(97)
	4t	6.5	8.11	1.83	32.55	-0.92	17.7(92)	17.7(84)
5	pre	9.7	7.42	3.03	43.55	-0.92	21.1(100)	24.6(100)
	2t	6.4	7.15	2.93	41.36	-0.95	20.4(97)	23.0(93)
	4t	6.8	6.26	2.39	37.06	-0.80	22.1(105)	22.1(90)
6	pre	7.6	5.67	3.66	43.28	-0.95	22.5(100)	25.2(100)
	2t	5.7	5.62	3.32	37.42	-0.92	18.8(84)	21.0(83)
	4t	6.0	4.95	3.06	33.61	-0.91	18.5(82)	18.5(73)

For explanation see Table 4.2

Table 4.2. Change in Hb concentration, TLC and D_{LCO}/V_A vs V_A relationship dependent on the stage of the chemotherapy in the patients, who did not develop a restrictive ventilatory defect.

Nr.	After bleomycine treatment and recovery	Hb (mmol.l^{-1})	TLC (l)	$D_{LCO}/V_A = a - bV_A$			D_{LCO}/V_A μmol.s^{-1}.kPa^{-1}.l^{-1} (% pretreatment)	
				b	a	r	At actual TLC	At equal lung volume *
1	pre	10.2	6.96	2.08	41.81	-0.97	27.3(100)	27.8(100)
	2t	7.8	7.12	2.56	41.22	-0.95	23.0(84)	24.0(86)
	4t	8.0	6.74	2.44	42.49	-0.98	26.0(95)	26.0(94)
	½y	9.6	6.70	2.05	39.69	-0.97	26.0(95)	25.9(93)
	1y	10.0	6.44	1.75	36.99	-0.88	25.7(94)	25.2(91)
2	pre	9.1	8.46	2.12	35.88	-0.96	17.9(100)	19.1(100)
	2t	8.5	8.23	2.08	33.92	-0.93	16.8(94)	17.5(92)
	4t	6.8	7.91	2.10	31.96	-0.93	15.4(86)	15.4(81)
	½y	9.6	8.26	2.34	35.58	-0.97	16.3(91)	17.1(90)
	1y	10.3	7.62	2.13	34.13	-0.98	17.9(100)	17.3(91)
3	pre	9.8	6.88	4.41	55.92	-0.96	25.6(100)	26.1(100)
	2t	9.1	6.90	4.96	53.34	-0.96	19.1(75)	19.8(76)
	4t	5.9	6.76	4.79	52.47	-0.91	20.1(79)	20.1(77)
	½y	8.9	6.41	5.01	55.08	-0.99	23.0(90)	21.2(81)
	1y	8.8	6.49	5.78	60.63	-0.99	23.1(90)	21.6(83)
4	pre	7.3	7.40	2.82	41.14	-0.91	20.3(100)	19.4(100)
	2t	6.4	8.09	2.95	41.68	-0.88	17.8(88)	19.0(98)
	4t	5.9	7.70	2.90	42.37	-0.88	20.0(99)	20.0(103)
	½y	8.0	7.46	2.99	42.80	-0.89	20.5(101)	19.8(102)
	1y	8.6	8.10	3.10	43.95	-0.93	18.8(93)	20.1(104)
5	pre	9.8	7.89	4.29	57.01	-0.96	23.2(100)	23.8(100)
	2t	8.0	8.59	3.83	52.35	-0.93	19.5(84)	22.7(95)
	4t	7.8	7.75	4.19	51.74	-0.91	19.3(83)	19.3(81)
	½y	9.4	7.61	4.60	60.22	-0.94	25.2(109)	24.6(103)

6	pre	8.1	6.30	3.04	38.52	-0.91	19.4(100)	18.2(100)
	2t	6.5	6.62	2.78	34.04	-0.92	15.6(80)	15.5(85)
	4t	5.5	6.67	2.42	32.47	-0.91	16.3(84)	16.3(90)
	½y	8.2	6.66	2.02	32.70	-0.88	19.3(99)	19.2(105)
7	pre	9.1	7.73	1.47	36.54	-0.91	25.2(100)	25.3(100)
	2t	8.3	7.55	1.18	31.08	-0.77	22.2(88)	22.0(87)
	4t	7.0	7.66	2.66	38.17	-0.94	17.8(71)	17.8(70)
	½y	8.4	7.19	1.87	35.45	-0.83	22.0(87)	21.1(83)

pre - *Before chemotherapy*

2t - *After two treatments with bleomycin*

4t - *After four treatments with bleomycin*

½y - *Half a year after the last (fourth) treatment with bleomycin*

1y - *A year after the last (fourth) treatment with bleomycin*

* - *Lung volume is TLC after four Bleomycin courses*

In Table 4.1 patients 2 and 4-6 and in Table 4.2 patients 5-7 the protocol is not finished yet.

Within parentheses we expressed D_{LCO}/V_A in percentage of pretreatment value at pretreatment TLC and at similar lung volume respectively.

In all patients we found a linear relationship between D_{LCO}/V_A and V_A (Tables 4.1 and 4.2). The slope *b* of the D_{LCO}/V_A vs V_A relationships, corrected to the initial Hb concentration, was not significantly changed by four chemotherapeutic treatments (paired t-test P-value=0.30). However, the relationships of D_{LCO}/V_A vs V_A shifted downwards, implying that D_{LCO}/V_A at TLC after four treatments with bleomycin was significantly decreased with respect to its pretreatment D_{LCO}/V_A at the same lung volume (paired t-test P-value= 0.001 and 0.01 in both groups respectively).

In the Tables 4.1 and 4.2 we presented the comparison of D_{LCO}/V_A at actual TLC during chemotherapy with the pretreatment D_{LCO}/V_A at pretreatment TLC and the D_{LCO}/V_A values before and during bleomycin treatment and recovery at a lung volume equal to the TLC after four treatments with bleomycin. In the patients who developed a volume restriction (Table 4.1) the comparison of D_{LCO}/V_A at equal lung volume resulted in a larger relative decrease in D_{LCO}/V_A than its comparison with D_{LCO}/V_A at pretreatment TLC (paired t-test P-value=0.0007). In the patients who did not develope a volume restriction (Table 4.2) both methods to evaluate the decrease in D_{LCO}/V_A lead to similar relative changes (paired

t-test P-value=0.86). After four bleomycin courses mean D_{LCO}/V_A was 77% and 85% of the pretreatment D_{LCO}/V_A at the same volume level in the patients with and without a development of a restrictive lung volume respectively. These percentual D_{LCO}/V_A values were not significantly different (P-value=0.19).

DISCUSSION

D_{LCO}/V_A vs V_A relationship

Bleomycin did not change the individual slopes of the D_{LCO}/V_A vs V_A relationships. In 12 out of 13 patients these relationships shifted significantly downwards with increasing bleomycin dose. An unchanged slope of D_{LCO}/V_A vs V_A is in support of our hypothesis that the effect of decreasing lung volume by bleomycin treatment is similar to the effect of voluntary reduction in lung volume in healthy volunteers. Furthermore, it might be an indication that alveolar membrane expansion is unchanged.

D_{LCO} and D_{LCO}/V_A at actual TLC after chemotherapy is usually compared with the diffusion indexes at TLC before treatment [14, 21]. In the patients of table 4.2 a decrease in level of the D_{LCO}/V_A vs V_A relationship was observed without a significant decrease in TLC. In such group of patients the usual comparison can be maintained. In the patients who developed a volume restriction (Table 4.1) the difference between D_{LCO}/V_A at actual TLC during chemotherapy and the pretreatment D_{LCO}/V_A at TLC before chemotherapy was significantly smaller than the difference in D_{LCO}/V_A at comparable lung volumes in those stages of the treatment where lung volume was decreased (Fig. 4.1). We concluded that the comparison of D_{LCO}/V_A at the disease limited TLC with the D_{LCO}/V_A at the initial or reference TLC implies an underestimation of the diffusion disorder.

To evaluate the individual decrease in total diffusing capacity we compared D_{LCO} after chemotherapy with the pretreatment D_{LCO} at initial TLC. The difference between both values reflects the total effect on D_{LCO} by reduction in hemoglobin, volume restriction and alveolar capillary diffusion disorder. To evaluate the alveolar capillary diffusion disorder, we eliminate the effect of hemoglobin by correction, and compare D_{LCO}/V_A after chemotherapy with pretreatment D_{LCO}/V_A at a lung volume equal to the symptom limited TLC.

For routine lungfunction testing the estimation of D_{LCO} and D_{LCO}/V_A at a large number of alveolar volumes is time consuming. Therefore, we would recommend to determine the relationship between D_{LCO}/V_A and V_A before chemotherapy and to estimate D_{LCO}/V_A during the courses of medication at the actual TLC only.

If the pretreatment diffusion indexes are already decreased (Fig. 4.2), a decrease as large as in patients with normal pretreatment values could imply a decrease to a critical level of gas exchange. In these patients it might be important to follow up D_{LCO} and D_{LCO}/V_A more frequently during the treatment and to compare them to their pretreatment as well as their reference values [12].

REFERENCES

1. Quanjer PhH. Standardized lung function testing. *Bull. Eur. Physiopathol. Respir.* 1983; 19: Suppl. 5.
2. Billiet L. De bepaling van de Pulmonaire Diffusie Capaciteit. (Thesis) Leuven 1965; University of Leuven.
3. Cadigan JB, Marks A, Ellicott MF, Jones RH, and Gaensler EH. An analysis of factors affecting the measurement of pulmonary diffusing capacity by the single breath method. *J. Clin. Invest.* 1961; 40: 1495-1514.
4. Cotton DJ, Taher F, Mink JT, and Graham BL. Effect of volume history on changes in D_{LCO}^{SB}-3EQ with lung volume in normal subjects. *J. Appl. Physiol.* 1992; 73(2): 434-439.
5. Gurtner GH, and Fowler WS. Interrelationships of factors affecting pulmonary diffusing capacity. *J. Appl. Physiol.* 1971; 30: 619-624.
6. Lipscomb DJ, Patel K, and Hughes JMB. Interpretation of increases in the transfer coefficient for carbon monoxide (TL_{CO}/V_A or K_{CO}). *Thorax* 1978; 33: 728-733.
7. Marks A, Cugell DW, Cadigan JB, and Gaensler EA. Clinical determination of the diffusing capacity of the lungs. *Am. J. Med.* 1957; 22: 51-73.
8. McGrath MW, and Thomson ML. The effect of age body size and lung volume change on alveolar-capillary permeability and diffusing capacity in man. *J. Physiol. Lond.* 1959; 146: 572-582.
9. Rosenberg E, and Young RC. Potential value of diffusing capacity per liter of lung volume (D_{LCO}/V_A) for early detection of alveolar capillary defects. *Lung* 1979; 157: 23-29.
10. Stam H, Versprille A, and Bogaard JM. The components of the carbon monoxide diffusing capacity in man dependent on alveolar volume. *Bull. Eur. Physiopathol. Respir.* 1983; 19: 17-22.
11. Stam H, Kreuzer FJA, and Versprille A. Effect of lung volume and positional changes on pulmonary diffusing capacity and its components. *J. Appl. Physiol.* 1991; 71: 1477-1488.

12. Stam H, Hrachovina V, Stijnen T, and Versprille A. Diffusing capacity dependent on lung volume and age in normal subjects. *J. Appl. Physiol.* 1994; 76(6): 2356-2363.
13. Blum R, Carter SK, Agre K. A clinical review of bleomycin: a new antineoplastic agent. *Cancer* 1973; 31: 903-914.
14. Ginsberg SJ, and Comis RL. The pulmonary toxicity of antineoplastic agents. *Semin. Oncol.* 1982; 9: 34-51.
15. Pascual RS, Mosher MD, Sikaud RS, de Conti RC, and Bouhuys A. Effects of bleomycin on pulmonary function in man. *Am. Rev. Respir. Dis.* 1973; 108:211-217.
16. Comis RL, Kuppinger MS, Ginsbert SJ, Crooke ST, Gilbert R,and Auchintloss JH. Role of single-breath carbon monoxide diffusing capacity in monitoring the pulmonary effects of bleomycin in germ cell tumour patients. *Cancer Res.* 1979; 39: 5076-5080.
17. Perez-Guerra F, Harkleroad LE, Walsh RE, and Costanzi JJ. Acute bleomycin lung. *Am. Rev. Respir. Dis.* 1972; 106: 909-913.
18. Lebecque P, Mwepu A, Veriter C, Rodenstein D, Nemery B, and Frans A. Hysteresis of the alveolar capillary membrane in normal subjects. *J. Appl. Physiol.* 1986; 60: 1442-1445.
19. Ferris BG. Epidemiology standardization project. *Am. Rev. Respir. Dis.* 1978; 118 (suppl.): 62-72.
20. Cotes JE. *Lung Function: Assessment and Application in Medicine* (4th ed.). 1979; Oxford, UK: Blackwell.
21. Sorenson PG, Rossing N, and Rorth M. Carbon monoxide diffusing capacity: A reliable indicator of bleomycin-induced pulmonary toxicity. *Eur. J. Respir. Dis.* 1985; 66: 333-340.

CHAPTER 5

ESTIMATION OF THE CO TRANSFER FACTOR OF THE LUNGS DURING SPONTANEOUS BREATHING

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This chapter has been submitted for publication.

The diffusing capacity of carbon monoxide (D_{LCO}) and D_{LCO} normalized to alveolar volume (D_{LCO}/V_A) are usually estimated with the single breath method at total lung capacity (TLC) [for references see 1]. With this method a breath holding period of 10 s at TLC-level is necessary. Not all severely ill patients are able to perform this procedure. Some cannot hold their breath for 10 s at TLC and others have a too small vital capacity (VC). For a proper single breath procedure a minimal VC of about 1.5 l is necessary [2, 3]. To study the diffusion variables in such patients rebreathing methods have been developed [4-11]. In these methods CO_2 was not absorbed and O_2 not supplied. Sølvsteen [12-14] described a system applicable during increased ventilation, in which O_2 was supplied, but CO_2 was not absorbed. As a consequence, the measurement period of these methods is short. Furthermore, the measurements are usually performed during voluntary hyperventilation to approximate one compartment for the alveolar volume, the dead space and the volume in the rebreathing device.

Patients who are too ill to perform a single breath test, also will have problems with a hyperventilation procedure. Therefore, we developed a rebreathing method at normal, spontaneous resting ventilation. Then CO_2 has to be absorbed and O_2 supplied. Because our rebreathing procedure is different from the other rebreathing methods mentioned above, we derived reference equations from our results in both adults between 20 and 70 years and children between 6 and 20 years of age.

To study whether the rebreathing method at resting ventilation is reliable to detect a diffusion disorder, we compared in different types of patients the diffusion indexes, expressed in percentage of the reference values, with those obtained with the single breath procedure, also in percentage of the corresponding reference values.

METHODS

Normal Subjects

In 196 healthy volunteers we determined D_{LCO} and D_{LCO}/V_A with the rebreathing method after informed consent. The protocol was approved by the Erasmus University review board for human studies. The population was recruited from citizens of Rotterdam and its suburbs, an industrial Dutch area. All were caucasians without any sign of a respiratory

disease. They had no history of chronic pulmonary or cardiac disease, thoracic surgery or any other disease which might influence the respiratory system or the general state of health. During three weeks prior to the investigation the volunteers did not experience an upper respiratory tract infection. They were nonsmokers and had no contact with harmful substances, which could affect the lung function. We separated the volunteers in two age groups: one from 6 to 20 years (n=103, 53♀ and 50♂) and the other from 20 to 70 years (n=93, 40♀ and 53♂). We selected normal adults with a weight range within 20% of ideal body weight. To estimate ideal body weight we used the modified Metropolitan Life Insurance Company charts [15]. All adults had normal lung volumes in % of the reference values of the European Community for Coal and Steel (ECCS) [3] and a normal single breath D_{LCO} in % of reference values at TLC [16], (mean ± SD); males: TLC = 104 ± 9%; VC = 108 ± 11%; FEV_1 = 102 ± 12%; D_{LCO} single breath = 98 ± 10% and females: TLC = 107 ± 9%; VC = 110 ± 13%; FEV_1 = 104 ± 11%; D_{LCO} single breath = 93 ± 11%.

For the group between 6 and 20 years of age we selected a representative sample of normal Dutch children. In 95% of the children weight and height were between the 3rd and 97th percentiles of the Health Interview Survey of Statistics Netherlands [17] respectively. The remaining volunteers were taller and the weight was larger. We compared their pulmonary function data with the references of Zapletal [18], (mean ± SD); boys: TLC = 93 ± 10%; VC= 98 ± 8%; FEV_1 = 98 ± 10%; D_{LCO} single breath = 101 ± 11% and girls: TLC = 93 ± 9%; VC= 101 ± 9%; FEV_1 = 103 ± 10%; D_{LCO} single breath = 94 ± 10%.

Patients

We compared the diffusion indexes obtained with the rebreathing method with those obtained with the single breath method in 33 patients. We determined the mean of three values with each method. Because the single breath and rebreathing method were performed at different lung volumes, TLC and FRC + ½V_T respectively, we expressed the diffusion indexes in percentage of the corresponding reference values. In the patients with a restrictive lung function, we used single breath reference values, in which the smaller TLC than reference TLC was taken into account [16].

We compared both methods in patients with equal as well as unequal distribution of

Table 5.1. Anthropometric and lung function data of a group of patients without ventilation distribution disturbances. The lung function data are expressed as a percentage of reference values [3].

no	sex m/f	Age (y)	H (m)	W (kg)	Hb (mmol.l^{-1})	Diagnosis	TLC (mb) (%)	VC (%)	RV (%)	FEV_1/VC (%)	TLC (sb/mb)
1	f	61	1.55	72	6.3	Hepato-Pulm. Syndrome.	99	112	90	69	0.98
2	f	34	1.58	50	8.2	Fibrosis	58	45	88	43	0.91
3	f	65	1.60	65	7.9	Fibrosis	59	73	46	99	0.97
4	m	32	1.76	63	6.2	Status after bleomycin	82	84	75	95	0.96
5	m	39	1.82	84	8.3	Status after Hodgkin	67	67	73	81	0.96
6	m	62	1.80	68	7.7	Post HTX	88	83	104	94	0.88
7	m	65	1.81	73	10.2	Emphysema + CHF	99	110	89	70	0.91
8	f	71	1.50	56	8.6	Sarcoidosis	71	80	71	98	0.98
9	m	63	1.98	95	10.3	Emphysema +Fibrosis	91	94	96	72	0.88
10	f	57	1.60	68	8.8	Sjögren Syndrome	90	115	57	105	1.00
11	m	20	1.85	77	9.7	Status after bleomycin	91	88	103	100	0.99
12	m	49	1.72	97	8.7	Post Tuberculosis	92	94	92	95	0.91
13	m	28	1.71	90	10.0	Status after bleomycin	98	101	86	85	1.00
14	f	46	1.64	72	6.4	LIP	55	55	58	106	0.97
15	f	73	1.54	63	6.4	CHF	107	123	104	90	0.90
16	m	29	1.87	74	6.4	Status after bleomycin	102	90	143	113	1.00
17	m	57	1.75	70	9.1	Pneumonectomy + RTh	83	79	96	100	0.92
18	m	42	1.86	94	9.0	Sarcoidosis	80	79	86	82	0.97
19	f	22	1.63	59	7.6	Alv. Proteinosis	69	64	80	102	0.93
20	m	69	1.72	50	7.8	COPD	68	62	84	98	0.91
21	m	58	1.78	95	8.5	Pleural Thickening	100	106	95	93	0.99
22	m	19	1.83	91	8.6	Status after bleomycin + RTh	96	103	73	96	1.00

Table 5.2. Anthropometric and lung function data of a group of patients with ventilation distribution disturbances. The lung function data are expressed as a percentage of reference values [3].

no	sex m/f	Age (y)	H (m)	W (kg)	Hb (mmol.l^{-1})	Diagnosis	TLC (mb) (%)	VC (%)	RV (%)	FEV_1/VC (%)	TLC (sb/mb)
1	m	63	1.74	76	8.4	Asthma	125	131	125	77	0.84
2	m	68	1.75	83	9.4	Emphysema	89	92	91	64	0.84
3	m	77	1.63	53	8.3	Status after thoracotomy	76	64	96	112	0.82
4	m	64	1.69	65	8.5	Status after RTh	78	72	92	88	0.85
5	m	64	1.71	61	7.6	COPD recurrent Aspir.	91	80	117	50	0.70
6	m	66	1.77	93	9.5	Status after HTX	88	55	152	64	0.62
7	m	70	1.85	98	8.6	Emphysema	96	75	143	58	0.73
8	f	64	1.53	44	8.0	Fibrosis	53	57	53	100	0.69
9	m	73	1.72	62	8.2	Emphysema	118	82	181	43	0.76
10	f	41	1.67	107	7.9	Status after HTX	107	85	156	78	0.84
11	f	64	1.52	62	..	Hepato-Pulm. Syndrome	108	126	97	85	0.84

ventilatory air. Ventilation distribution was evaluated on basis of the ratio between TLC determined with the single breath test (TLC_{sb}) and TLC determined with the multiple breath He washin method (TLC_{mb}). A TLC_{sb}/TLC_{mb} ratio larger than 0.85 has been regarded as an indication for normal ventilation distribution [19]. In the group of patients we corrected the diffusion indexes for abnormal Hb concentrations. This correction was performed according to the procedure described by Cotes and recommended by the ECCS [3, 20]. We used 9.2 $\pm$ 0.5 (SD) mmol.l^{-1} (n=120) and 8.3 $\pm$ 0.5 (SD) mmol.l^{-1} (n=120) as reference Hb concentration in men and women respectively. These reference values for Hb concentration in a group of healthy volunteers with the same demographic background, have been determined in the Laboratory for Clinical Chemistry of our hospital. The lung function data of these patients are presented in Tables 5.1 and 5.2.

Single breath method

We followed the single breath procedure as recommended by the ECCS [3]. The subjects expired to residual volume (RV) and inspired vital capacity (VC) of a gas mixture containing about 0.25% CO, 5% He, 20,9% O_2 and balance N_2, and held their breath at TLC. Breath holding time was taken to start when 30% of the volume had been inspired, and to end when half of the expired sample had been expired [21]. Overall breath holding time slightly exceeded 10 s. Inspirations and expirations were performed rapidly.

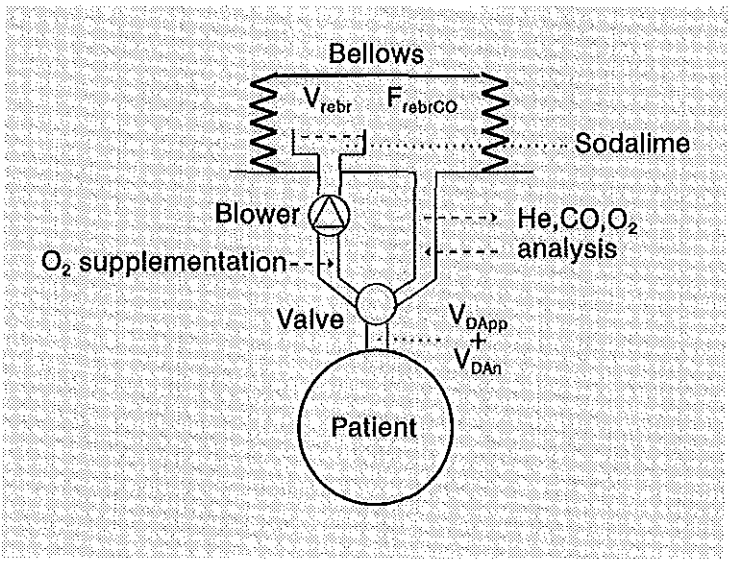

Fig. 5.1. *Rebreathing circuit used to measure FRC and diffusing capacity. The valve near the mouth permitted the volunteers to breath room air before connection to the rebreathing system. A further description is given in the text. V_{rebr}: Volume of rebreathing system before the connection of the patient; F_{rebrCO}: CO fraction in the rebreathing system; V_{DApp}: Dead space of the apparatus; V_{DAn}: Anatomical dead space.*

Alveolar fractions of CO and He were obtained from expired gas after discarding 800 ml for washout of airways and apparatus dead space. The size of the alveolar sample was 800 ml. Maneuvers were performed with a "Masterlab Transfer" (Jaeger, Würzburg, Germany). The interval between consecutive measurements was 5 min. To minimize CO back tension, we restricted the number of consecutive measurements to six. In the single breath procedures back tension was ignored, because it was less than 1% of the alveolar CO tension at the start of breath holding. We used a heat conductivity type He analyzer, which is sensitive to CO_2. Therefore, we absorbed CO_2 prior to both He and CO analysis.

The remaining gas concentrations were corrected for an absorbed volume corresponding to 5% CO_2 [22].

D_{LCO} and D_{LCO}/V_A were expressed in $\mu mol.s^{-1}.kPa^{-1}$ and $\mu mol.s^{-1}.kPa^{-1}.l^{-1}$ respectively.

Rebreathing method

Our rebreathing system (Fig. 5.1) consisted of a bellows, which was compensated for its weight by a rolling string, a soda lime container, a blower, a tubing system and a valve at the mouthpiece. During a rebreathing procedure CO_2 was fully absorbed by soda lime. O_2 concentration was kept between 20 and 22%, guided by measurements of O_2 concentration. O_2 was supplied to the tube through which the patient's expiratory air returned to the bellows, where mixing occurred before the next inspiration. The minimum volume of bellows, ventilator, tubes and valve was about 2.5 l and was estimated before each observation by means of He dilution. The ventilation of a volunteer or a patient was measured with a displacement transducer (Schaevitz Type 3002 XS-D), connected to the bellows. Because we intended to analyse only slowly changing gas concentrations in the rebreathing system, we used relatively slow gas analyzers. He, CO and O_2 concentrations were analyzed continuously, using a heat conductivity type He analyzer, an infrared CO analyzer and a paramagnetic O2 analyzer (Jaeger, Würzburg, Germany). The gases returned to the system after analysis. A computer sampled the signals of He, CO, O_2 and volume at a frequency of 20 Hz. Before each measurement the rebreathing system was filled with 5% He, 0.3% CO, 20.9% O_2 and balance N_2. The apparatus dead space between patient and valve was 20 ml. The patient was connected via this valve to the system at end-expiration. Functional residual capacity, FRC, was estimated by the distribution of He. In a pilot study we measured the temperature and relative humidity during the rebreathing procedure in the bellows system. After a few minutes of rebreathing temperature stabilized at 25°C and the relative humidity appeared to be 100%. We calculated FRC from the He mass balance and corrected it to BTPS conditions, assuming an equilibrium temperature equal to 25°C and a relative humidity of 100%. After He dilution was completed the exponential decay in CO fraction in the bellows was determined until CO fraction had decreased to about 3 % of the initial F_{CO} as a measure for the total gas transport from mouth to capillary blood (Fig. 5.2), according to:

$$F_{CO_t} = F_{CO_0} e^{-kt} \quad (5.1)$$

giving:

$$\ln (F_{CO_t}) = \ln (F_{CO_0}) - kt \quad (5.2)$$

The whole procedure lasted 3 to 5 minutes in healthy volunteers and 3 to 8 minutes in the patients with a diffusion disorder. The $\ln(F_{CO})$ vs time relationship needed to be linear and the slope k was used to calculate the diffusion parameters.

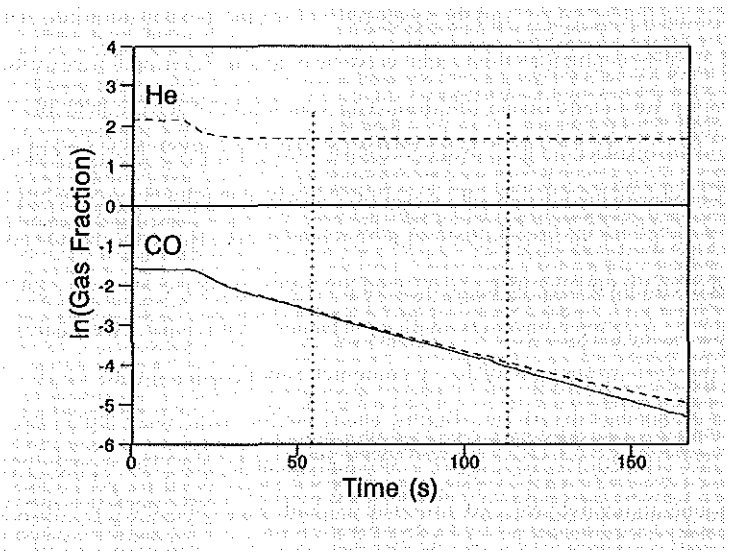

Fig. 5.2. A logarithmic recording of He- and CO-concentrations (dotted lines) and the $\ln(F_{CO})$ vs time relationship corrected for back pressure (continuous line). The exponential decay in F_{CO} between both vertical dotted lines was used for analysis.

If at low CO fractions the $\ln(F_{CO})$ vs time relationship curved upwards, we linearized it by substraction of a small CO fraction (F_{COcor}) from the measured F_{CO}, assuming that the deviation from linearity was caused by back tension. With an iterative procedure an optimal correction was found if the correlation coefficient for the linear $\ln(F_{CO}-F_{COcor})$ vs time relationship was maximal. A too large F_{COcor} deflects the $\ln(F_{CO})$ vs time relationship

downwards at low CO fractions. To test the reliability of this mathematical procedure we compared the calculated F_{COcor} with the CO fraction due to back tension (F_{COback}). Directly after the measurement of the diffusing capacity we measured the back tension by a rebreathing procedure without addition of He and CO for at least 5 minutes, according to the procedure described by Cotes [20] and recommended by the ECCS [3].

The calculated diffusion constant k for the exponential decay in CO represents the CO disappearence from the total volume of lungs and rebreathing system together (FRC+ V_{rebr}). However, with a mean tidal volume (V_T) and an anatomical dead space (V_{DAn}) CO uptake occurred in a mean alveolar volume, equal to: FRC-V_{DAn}+½V_T. Accordingly, we corrected the measured diffusion constant k according to:

$$K_{CO} = k \left(\frac{V_{rebr} + FRC}{FRC - V_{DAn} + \frac{1}{2} V_T} \right) \tag{5.3}$$

where V_{rebr} is the volume of the rebreathing system before connection of patient or volunteer, including an apparatus dead space of 0.020 liters; V_{DAn} is the reference value for the anatomical dead space in liters, found from body weight in kg times 0.0022. All volumes in this formula were recalculated to STPD conditions. The ratio between FRC and V_{rebr} determined the correction factor, which was in our system approximately 2.

At infinite ventilatory rate FRC and V_{rebr} are one compartment theoretically. At normal ventilatory rate this is not true, due to delay of mixing between both compartments. Therefore, we also studied the influence of alveolar ventilation V_A' on the rebreathing diffusing capacity. V_A' was found from total minute ventilation corrected for the reference anatomical dead space and the dead space of the apparatus (0.020 liters). We compared rebreathing D_{LCO}/V_A at various alveolar ventilations with the relationship between single breath D_{LCO}/V_A and V_A. D_{LCO}/V_A vs V_A yields a linear relationship in normals [1, 16, 23]. We varied minute ventilation in these measurements by varying the breathing frequency, while V_T was kept as constant as possible. Although we examined patients during resting ventilation, a considerable variation in ventilation occurred between subjects. Therefore, we determined reference values of the rebreathing diffusing capacity at least at three different V_A' levels, containing one measurement at resting ventilation, in each normal volunteer.

D_{LCO} and D_{LCO}/V_A were expressed in $\mu mol.s^{-1}.kPa^{-1}$ and $\mu mol.s^{-1}.kPa^{-1}.l^{-1}$ respectively.

Statistics

We performed stepwise multiple regression analysis using the SAS PROC MIXED program [24], assuming a compound symmetry covariance structure for the observations of each individual. Differences between two groups of data were regarded as significant at P-value < 0.05.

RESULTS

Mathematical correction for back tension

F_{COcor}, calculated with the iterative method (Fig. 5.2), was compared in 31 subjects with F_{COback}. We determined F_{COback} immediately after the measurement of the rebreathing diffusing capacity.

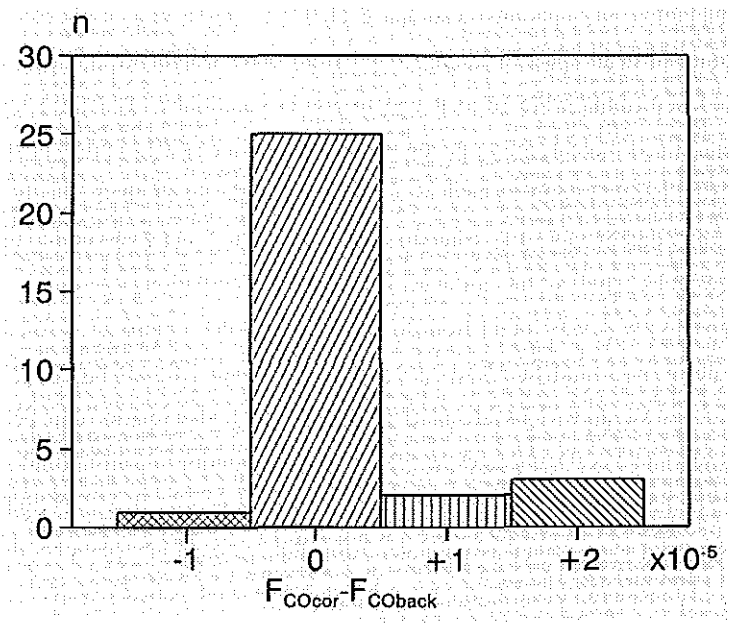

Fig. 5.3. Frequency histogram for the difference between the mathematically obtained linearization factor F_{COcor} and backpressure F_{COback} (n=31).

Mean F_{COcor} was 0.000029 with a SD of 0.000011 and mean F_{COback} 0.000027 with a SD of 0.000013. F_{COcor} was not different from F_{COback} at P-value=0.47, paired t test. The frequency histogram of the differences between the mathematically obtained correction value and the measured back tension (Fig. 5.3) illustrates that in 25 of the 31 observations F_{COcor} and F_{COback} were identical. The correction with use of F_{COback} increased D_{LCO} and D_{LCO}/V_A on average by 7 ± 4 (SD)% and the correction with use of F_{COcor} resulted in an increase of 8 ± 4 (SD)% of the uncorrected D_{LCO} and D_{LCO}/V_A values. In normal volunteers a correction for F_{COback} of 0.00001 appeared to increase D_{LCO} and D_{LCO}/V_A by 2.6 ± 0.7 (SD)%. Therefore, the maximum difference F_{COcor} - F_{COback} of 0.00002 resulted in an overestimation of D_{LCO} and D_{LCO}/V_A of about 5.2%.

Comparison of rebreathing and single breath D_{LCO}/V_A

In 7 healthy individuals we determined D_{LCO}/V_A with the single breath method at various alveolar volumes as well as D_{LCO}/V_A with the rebreathing method at various minute ventilations (Table 5.3).

A typical example of the single breath D_{LCO}/V_A vs V_A relationship and the rebreathing D_{LCO}/V_A as function of V_A' in one subject is illustrated in Fig. 5.4, where mean alveolar volume during rebreathing ($V_{A,r}$) was 3.2 ± 0.8 liters (mean ± 2SD). Above an alveolar ventilation of 35 l.min^{-1} D_{LCO}/V_A determined with the rebreathing method was similar to D_{LCO}/V_A obtained with the single breath method at the corresponding alveolar volume of 3.2 ± 0.8 liters (2SD). This result illustrates the dependence of the rebreathing D_{LCO}/V_A on the alveolar ventilation V_A'. The two smallest D_{LCO}/V_A and alveolar ventilation values in Fig. 4b were obtained during resting ventilation. If alveolar ventilation increases, D_{LCO}/V_A increases linearly with V_A' up to a value of 20 l.min^{-1}. The same was found in all 7 volunteers as shown in Table 5.3. Above an alveolar ventilation of 30 l.min^{-1} the mean of the absolute values of the rebreathing D_{LCO}/V_A was 97 ± 7(SD)% of the single breath D_{LCO}/V_A at a comparable level of V_A. Although this value was significantly different from 100% (P-value=0.03) the D_{LCO}/V_A values during hyperventilation can be regarded similar to the single breath values.

Table 5.3. Comparison of single breath and rebreathing D_{LCO}/V_A at the same alveolar volume and at normal as well as hyperventilation.

Subj. nr.	Age	Single Breath $D_{LCO}/V_A=-aV_A+b$			$V_A'<20$ l.min^{-1} Rebreathing $D_{LCO}/V_A=cV_A'+d$			$V_A'>30$l.min^{-1} Equal V_A		
		a	b	r	c	d	r	V_A rebr l ±1SD	D_{LCO}/V_A rebr μmol.s^{-1}.kPa^{-1}.l^{-1} ±1SD	D_{LCO}/V_A sb μmol.s^{-1}.kPa^{-1}.l^{-1} ±1SD
1	45	2.95	45.2	-0.92	0.84	8.32	0.94	3.2±0.4	35.7±2.4	35.8±1.0
2	23	4.52	62.2	-0.89	0.53	17.5	0.66	4.3±0.3	40.0±4.2	42.6±1.5
3	23	4.58	63.4	-0.95	1.06	8.80	0.87	3.7±0.1	45.9±1.1	46.3±0.7
4	38	2.52	45.2	-0.84	0.89	9.74	0.99	3.2±0.1	37.2±2.2	37.1±0.3
5	57	1.65	35.0	-0.89	0.40	15.4	0.98	4.0±0.5	25.2±1.6	28.4±0.9
6	55	2.62	42.8	-0.91	16.5	0.73	0.98	2.8±0.1	36.0±1.3	35.4±1.3
7	26	3.67	53.0	-0.92	11.8	0.62	0.90	4.2±0.02	33.6±0.6	37.7±2.7

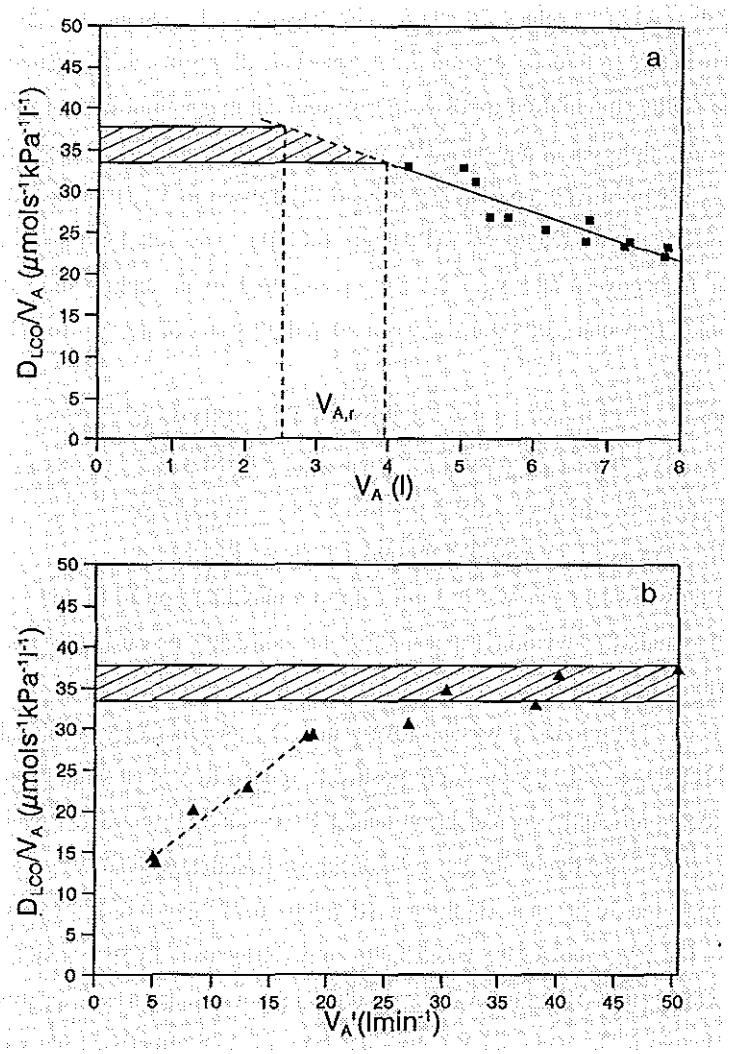

Fig. 5.4. *The single breath D_{LCO}/V_A as function of V_A (a) and the rebreathing D_{LCO}/V_A as function of V_A' (b) in a normal volunteer. $V_{A,r}$ is the alveolar volume range between the mean $\pm$ 2 SD obtained from the rebreathing maneuvers. The hatched area in a represent the range in single breath D_{LCO}/V_A corresponding to the volume range of $V_{A,r}$. This area corresponds with the hatched area in b. The dashed line in b is the linear regression line for the D_{LCO}/V_A vs. V_A' relationship up to a V_A' of 20 l.min^{-1}.*

Reference values

We estimated reference values for D_{LCO} and D_{LCO}/V_A, in which V_A and V_A' were variables. To derive reference equations we selected all results at V_A' smaller than 20 l.min^{-1} in the adults. In children (6-18 years) we took an upper limit of 15 l.min^{-1}.

In the group of adults D_{LCO} and D_{LCO}/V_A decreased significantly (P-value$<$0.0001) with age (A). D_{LCO} was positively and D_{LCO}/V_A negatively related to V_A (P-value$<$0.0001). Both were positively related to V_A' (P-value$<$0.0001). The relationships between D_{LCO} and D_{LCO}/V_A respectively and V_A, V_A' and A appeared to be significantly different for men and women (P-value$<$0.0001). Between 20 and 70 years of age we found:

Males: $D_{LCO} = 9.5V_A+3.2V_A'-0.39A+16.3$ RSD=8.0 (5.4)

$D_{LCO}/V_A = -2.7V_A+0.8V_A'-0.09A+24.6$ RSD=2.2 (5.5)

Females: $D_{LCO} = 6.2V_A+2.0V_A'-0.30A+26.9$ RSD=7.5 (5.6)

$D_{LCO}/V_A = -3.7V_A+0.6V_A'-0.09A+27.1$ RSD=2.4 (5.7)

where V_A in l, A in years and V_A' in l.min^{-1} up to a maximum of 20 l.min^{-1}.

The residual standard deviations appeared to be constant, because the residuals were equally distributed around the model independent of V_A, V_A' and A.

In the group of children D_{LCO} was positively and D_{LCO}/V_A negatively related to V_A (P-value$<$0.0001). D_{LCO} and D_{LCO}/V_A were positively related to V_A' (P-value$<$0.0001). D_{LCO} appeared to be significantly dependent on height H (P-value=0.002 and P-value$<$0.0001) in boys and girls respectively. In the relationship between D_{LCO} and V_A, V_A' and H the age effect appeared to be not significant (P-value=0.77 and 0.69) for boys and girls respectively. D_{LCO}/V_A, however, was significantly influenced by interaction between H and V_A (P-value=0.0001 and P-value$<$0.0001) in boys and girls respectively. In the relationship between D_{LCO}/V_A and V_A, V_A' and HV_A the age effect was not significant (P-value=0.06 and 0.40) for boys and girls respectively.

Boys: $D_{LCO} = 7.8V_A+2.8V_A'+23.1H-28.4$ RSD=5.2 (5.8)

$D_{LCO}/V_A = 27.9-(19.2-7.5H)V_A+1.3V_A'$ RSD=2.8 (5.9)

Girls: $D_{LCO} = 6.4V_A+2.7V_A'+28.0H-32.7$ RSD=4.5 (5.10)

$D_{LCO}/V_A = 31.7-(29.8-12.4H)V_A+1.5V_A'$ RSD=2.7 (5.11)

where H in m. In these regression equations for children the residual standard deviations

were constant and independent on V_A, V_A' and H. Both models for D_{LCO} and D_{LCO}/V_A appeared to be not different between boys and girls (P-value=0.22 and 0.13 respectively).

Application to patients

In all 33 patients the single breath and the rebreathing D_{LCO}/V_A were compared, both expressed in percentage of the corresponding reference values. The data were closely scattered around the line of equality both in patients with or without unequal ventilation (Fig. 5.5).

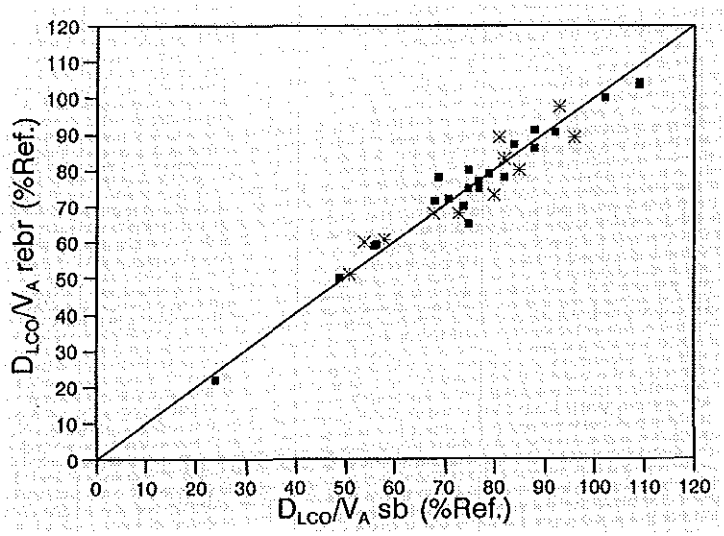

Fig. 5.5. *Comparison of rebreathing and single breath D_{LCO}/V_A, both expressed in percentage of the corresponding reference values, in patients. The solid line is the line of equality.*
- ▪ *Patients without ventilation distribution disturbances.*
- * *Patients with ventilation distribution disturbances.*

Regression equation of the first group:
D_{LCO}/V_A *rebr* $= 4.7 + 0.94(D_{LCO}/V_A$ *sb)* $r^2=0.96$
Without point (24,22) this equation is:
D_{LCO}/V_A *rebr* $= 9.3 + 0.88(D_{LCO}/V_A$ *sb)* $r^2=0.93$
Regression equation of the second group:
D_{LCO}/V_A *rebr* $= 8.1 + 0.89(D_{LCO}/V_A$ *sb)* $r^2=0.88$

The regression equation for these data in the group of patients without ventilation distribution disturbances was closer to the line of identity, than that of the patients with ventilation distribution disturbances. This was mainly caused by the results of one patient who had a D_{LCO}/V_A value of 24% of the single breath and 22% of the rebreathing

reference value. If this patient is eliminated the regression equations for both groups of patients are similar.

Table 5.4. Data on D_{LCO}/V_A estimated with the single breath and rebreathing method in patients without ventilation distribution disturbances.

patient	Single Breath Method		Rebreathing Method	
	D_{LCO}/V_A (%Ref ± SD)	SD below Ref.	D_{LCO}/V_A (%Ref ± SD)	SD below Ref.
1	49 ± 1.5	>2	50 ± 2	>2
2	74 ± 1	>2	70 ± 5	>2
3	77 ± 2	1.7	77 ± 6	>2
4	71 ± 2.5	>2	72 ± 2.5	>2
5	79 ± 1	1.9	79 ± 1	1.8
6	69 ± 1	2.0	78 ± 2.5	1.3
7	24 ± 0.5	>2	22 ± 0.5	>2
8	75 ± 5	1.8	65 ± 0.9	>2
9	56 ± 1	>2	59 ± 4	>2
10	75 ± 0.6	1.7	75 ± 3	1.9
11	82 ± 3	1.6	78 ± 5	1.4
12	88 ± 3	0.9	91 ± 2	0.7
13	92 ± 2.1	0.8	91 ± 3.8	0.9
14	56 ± 1.5	>2	59 ± 2.6	>2
15	68 ± 3	>2	71 ± 2	1.8
16	84 ± 2.7	1.2	87 ± 3.6	1.0
17	88 ± 4	0.8	86 ± 0.8	1.2
18	102 ± 2	-0.1	100 ± 1.3	0
19	75 ± 0.3	>2	80 ± 3	>2
20	77 ± 6	1.5	75 ± 2	1.7
21	109 ± 2.7	-0.6	104 ± 0.7	-0.3
22	109 ± 3	-0.7	104 ± 6.4	-0.4

Table 5.5. Data on D_{LCO}/V_A estimated with the single breath and rebreathing method in patients with ventilation distribution disturbances.

patient	Single Breath Method		Rebreathing Method	
	D_{LCO}/V_A (%Ref ± SD)	SD below Ref.	D_{LCO}/V_A (%Ref ± SD)	SD below Ref.
1	81 ± 4	1	89 ± 5	0.8
2	85 ± 4	1	80 ± 1	1.8
3	54 ± 8	>2	60 ± 8	>2
4	96 ± 3.5	0.4	89 ± 1.6	0.9
5	51 ± 3.6	>2	51 ± 1	>2
6	82 ± 1	1.1	83 ± 5.7	1.4
7	68 ± 2.6	1.8	68 ± 5.3	2
8	80 ± 3	1.6	73 ± 0.9	2
9	73 ± 3.2	1.4	68 ± 0.7	1.9
10	93 ± 2.7	0.6	98 ± 2.1	0.2
11	58 ± 5	>2	61 ± 0.8	>2

In Tables 5.4 and 5.5 we compared the relative rebreathing diffusion indexes with those of the single breath method. Paired testing of the mean values revealed that rebreathing and single breath D_{LCO}/V_A were not significantly different (P-value=0.76 and 0.96 respectively) in both groups of patients. Also the variation coefficients of the single breath and rebreathing D_{LCO}/V_A (Table 5.4: 2.9 ± 1.8 % and 3.7 ± 2.1 % and Table 5.5: 5.4 ± 3.7 % and 4.0 ± 3.9 % respectively) were not significantly different (paired t-test P-value=0.26 and 0.24 respectively) in both groups of patients.

DISCUSSION

General aspects

For the benefit of patients with limited breathing ability, who are not able to perform a single breath test or to hyperventilate during a rebreathing procedure, we developed a

rebreathing test, which can be done during resting ventilation. The decrease in CO fraction in the rebreathing system, certainly in resting conditions, depends on two components: [1]) the transfer of CO from the rebreathing system into the alveolar compartment and [2]) the CO uptake from the alveolar compartment into the capillary blood. We assumed alveolar volume as one compartment in which gas mixing is instantaneous. Also the rebreathing system was regarded as one compartment, because the gas was vigorously mixed by the ventilator. However, at normal ventilation rate even after complete washin CO concentration in lungs and rebreathing system will be different. The CO fraction in the rebreathing system will be higher than that in the alveolar compartment, where CO uptake occurs. The difference depends on alveolar ventilation. We limited our analysis to the mono exponential decay in CO after the washin of He was completed, to minimize the effect of dilution on the decay of F_{CO}.

The influence of back tension was evident, especially when several rebreathing measurements were performed consecutively. The comparison with the measured back tension revealed, that the mathematical correction is a reliable procedure for accurate estimation of the diffusion indexes.

Effect of alveolar volume and alveolar ventilation

If V_A is decreased in normal subjects, the single breath D_{LCO} will be decreased due to a smaller total alveolar surface area, and D_{LCO}/V_A will be increased due to a better alveolar surface to volume ratio [1]. Rebreathing D_{LCO} and D_{LCO}/V_A, determined at FRC, were consistently lower than these indexes obtained from the single breath test at TLC. An important reason will be the difference in alveolar volume at which both techniques are performed. Another reason might be the influence of the gas mixing between inspired air and alveolar gas. With increasing alveolar ventilation mixing between inspired air and resident gas occurs faster and therefore, D_{LCO} and D_{LCO}/V_A, determined with the rebreathing method, will be increased. Above an alveolar ventilation of 35 $l.min^{-1}$ the rebreathing indexes were equal to those of the single breath procedure at the same alveolar volume of $FRC+1/2V_T$ (Fig. 5.4). In seven healthy volunteers we found similar results (Table 5.3). No differences between the indexes from the single breath and rebreathing technique existed if V_A was the same and V_A' large enough. These results confirm those of Clark et al. [4], Felton et al. [6] and Rose et al. [9], who found similar values of

diffusion indexes with single breath and rebreathing technique if alveolar volume was the same, and at high levels of alveolar ventilation during rebreathing.

It will be obvious that a comparison of the rebreathing indexes D_{LCO} and D_{LCO}/V_A with those of the single breath test [5, 10, 25] will be acceptable only if V_A in both procedures is the same. Consequently, we regarded the results of those studies, in which D_{LCO}/V_A, determined with the rebreathing method at $FRC+\frac{1}{2}V_T$, was similar to D_{LCO}/V_A at TLC obtained with the single breath procedure [4, 5, 9, 25], to depend on a coincidence of a positive effect of the low alveolar volume and a negative effect of alveolar ventilation on D_{LCO}/V_A.

Reference values

The values which we obtained in four groups of volunteers, male and female children and adults, can be used as reference values within the limits of normal ventilation to a maximum of 20 $l.min^{-1}$ for adults and 15 $l.min^{-1}$ for children. In the reference equations alveolar ventilation is a parameter to adapt the reference value to a subject's ventilation. For a reliable estimation of the diffusion indexes it is important that alveolar ventilation is approximately constant, because an increase (or decrease) will increase (or decrease) the slope in the $\ln(F_{CO})$ vs time relationship, resulting in a larger (or lower) value of D_{LCO} and D_{LCO}/V_A. Effects of irregular respiration can be avoided by the selection of one or more shorter periods with regular ventilation during the CO decay.

Corresponding to our former paper [16] we did not correct for the normal variability in Hb concentration when estimating reference equations of D_{LCO} and D_{LCO}/V_A, because Hb concentration was normally distributed in our healthy volunteers, resulting in equal positive and negative corrections. Standard deviations were not changed by such correction, due to a low variation coefficient in the Hb concentration of about 5 %, compared to the coefficients of vatiation of the diffusion indexes, which were more than 10% [16].

Applicability

Since many years the single breath method serves to evaluate the diffusion disorders in patients. We regard the rebreathing method at normal ventilation as useful for assessing the diffusion indexes in patients who cannot perform the single breath test. We tested the

method in patients who could perform both tests. Because of the differences in lung volume, causing different values of the diffusing indexes, we compared the values relative to the corresponding reference values. The D_{LCO}/V_A values in percentage of their reference values obtained with the rebreathing method were similar to those of the single breath test in the majority of the patients both with or without ventilation distribution disturbances (Tables 5.4 and 5.5 and Fig. 5.5). Furthermore, the coefficients of variation of both methods were similar. A comparison of total diffusing capacity would have led to the same conclusions.

In conclusion, we regard the rebreathing method at resting ventilation as a reliable method to determine the diffusion indexes, D_{LCO} and D_{LCO}/V_A, for the assessment of a patient's diffusion disorder.

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REFERENCES

1. Stam H, Versprille A, and Bogaard JM. The components of the carbon monoxide diffusing capacity in man dependent on alveolar volume. *Bull. Eur. Physiopathol. Respir.* 1983; 19: 17-22.
2. Crapo RO and Gardner RM. Single breath carbon monoxide diffusing capacity (transfer factor). Recommendations for a standard technique. *Am. Rev. Respir. Dis.* 1987; 136: 1299-1307.
3. Quanjer PhH. Standardized lung function testing. *Bull. Eur. Physiopathol. Respir.* 1983; 19, Suppl.: 5.
4. Clark EH, Jones HA and Hughes JMB. Bedside rebreathing technique for measuring carbon monoxide uptake by the lung. *Lancet* 1978; 791-793.
5. Dujic Z, Eterovic D, Denoble P, Krstacic G and Tocilj J. Lung diffusing capacity in hyperbaric environment: assessment by a rebreathing technique. *Brit. J. Ind. Med.* 1992; 49: 254-259.
6. Felton C, Rose GL, Cassidy SS and Johnson RL. Comparison of lung diffusing capacity during rebreathing and during slow exhalation. *Resp. Physiol.* 1981; 43: 13-22.
7. Kreukniet J. Relation between rebreathing CO-diffusing capacity of the lung and unequal ventilation. *Scand. J. Resp. Dis.* 1970; 51: 49-54.
8. Lewis BM, Lin TH, Noe FE and Hayford-Welsing EJ. The measurement of pulmonary diffusing capacity for carbon monoxide by a rebreathing method. *J. Clin. Invest.* 1959; 38: 2073-2086.
9. Rose GL, Cassidy SS and Johnson RL. Diffusing capacity at different lung volumes during breath holding and rebreathing. *J. Appl. Physiol.* 1979; 47(1): 32-37.
10. Russell NJ, Bagg LR, Dobrzynski J and Hughes DTD. Clinical assessment of a rebreathing method for measuring pulmonary gas transfer. *Thorax* 1983; 38: 212-215.

11. Sackner MA, Greeneltch D, Heiman MS, Epstein S and Atkins N. Diffusing capacity, membrane diffusing capacity, capillary blood volume, pulmonary tissue volume and cardiac output measured by a rebreathing technique. *Am. Rev. Resp. Dis.* 1975; 111: 157-165.

12. Sølvsteen P. Measurement of lung diffusing capacity by means of $C^{14}O$ in a closed system. *J. Appl. Physiol.* 1964; 19(1): 59-74.

13. Sølvsteen P. Lung diffusing capacity: cyclically and continuously ventilated closed systems. *J. Appl. Physiol.* 1965; 20(1): 92-98.

14. Sølvsteen P. Lung diffusing capacity: rebreathing method, applicability in nonuniform ventilation. *J. Appl. Physiol.* 1965; 20(1): 99-102.

15. Blackburn GL, Benotti PN, Bistrian BR, Bothe A, Maini BS, Schlamm HT, and Smith MF. Nutritional assessment and treatment of hospital malnutrition. *Infusionstherapie* 1979; 6: 238-250.

16. Stam H, Hrachovina V, Stijnen T and Versprille A. Diffusing capacity dependent on lung volume and age in normal subjects. *J. Appl. Physiol.* 1994; 76(6): 2356-2363.

17. Verweij GCG. Health Interview Surveys. Height and weight of children and adolescents up to 21 years, 1981-1992. Report Netherlands Central Bureau of Statistics, 1994.

18. Zapletal A, Samánek M and Paul T. Lung function in children and adolescents. Methods, Reference values. Karger, Basel 1987.

19. Roberts CM, McRae KD and Seed WA. Multiple breath and single breath helium dilution lung volumes as a test of obstruction. *Eur. Resp. J.* 1990; 3: 515-520.

20. Cotes JE. Lung Function: Assessment and Application in Medicine (4th ed.). Oxford, UK: Blackwell, 1979.

21. Jones RS and Meade F. A theoretical and experimental analysis of anomalies in the estimation of pulmonary diffusing capacity by the single breath method. *Q. J. Exp. Physiol.* 1961; 46: 131-143.

22. Ferris BG. Epidemiology standardization project. *Am. Rev. Respir. Dis.* 1978; 118, Suppl.:62-72.

23. Stam H, Kreuzer FJA, and Versprille A. Effect of lung volume and positional changes on pulmonary diffusing capacity and its components. *J. Appl. Physiol.* 1991; 71: 1477-1488.

24. SAS Technical Report P-229, SAS/STAT Software Release 6.07. The Mixed Procedure. Chapter 16: 289-366. SAS Institute Inc., Cary, NC, 1992

25. DeMuth GR and Howatt WF. The growth of lung function. Part III. Pulmonary diffusion. *Pediatrics* 1965; 35: 185-193.

CHAPTER 6

EFFECT OF LUNG VOLUME AND POSITIONAL CHANGES ON THE PULMONARY DIFFUSING CAPACITY AND ITS COMPONENTS

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Pulmonary diffusing capacity for CO, D_{LCO}, is usually estimated in the sitting position. However, in some patients measurements can only be made in the supine position. Healthy subjects have a larger diffusing capacity normalized per liter alveolar volume (BTPS), D_{LCO}/V_A, in the supine position as compared with the sitting position [1-5]. In these studies D_{LCO}/V_A was determined at total lung capacity (TLC). An increase in D_{LCO}/V_A was also observed at functional residual capacity (FRC) when healthy subjects changed from sitting to supine positions [6-8]. In patients with various pulmonary and cardiac diseases without a positional change in D_{LCO}/V_A the capillaries in the upper lung zones were assumed to be fully recruited in both positions [1, 5]. We doubt whether similar values in both positions at one level of V_A imply similar values at another V_A. Therefore, we wonder whether measurements at TLC (Single Breath method) and those at FRC (Steady State or Rebreathing method) are comparable. We studied D_{LCO} and D_{LCO}/V_A as functions of V_A in both body positions.

To study the mechanisms involved in the changes of D_{LCO} and D_{LCO}/V_A elicited by changing from sitting to supine, we also analyzed the membrane conductance, D_m, and the capillary blood volume, Q_c, as functions of V_A, based on the linear relationships between D_{LCO}/V_A and V_A for ambient air and oxygen breathing respectively [9].

The objectives of this study were:

1. to analyze the effect of changing body position on D_{LCO}/V_A, D_{LCO}, D_m and Q_c over a wide range of V_A;
2. to evaluate the reliability of the derived variables D_m and Q_c by application of random noise to the estimates of D_{LCO}/V_A;
3. to determine the relationships of age, sex and smoking habits with the response of the diffusion variables to a change in body position;
4. to evaluate the applicability of the various models of lung deployment [9] in explaining the diffusion variables in both positions, sitting and supine.

SUBJECTS AND METHODS

Subjects

Thirty-seven healthy subjects (20 males; 4 smokers, 17 females; 4 smokers) without any

history of pulmonary or cardiac disease were studied. They had normal respiratory function: VC=111 ± 20(SD)% ref.; FEV_1=111 ± 10(SD)% ref.; PEF=124 ± 17(SD)% ref. and MEF_{50}=91 ± 17(SD)% ref. The age ranged from 16 to 79 years. Informed consent was obtained prior to the experiments.

Procedure

In a series of single breath maneuvers both in sitting and supine position D_{LCO}/V_A was measured at various levels of alveolar volume and using low oxygen (0.25% CO, 5% He, 20% O_2) and high oxygen (0.25% CO, 5% He, 94.75% O_2) concentrations [9]. We followed the single breath procedure recommended by the European Community for Coal and Steel [10]. The subjects expired to residual volume (RV) and inspired volumes ranging from 1.5 l to vital capacity (VC) holding their breath at $V_{A,max}$. The D_{LCO}/V_A values were determined in random order at various alveolar volumes. We used in our calculations a linear regression equation between the D_{LCO}/V_A and V_A values. From such a relationship data at specific alveolar volumes could be derived.

Breath holding time was taken to start when 0.3 part of the volume had been inspired, and to end when half of the expired sample had been expired [11]. The average breath hold time exceeded 10 s slightly. Inspirations and expirations were performed rapidly.

Alveolar fractions of CO and He were measured in the expired gas after discarding 600 ml for washout airways and apparatus dead space. Because this discard volume is smaller than recommended, we checked whether this volume was large enough in our experiments by calculating RV at high and low V_A levels. RV was unchanged by target volume, so errors due to gas sampling from the dead space seemed unlikely. The size of the alveolar sample was 800 ml. Maneuvers were performed with a slightly modified version of the << alveo-diffusion test >> manufactured by Jaeger (Würzburg). High oxygen measurements were done after an equilibration period of 5 min breathing pure oxygen.

The interval between measurements was 5 min. To minimize CO back tension, we performed six or less measurements in a day. Back tension was estimated before and after such a series of measurements by rebreathing in a closed system [12]. To correct for the effect of CO back tension linear interpolation between the back tension before and after the series of measurements was performed. Back tension was subtracted from both alveolar CO pressures at the beginning and at the end of the single breath maneuver

respectively. Back tension was neglected in the non-smokers when breathing low oxygen, because it was less than 1% of the initial alveolar CO tension.

Four series of measurements were performed on four different days. In the first and second session, diffusion variables were measured with 20% O_2 in the test gas in both positions. The third and fourth day were used to measure during 95% oxygen breathing.

Functional residual capacity, FRC, was estimated the first day in both sitting and supine positions using a closed He-dilution method [12].

Analysis

D_{LCO} is related to the variables D_m and Q_c according to the equation [13]:

$$\frac{1}{D_{LCO}} = \frac{1}{D_m} + \frac{1}{\theta Q_c F_{Hb}} \qquad (6.1)$$

where, according to Forster [14], D_{LCO} is the diffusing capacity of the whole lung and D_m is the analogous diffusing capacity of the membrane, including alveolar epithelium, interstitium and capillary endothelium. By analogy $\theta Q_c F_{Hb}$ is the diffusing capacity of the total mass of red cells in the capillary bed of the lung at any instant, where Q_c is the volume of the capillary bed in ml and θ is the standard rate at which 1 ml of whole blood will take up the gas CO. F_{Hb} is the hemoglobin concentration as a fraction of the normal concentration.

θ was calculated from the original data of Roughton and Forster [13] after correction to pH 7.4 (see Appendix for explanation):

$$\frac{1}{\theta} = 0.059 + 0.0073\ P_{O_2} \qquad (6.2)$$

where P_{O_2} = ideal alveolar oxygen tension.

Further assumptions were a normal hemoglobin concentration (F_{Hb} = 1) and an infinite permeability of the red cell membrane [15-17]. As normal hemoglobin concentration we assumed 8.3 and 9.2 ± 0.5 (SD) $mmol.l^{-1}$ for women and men respectively.

The D_{LCO} values obtained from linear regressions of D_{LCO}/V_A vs V_A in both air and oxygen were used to estimate D_m and Q_c at discrete levels of V_A. The calculations are described in detail in our previous study [9].

The change in membrane conductance with V_A is described by $D_m = kV_A^x$, where k and x are constants and x depends on the mode of alveolar expansion [9]. We also calculated x in both positions to analyze whether a characteristic difference in the mode of alveolar expansion will occur.

Reliability of the analysis

Random noise

The effect of random variation in D_{LCO}/V_A was studied in 4 subjects to evaluate the consequences of variations in D_{LCO}/V_A on the derived variables D_m and Q_c. The distribution of D_{LCO}/V_A at TLC was determined by measuring the low oxygen D_{LCO}/V_A at least six times at TLC. We used TLC because this volume could easily be reproduced by the volunteers. We averaged the D_{LCO}/V_A values and determined the standard deviation. We assumed the standard deviation at TLC to be representative for the distribution of D_{LCO}/V_A values at all levels of V_A. From normally distributed random noise with a mean value of zero and a standard deviation equal to the standard deviation at TLC, we selected numbers randomly to add to or subtract from each measured D_{LCO}/V_A value. Thus, the chance to add or subtract random noise equal to 2 SD was 2.5 %. A new regression equation of D_{LCO}/V_A vs V_A was then calculated. This sequence was repeated 5 times giving 6 equations, including the original one. In each of 4 volunteers we derived two sets of 6 regression equations, for air and oxygen breathing in sitting and supine position respectively.

In each position the six equations in air as well as in oxygen were randomly paired and used for the derivation of D_m and Q_c respectively as a function of V_A. At 5 - 9 levels of V_A with steps of 0.5 l we calculated averages and standard deviations for D_m and Q_c.

RESULTS

Diffusing capacity D_{LCO}

Plots of D_{LCO}/V_A vs V_A yielded a linear relationship as the best mathematical description. The numerical data for these regression lines are presented in Table 6.1. A linear fit was

Table 6.1. Regression equations for D_{LCO}/V_A as function of V_A in sitting and supine position in air and 95% O_2

SUBJECT	SEX M/F	AGE Y	HEIGHT cm	WEIGHT kg	SITTING										SUPINE									
					AIR $D_{LCO}/V_A = -aV_A + b$				95% O_2 $D_{LCO}/V_A = -cV_A + d$				Q_{max} (ml)	$D_m = kV_A^x$	AIR $D_{LCO}/V_A = -eV_A + f$				95% O_2 $D_{LCO}/V_A = -gV_A + h$				Q_{max} (ml)	$D_m = kV_A^x$
					n	a	b	r	n	c	d	r		x	n	e	f	r	n	g	h	r		x
1	M	37	183	83	26	3.58	50.61	-0.95	6	1.64	22.20	-0.98	67	0.56	11	3.19	47.96	-0.95	8	2.11	27.33	-0.96	95	0.58
2	F	23	172	63	19	4.99	48.69	-0.93	6	2.36	22.34	-0.94	50	0.54	11	5.20	52.56	-0.90	6	2.44	28.17	-0.83	109	0.48
3	M	31	186	72	12	2.67	41.32	-0.91	6	1.07	17.73	-0.90	70	0.56	12	2.95	46.54	-0.94	6	2.70	31.30	-0.98	105	0.78
4	F	36	169	63	11	4.73	50.52	-0.91	6	3.27	29.75	-0.97	74	0.65	11	3.45	54.50	-0.97	6	2.14	29.89	-0.85	101	0.81
5	M	26	184	70	12	4.43	50.76	-0.96	6	2.58	27.57	-0.94	78	0.57	15	6.63	66.18	-0.93	8	4.14	37.54	-0.99	92	0.49
6	M	25	184	75	11	3.41	49.93	-0.93	6	2.69	28.11	-0.94	69	0.89	8	3.92	52.67	-0.95	6	3.05	34.03	-0.94	114	0.55
7	M	23	168	72	11	5.68	55.18	-0.95	6	3.97	29.89	-0.96	53	0.84	14	10.40	78.18	-0.96	6	5.93	43.09	-0.95	87	0.37
8	M	25	186	75	10	3.57	54.95	-0.85	6	3.41	34.60	-0.97	89	0.99	10	3.86	63.82	-0.96	6	4.58	44.43	-0.91	118	1.01
9	F	38	169	70	6	2.11	32.68	-0.79	6	2.85	21.13	-0.99	37	1.24	6	5.42	46.87	-0.98	6	3.94	28.83	-0.99	59	0.52
10	M	22	185	76	6	4.10	55.40	-0.93	6	2.86	33.76	-0.97	115	0.57	9	4.48	60.33	-0.87	6	3.51	37.45	-0.98	109	0.67
11	M	25	184	84	6	4.43	57.59	-0.93	6	3.01	34.47	-0.97	113	0.59	6	5.02	64.47	-0.97	6	3.13	38.78	-0.99	148	0.53
12*	M	48	177	72	6	0.17	19.46	-0.19	6	0.63	14.19	-0.60	76	1.11	6	0.69	23.59	-0.62	6	0.10	11.96	-0.13	96	0.76
13*	M	25	186	80	6	4.50	55.06	-0.97	6	1.95	23.76	-0.95	67	0.57	6	4.46	53.44	-0.98	6	3.35	33.60	-0.99	99	0.55
14	M	46	178	79	6	2.51	40.02	-0.98	6	2.29	24.35	-0.92	65	1.01	6	3.79	47.92	-0.99	6	2.34	25.66	-0.92	71	0.55
15	M	53	182	102	6	1.68	34.43	-0.92	6	1.84	23.26	-0.91	76	1.00	6	2.51	42.35	-0.95	6	1.43	21.67	-0.85	77	0.62
16	M	52	164	66	6	1.68	29.91	-0.85	6	1.81	20.09	-0.91	60	0.94	6	3.25	38.88	-0.96	6	2.11	20.89	-0.93	50	0.66
17	M	52	181	71	6	3.46	43.55	-0.99	6	0.91	15.78	-0.71	64	N.M.	6	2.92	44.62	-0.93	6	1.32	20.79	-0.76	78	0.61
18*	M	56	178	80	6	1.60	34.43	-0.86	6	0.37	12.41	-0.51	56	0.57	6	2.11	37.78	-0.89	6	0.68	13.75	-0.78	53	0.60
19*	F	79	170	76	6	0.10	15.95	-0.06	6	0.00	4.72	0.00	16	0.91	6	0.48	19.13	-0.39	6	0.23	8.01	-0.14	29	0.91
20	F	74	175	61	6	0.40	25.01	-0.25	7	0.51	9.81	-0.25	26	1.27	7	3.43	38.66	-0.85	6	0.72	12.36	-0.39	33	0.38
21*	M	58	172	66	6	1.69	27.87	-0.91	6	1.48	16.05	-0.97	41	0.99	6	2.26	31.07	-0.97	6	1.51	16.57	-0.91	43	0.76
22	M	49	186	99	6	1.87	35.04	-0.91	6	1.48	20.88	-0.98	71	0.79	6	1.75	35.24	-0.90	6	1.30	19.47	-0.94	69	0.84
23	F	75	167	70	6	1.37	23.80	-0.96	6	0.79	11.47	-0.77	36	0.75	6	0.79	21.32	-0.92	6	1.12	13.77	-0.86	40	1.12
24	F	73	169	73	6	- 2.50	12.50	0.73	6	1.15	13.70	-0.89	34	1.88	6	-0.73	20.57	0.35	6	-0.51	6.00	0.19	30	0.86
25	F	24	174	57	6	5.62	53.41	-0.98	6	2.99	27.78	-0.97	68	0.56	6	3.96	47.92	-0.96	6	1.94	25.32	-0.94	94	0.55
26	F	25	174	63	6	2.99	42.24	-0.94	6	1.68	19.09	-0.93	45	0.84	6	3.93	46.29	-0.98	6	1.98	23.92	-0.84	80	0.52
27*	F	42	167	75	6	1.39	25.04	-0.88	6	0.51	8.49	-0.58	22	0.83	7	3.08	33.22	-0.97	6	1.83	17.24	-0.93	40	0.66
28	F	47	168	77	6	0.25	25.91	-0.29	6	1.06	17.11	-0.92	60	1.28	6	1.99	38.26	-0.90	6	1.65	21.98	-0.93	70	0.89
29	M	16	175	55	6	5.07	53.92	-0.90	6	3.62	28.21	-0.98	50	0.88	6	7.25	64.38	-0.97	6	1.40	22.45	-0.50	69	0.37
30*	F	52	168	69	6	1.77	29.56	-0.71	6	0.82	12.15	-0.62	37	0.72	6	2.01	33.99	-0.92	6	1.86	19.90	-0.81	50	1.00
31*	F	58	168	60	6	1.65	26.54	-0.81	6	1.09	12.34	-0.83	27	1.06	7	4.05	40.36	-0.94	6	1.21	15.07	-0.62	45	N.M.
32	M	57	182	93	6	2.51	39.66	-0.96	6	2.12	24.03	-0.95	69	0.86	6	3.84	48.62	-0.99	6	2.87	31.21	-0.96	100	0.56
33	M	53	176	95	6	1.59	35.59	-0.90	8	0.93	15.76	-0.82	53	0.91	6	2.35	40.22	-0.96	6	2.25	27.67	-0.99	98	0.80
34	F	27	168	63	7	3.06	39.73	-0.80	6	4.32	29.64	-0.91	60	1.29	6	5.67	52.15	-0.98	6	3.46	29.54	-0.83	69	0.51
35	F	31	170	63	7	5.23	52.37	-0.99	7	5.07	35.88	-0.98	76	0.88	7	5.65	56.12	-0.93	6	4.63	39.63	-0.82	118	0.55
36	F	70	155	60	12	4.78	46.25	-0.96	6	1.35	16.65	-0.81	47	N.M.	7	2.47	37.74	-0.86	7	1.62	18.80	-0.53	48	0.85
37	F	71	165	68	7	0.63	38.44	-0.17	7	-0.03	11.69	0.01	41	0.73	6	3.69	48.05	-0.99	8	0.39	14.70	-0.14	53	N.M.

*Where x is a constant depending on the mode of alveolar expansion. D_{LCO}/V_A in $\mu mols^{-1}kPa^{-1}l^{-1}$. N.M., not model ($D_m=kV_A^x$). * Smokers.*

best in 79% of equations. In 21% a hyperbolic or biphasic description provided a negligible improvement. In these cases deviation from the linear relationship was smaller than the standard deviation of D_{LCO}/V_A at TLC (Table 6.2).

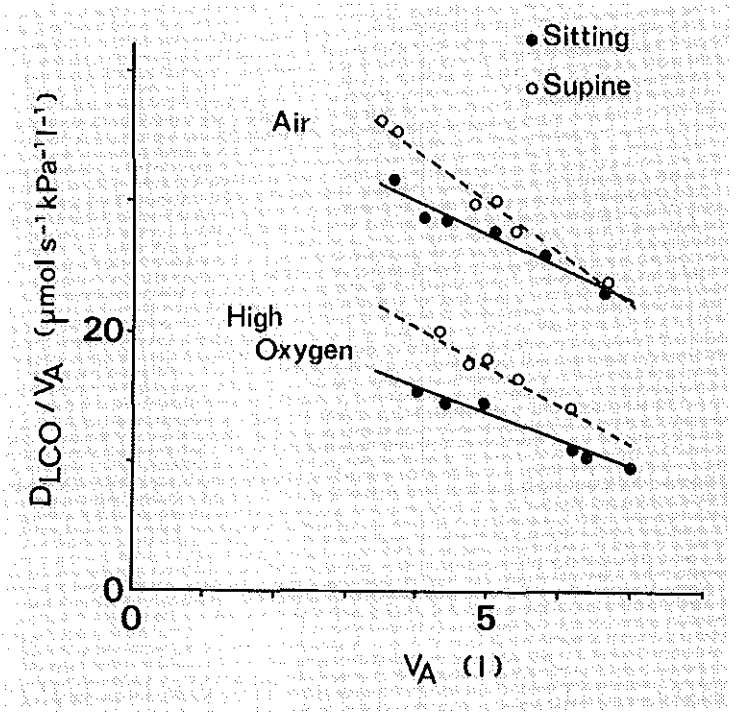

Fig. 6.1. *A typical individual example (subject 32) of linear relationship between D_{LCO}/V_A and V_A in sitting and supine positions in low and high O_2 conditions. In supine position relationships are shifted upward with a steeper slope.*

Therefore, we used a linear relationship between D_{LCO}/V_A and V_A for the further analysis of our data.

The example in Figure 6.1 where the relationship is shifted upward when moving from sitting to supine position in both low and high oxygen is representative for 67% of all subjects. We have illustrated all individual responses of the D_{LCO}/V_A vs V_A relationship to the change in body position from sitting to supine in an X-Y diagram with four quadrants (Fig. 6.2). In this diagram we compared the change of D_{LCO}/V_A at 50% of TLC with that at TLC, both derived from the regression lines. The results in quadrant I indicate an increase in D_{LCO}/V_A at both 50% of TLC and TLC, implying an upward shift of the

D_{LCO}/V_A vs V_A relationship. In the results left of the 45° line in this quadrant the

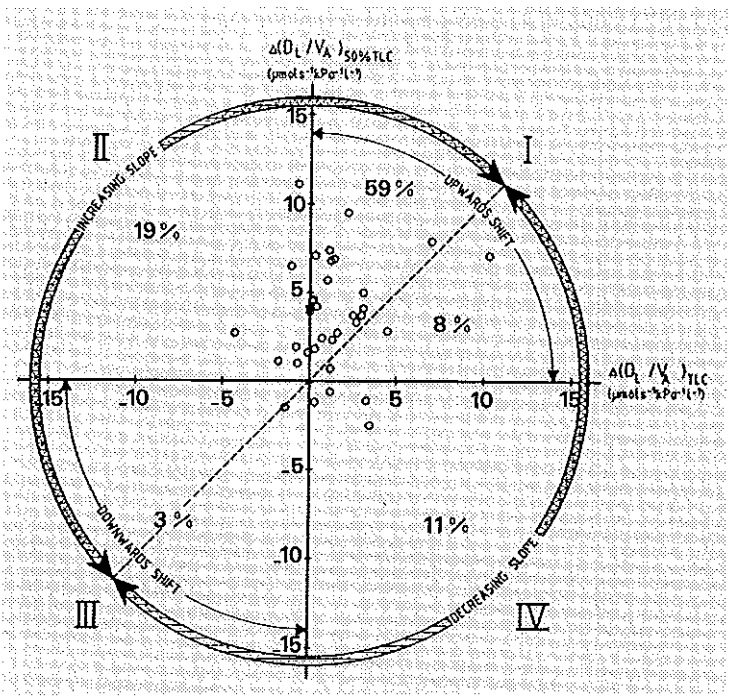

Fig. 6.2. *Comparison of response of D_{LCO}/V_A at 50 and 100% of TLC on change in body position from sitting to supine. Dashed line, parallel shift of regression line.*

increase of D_{LCO}/V_A at TLC was smaller than at 50% of TLC, giving an increase in slope of the regression line in the supine position. In the results right of this line a decrease in slope occurred.

In quadrant II D_{LCO}/V_A increased at 50% of TLC, but decreased at TLC, leading to a steeper slope of the D_{LCO}/V_A vs V_A relationship in supine position and a crossing of both regression lines. The single result in quadrant III indicates a parallel downward shift of the D_{LCO}/V_A vs V_A relationship. The data in quadrant IV decreased at 50% of TLC and increased at TLC, giving a less steep D_{LCO}/V_A vs V_A relationship. In 67% of the subjects we found an upward shift, with an increasing slope in 59%. In 30% of the subjects we observed no shift but only a change in slope . In 19% the slope was increased and in 11% it was decreased.

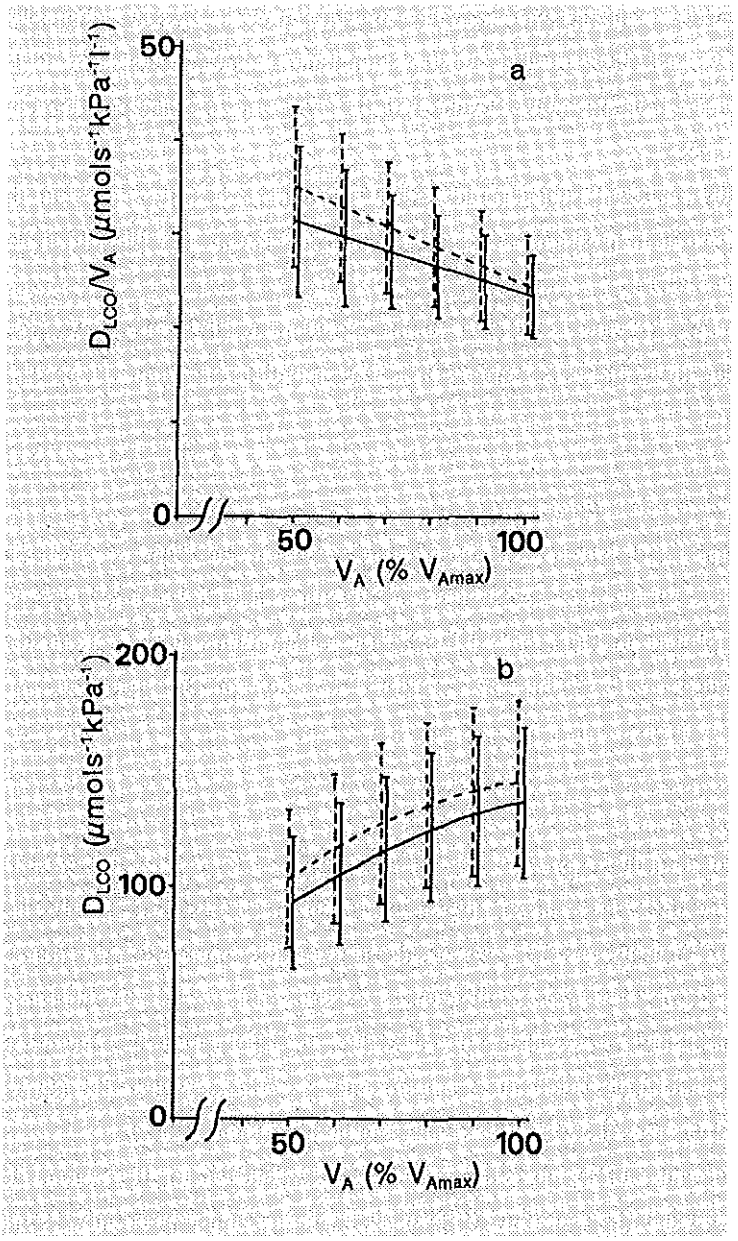

Fig. 6.3. *D_{LCO}/V_A (a) and D_{LCO} (b) vs. V_A in sitting (solid lines) and supine (dashed lines) positions (means ± SD).*

In Fig. 6.3 we present the average values of D_{LCO}/V_A and D_{LCO} at volume levels of 50, 60, 70, 80, 90 and 100% of TLC for the sitting and supine positions. At all levels of V_A D_{LCO}/V_A increased significantly (P-value$<$0.01, paired t-test) when position changed from sitting to supine.

We separately analyzed subjects older than 50 years. The differences in the D_{LCO}/V_A values between both positions were also significant except for the difference at TLC, where P-value=0.14.

D_{LCO} was higher (P-value$<$0.01) in supine position at all levels of V_A. Again in the subjects older than 50 years supine D_{LCO} was not different from sitting D_{LCO} (P-value=0.10) at TLC.

Application of random noise

The four subjects, in which we applied random noise to the data, were chosen randomly from four different age ranges. The data are listed in Table 6.2. The average values of D_m and Q_c are plotted in Figs. 6.4 and 6.5. The relationships derived from the originally measured 'values were within one standard deviation of the mean D_m and Q_c after application of random noise.

Membrane conductance

We analyzed x in the expression $D_m = kV_A^x$ to characterize alveolar expansion in both positions. The differences in x between both body positions were highly significant (P-value$<$0.001) in many cases. However, the individual responses did not imply any typical pattern (Fig. 6.6). There was no difference between smokers and non-smokers. Because of the non-typical pattern we also derived x after the application of random noise (Table 6.2). The standard deviation of x appeared to be 7-17%, which did not eliminate the significant differences between the x values in both positions.

The average values of D_m in both positions were calculated for the volume levels of 50, 60, 70, 80, 90 and 100% of TLC (Fig. 6.7). D_m in the supine position was smaller than in the sitting position at all levels of V_A in most subjects. There were however positive as well as negative responses to the positional changes at all levels of V_A. Only the responses at 80, 90 and 100% of TLC were significantly different (P-value$<$0.05, paired t-test). There was no specific relationship between the positional response of D_m and age.

Table 6.2 D_{LCO}/V_A in sitting position at TLC during low O_2 breathing and derived variables.

Subject	Sex	Age y	Height cm	Weight kg	Sitting in air at TLC D_{LCO}/V_A (μmol.s^{-1}.kPa^{-1}.l^{-1})	SD	n	x Sitting	SD	x Supine	SD	$Q_{c,max}$ (ml) Sitting	SD	$Q_{c,max}$ (ml) Supine	SD	V_A at $Q_{c,max}$ (% of TLC) Sitting	SD	V_A at $Q_{c,max}$ (% of TLC) Supine	SD
1	M	37	183	83	25.3	0.6	29	0.54	0.04	0.62	0.07	66	2	94	2	84	5	79	6
2	F	23	172	63	26.2	1.0	10	0.50	0.05	0.47	0.05	50	2	109	7	87	9	98	6
17	M	52	181	71	22.5	0.5	6	n.f.		0.56	0.07	63	6	82	6	>100		>100	
36	F	70	155	60	24.9	0.7	6	n.f.		0.83	0.14	47	4	49	2	>100		98	5

Values are means ± SD; n.f. means no fit possible according to model $D_m=kV_A^x$.

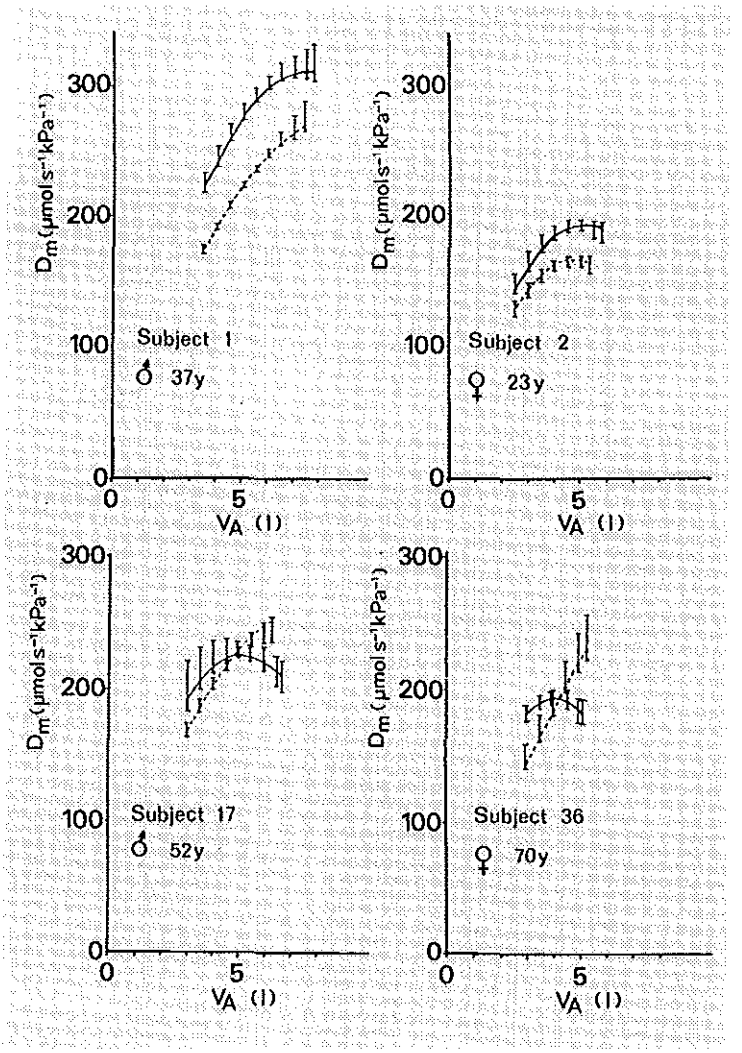

Fig. 6.4. *Variability in relationship between membrane conductance (D_m) and V_A after application of random noise in 4 volunteers. Solid and dashed lines sitting and supine positions respectively. Values are means ± SD.*

Pulmonary capillary blood volume

The relationship between Q_c and V_A could be described again [9] by a second order polynomial with a V_A related maximum in Q_c, $Q_{c,max}$, above 60% of TLC. In the supine

position the characteristics of this relationship were similar (Fig. 6.5).

Q_c was significantly larger (P-value < 0.001, paired t-test) in supine position than in sitting position at all levels of V_A (Fig. 6.8).

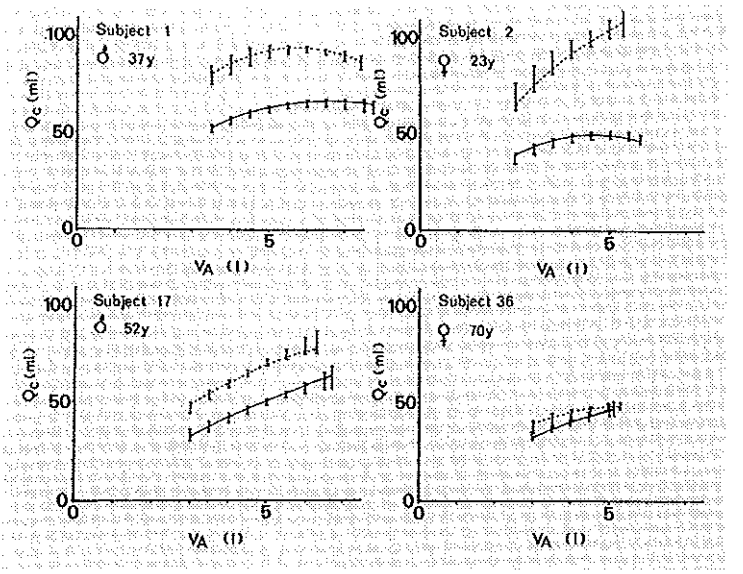

Fig. 6.5. *Variability in relationship between capillary blood volume (Q_c) and V_A after application of random noise in the same subjects as in Fig. 6.4. Response to change in body position decreases with age. Solid and dashed lines, sitting and supine positions, respectively. Values are means ± SD.*

After the addition of noise the smallest SD in Q_c was seen at $Q_{c,\,max}$, implying a lower sensitivity of $Q_{c,\,max}$ to variability in D_{LCO}/V_A (Table 6.2). Therefore, we used the responses of $Q_{c,max}$ to changes in body position as an indicator of change in pulmonary blood volume.

In all subjects $Q_{c,max}$ normalized per m^2 body surface area, $Q_{c,max}$/BSA, decreases with age in women and men in both body positions (Figs. 6.9a and 6.9b). In younger subjects $Q_{c,max}$/BSA was significantly higher in the supine position. Differences in $Q_{c,max}$/BSA between supine and sitting decreased with age up to about 50 years. Above this age the differences in $Q_{c,max}$/BSA were small and distributed around zero. The level of $Q_{c,max}$/BSA in sitting and supine position and the response to the change in body position were not

different for the smoking subjects, but no heavy smokers were studied.

Finally we observed that the alveolar volume at which $Q_{c,max}$ was detected ($V_{AQc,max}$), shifted to higher alveolar volume levels with age in sitting as well as in supine position (P-value=0.02, paired t-test). For the subjects younger than 50 years $Q_{c,max}$ was detected at a

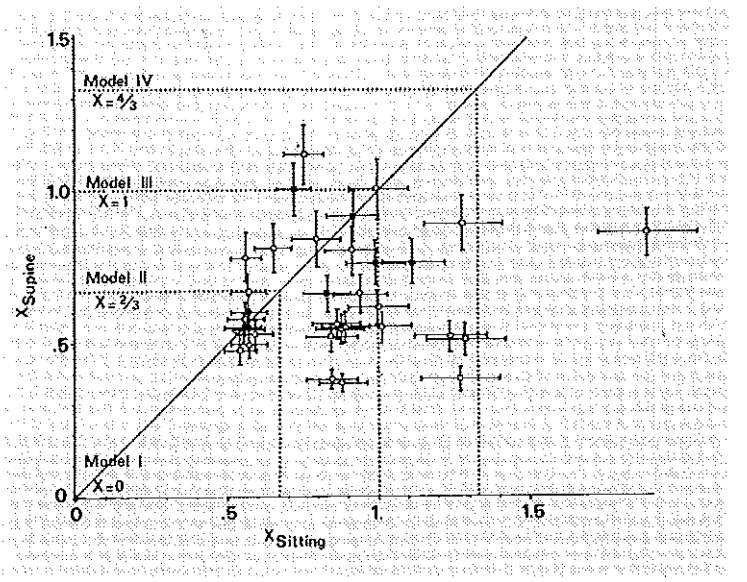

Fig. 6.6. *Effect of body position of males (○) and females (□) on exponent x of relationship $D_m=kV_A^x$ and comparison with functional models.*
Model I, bellows model (x=0).
Model II, isotropic expansion with constant barrier thickness (x=2/3).
Model III, a proportional increase of alveolar surface area with increasing V_A and constant membrane thickness (x=1).
Model IV, isotropic expansion with proportional decrease in barrier thickness (x=4/3).
Response of x to change in body position is not consistent, but comparison of values in sitting and supine position creates impression of a more isotropic expansion with constant barrier thickness in supine position (x=2/3, Model II). Closed symbols, smokers.

mean $V_{AQc,max}$ = 75 ± 15 (SD) % TLC in sitting and $V_{AQc,max}$ = 81 ± 15 (SD) % TLC in supine position respectively. For the subjects above 50 years $V_{AQc,max}$ = 88 ± 17 (SD) % TLC in sitting and $V_{AQc,max}$ = 89 ± 13 (SD) % TLC in supine position respectively.

The alveolar volume level at which $Q_{c,max}$ was detected, was not significantly different for sitting and supine positions (P-value=0.25, paired t-test).

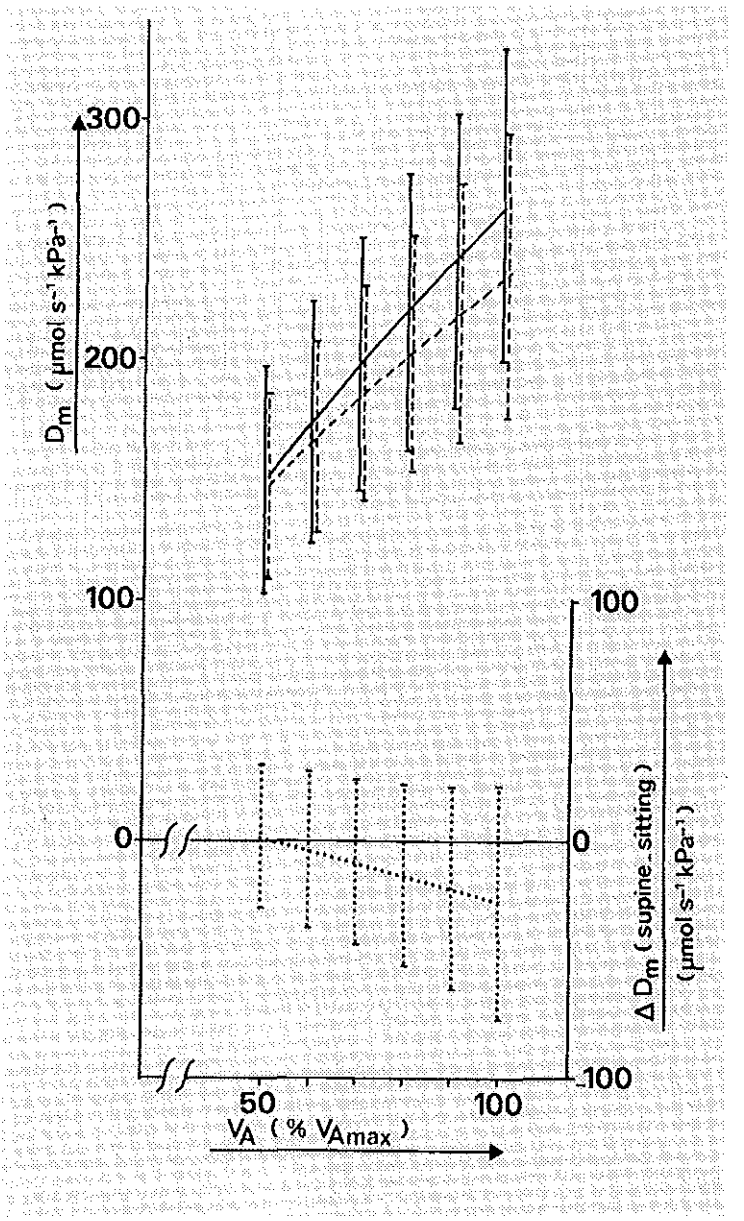

Fig. 6.7. *D_m and ΔD_m (dotted line; supine - sitting) as a function of V_A. Solid and dashed lines sitting and supine positions respectively. Values are means ± SD of all subjects.*

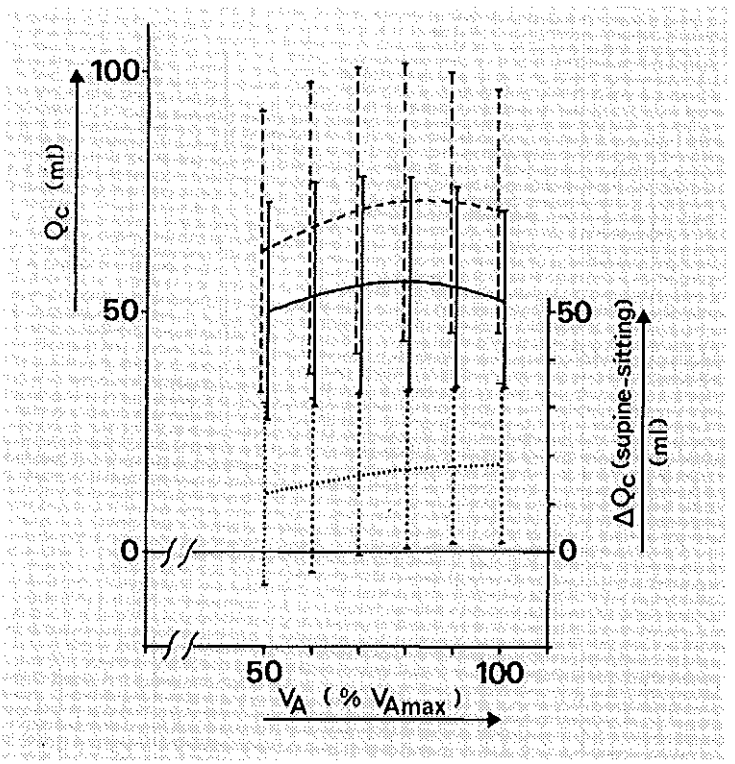

Fig. 6.8 *Q_c and ΔQ_c (dotted line; supine - sitting) as a function of V_A. Solid and dashed lines, sitting and supine positions, respectively. Values are means ± SD of all subjects.*

DISCUSSION

Evaluation of the method

Volume history

Lebecque et al. [18] described an immediate increase up to 16% in D_{LCO}/V_A after an inflation-deflation maneuver (IDM). During the first 5 minutes after IDM 62% of this increase was lost; D_{LCO}/V_A remained significantly increased even in the 7th minute after an

IDM. We did not control volume history. Between the successive measurements in air we visually observed ventilation to avoid measurements after sighs. In the high oxygen measurements we continuously monitored ventilation in between the successive measurements.

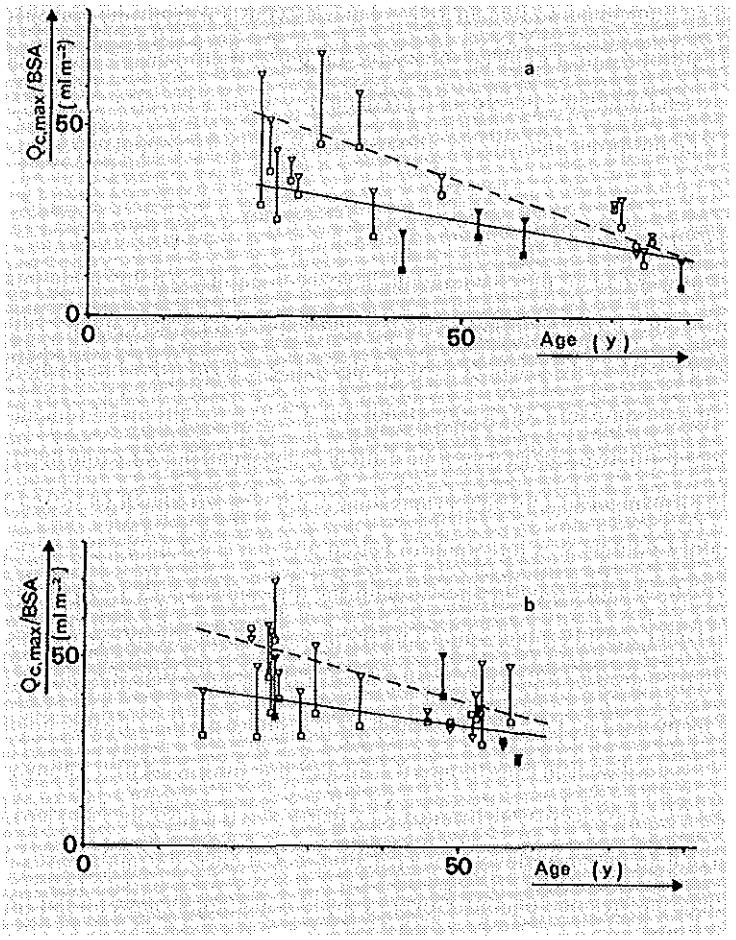

Fig. 6.9. *Relationship between effective capillary blood volume normalized per m² body surface area ($Q_{c,max}/BSA$) and age in females (a) and males (b). Solid and dashed lines, regression lines in sitting (□) and supine positions (▽), respectively. Closed symbols, smokers.*

Females: $Q_{c,max}/BSA = 41.68 - 0.33\ (Age)$, $r=-0.63$.
$Q_{c,max}/BSA = 68.17 - 0.65\ (Age)$, $r=-0.80$.

Males: $Q_{c,max}/BSA = 46.06 - 0.27\ (Age)$, $r=-0.46$.
$Q_{c,max}/BSA = 65.18 - 0.52\ (Age)$, $r=-0.65$.

Irregularities in tidal volume were observed, but we never found sighs larger than twice the tidal volume. To verify whether an IDM influenced our data we did a pilot study in three subjects, in which we repeated the D_{LCO}/V_A estimation at a constant V_A of about 70% of TLC at various times (0 to 8 minutes) after a vital capacity maneuver. D_{LCO}/V_A was 5% higher immediately after an IDM, but was constant from the fourth minute after an IDM. Therefore, an influence of sighs in our experiments seems unlikely. This assumption is supported by the good reproducibility of the D_{LCO}/V_A values, which were estimated in random order, and by the linearity between D_{LCO}/V_A and V_A.

Intrapulmonary pressure during breath holding

We checked also whether our data might have been influenced by high intrapulmonary pressure during the breath holding period. Such a pressure was only possible by glottis occlusion because the valve, which is normally closed during the breath holding period, opens at a mouth pressure of a few tenth of a kPa. In all measurements this valve remained closed. In two subjects we measured esophageal pressure during the measurement of the diffusing capacity at various lung volumes. At the lowest V_A levels, even when inspiratory volume was too small to get an alveolar gas sample, P_{es} varied between 0 and 0.2 kPa above atmospheric pressure during the apnea period. In both subjects P_{es} increased to 1 and 2.5 kPa above atmospheric pressure respectively when breath was hold at TLC level. Because we observed the highest pressures at TLC we studied the effect of Valsalva maneuvers on D_{LCO}/V_A at various pressures during breath holding in four subjects at TLC in the sitting position. At a mouth pressure of 2.5 kPa above atmospheric pressure D_{LCO}/V_A was decreased by no more than 4%. Because the standard deviation in D_{LCO}/V_A is comparable at all levels of V_A and at TLC between 2 and 4% (Table 6.2), we concluded that our results obtained over a wide range of V_A were not influenced by possible differences in alveolar pressure throughout the series of observations.

Sequential filling and emptying

The data estimated by means of the single breath diffusion test are a combined result of all lung areas. Application of xenon techniques [19] revealed that most of the gas is distributed to the upper lobes during early inspiration and more to the lower lobes during late inspiration. The same authors reported a decrease in regional D_{LCO}/V_A from base to apex.

If sequential filling of the lung had affected our results, it should have decreased the negative slope of the D_{LCO}/V_A vs V_A relationship. In three subjects we estimated D_{LCO}/V_A in air at TLC starting inspiration of the He, CO and air mixture from different alveolar volumes between RV and TLC. To avoid sampling of expiratory air from one lung region, we sampled this air early and late in expiration: between 0.7 - 1.7 l, 2.3 - 3.5 l and 3.9 -5.4 l of VC respectively. No statistically significant differences were found in D_{LCO}/V_A as a function of starting volume or alveolar sampling time. This is in agreement with other studies where nonsequential emptying of the lung is described [11, 20].

Reliability of the measurements

The addition of random noise did not change the estimates of D_m and Q_c significantly (Figs. 6.4 and 6.5). These results indicated that the reliability of our analysis of D_m and Q_c was satisfactory over the whole range of V_A in the four subjects of Table 6.2. At younger ages the response in D_m and Q_c to change in body position far exceeds the variability in the measurement (Figs. 6.4 and 6.5). At older ages the response in D_m and Q_c is not significantly different from the variability in the D_m and Q_c values in one position.

Diffusing capacity

D_{LCO}/V_A and alveolar volume

It has been generally accepted in the literature that D_{LCO}/V_A decreases with increasing V_A [9, 21-23]. However, some authors [21-23] found a fall in D_{LCO}/V_A when alveolar volume increased up to about 80% of TLC, whereas D_{LCO}/V_A became constant at higher levels. To find the best and most simple mathematical description we fitted a multiple degree polynomial to our data. In the majority of our subjects the fit of a linear relationship between D_{LCO}/V_A and V_A was satisfactory (Table 6.1) and was not improved by a higher degree equation.

Overall responses

An increase in D_{LCO}/V_A in normal subjects when body position changed from sitting to supine has been described by many authors [1-8]. Our results are in agreement with these

reports. The average values of D_{LCO}/V_A and D_{LCO} for all subjects at volume levels of 50, 60, 70, 80, 90 and 100% of TLC (Fig. 6.3) had large standard deviations, because they include the values of the younger and older subjects. When testing the individual positional responses of each subject in pairs, only in the subjects older than 50 years at TLC the increase in D_{LCO}/V_A and D_{LCO} was not significant. In spite of differences between individual responses our overall conclusion is that D_{LCO}/V_A and D_{LCO} increase if position is changed from sitting to supine. At older ages these responses decrease especially at the larger alveolar volumes.

Individual responses

In the majority of the subjects (67%) an upward shift of the complete D_{LCO}/V_A vs V_A relationship was observed (Fig. 6.2). In 33% only a change in slope or in one case even a downward shift was observed, indicating a lack of recruitment of capillaries.

A lack of response of D_{LCO}/V_A to changes in body position (5, 35) has been interpreted as an indication for pulmonary hypertension or pulmonary capillary restriction. However, the responses of D_{LCO}/V_A were estimated either at TLC [1-5] or at FRC [6-8], whereas our study revealed that a lack of response at one level of V_A does not necessarily imply a lack of response at another level. As illustrated in the individual example of Fig. 6.1, the response of D_{LCO}/V_A to a change in body position varied with alveolar volume. In this example the highest increase in D_{LCO}/V_A was observed at the lowest V_A, whereas at the highest V_A no response was found. In other volunteers we found the highest response at TLC (Fig. 6.2). This illustrates that a response in D_{LCO}/V_A to a change in body position at one level of V_A yields insufficient information for the responses at other levels and that such responses will be different among different individuals.

Accidental variation in V_A

If studying the positional responses at TLC level, measurements accidentally performed at a slightly lower volume level in one of the positions will lead to a misleading result. At similar but 20% lower levels than TLC the responses of D_{LCO}/V_A to the change in body position are not significantly different from the response at TLC, which we tested in the overall results of Fig. 6.3 (P-value=0.05, paired t-test).

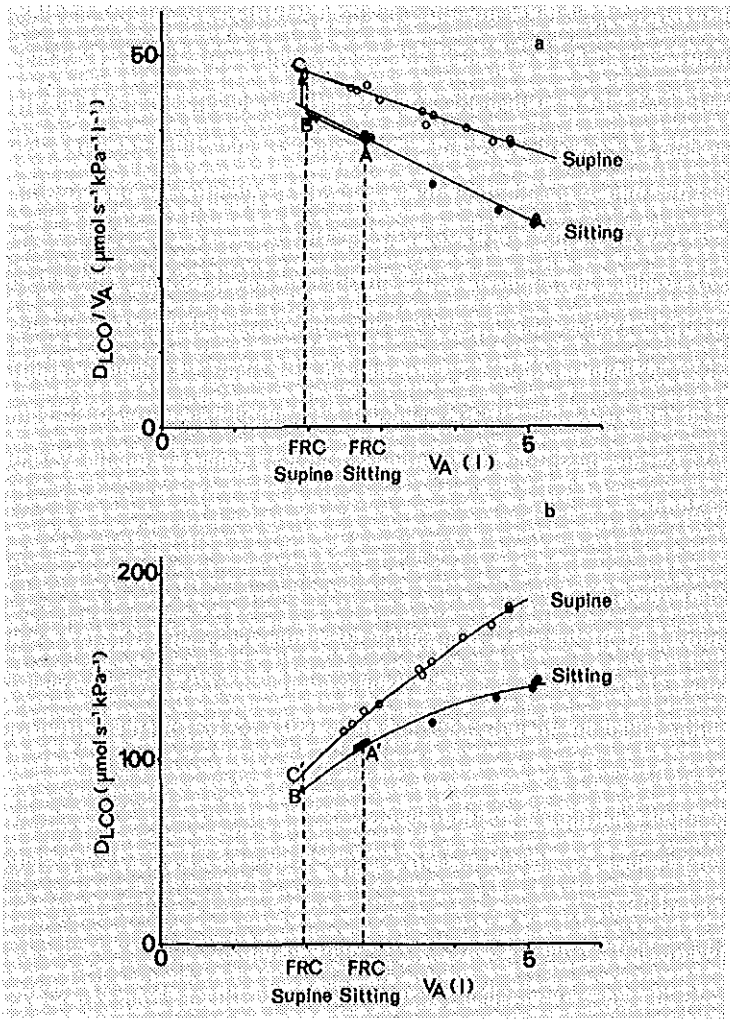

Fig. 6.10. *a: Typical example of linear relationship between D_{LCO}/V_A and V_A in sitting and supine position in air (subject 4). Sitting: $D_{LCO}/V_A = -aV_A + b$. Supine: $D_{LCO}/V_A = -cV_A + d$ where a, b, c and d are constants. Because of negative slope in D_{LCO}/V_A vs. V_A relationship, decreased FRC in supine position always results in an increased D_{LCO}/V_A value (point B). Only additional increase in D_{LCO}/V_A (point B to C) is due to a more equal perfusion in supine position.*

b: Linear relationships between D_{LCO}/V_A and V_A involve second-order relationships between D_{LCO} and V_A in both positions with the same constants a, b, c and d. Sitting: $D_{LCO} = -aV_A^2 + bV_A$. Supine: $D_{LCO} = cV_A^2 + dV_A$. Points A', B' and C' correspond with points A, B and C of 6.10A. An increased D_{LCO}/V_A value in supine position will not necessarily imply an increased D_{LCO} value, because D_{LCO} also depends on V_A.

Comparison of D_{LCO}/V_A at TLC in sitting position with D_{LCO}/V_A at 80 and 90% of TLC in supine position resulted in a response which was significantly larger (P-value <0.001 and P-value $=0.001$, paired t-test) than the response at TLC level. Comparison of the D_{LCO}/V_A in sitting position at alveolar volume levels of 80 and 90% of TLC with D_{LCO}/V_A measurements in supine position at TLC showed a significant underestimation of the response in D_{LCO}/V_A (P-value <0.01, paired t-test).

An accidental volume variation of 10% in one of the positions caused no significant difference in the response of D_{LCO} (P-value $=0.25$, paired t-test) with respect to the response at TLC in both positions. A variation of 20% caused a significant difference with the response at TLC (P-value <0.01, paired t-test), implying an overestimation when the variation of V_A occurs in sitting position and an underestimation for the variation of V_A in the supine position.

Diffusing capacity at FRC level

When a healthy person changes from sitting to supine D_{LCO} and D_{LCO}/V_A will change for two reasons: a change in FRC and a change in the D_{LCO}/V_A vs V_A relationship. Both changes are illustrated in an individual example (Fig. 6.10) obtained from quadrant I of Fig. 6.2.

D_{LCO}/V_A was higher in supine position due to:

1. the decrease in FRC and the negative slope in the D_{LCO}/V_A vs V_A relationship (A to B in Fig. 6.10a).
2. the upward shift of the D_{LCO}/V_A vs V_A relationship.

When D_{LCO}/V_A increases more than predicted from the linear D_{LCO}/V_A vs V_A relationship in the sitting position we conclude that this is an indication for recruitment of capillaries in the supine position (B to C).

In spite of an increased D_{LCO}/V_A, D_{LCO} was lower in the supine position (point C') than in the sitting position (point A') (Fig. 6.10b). The increase in D_{LCO}/V_A was smaller than the fall in FRC, leading to a decrease in the product $(D_{LCO}/V_A) \times V_A$.

In 56% of all volunteers D_{LCO} at FRC was smaller in the supine position. In 15% D_{LCO} at FRC was larger in the supine position. In the remaining 29% D_{LCO} did not change, indicating proportional opposite changes in D_{LCO}/V_A and V_A.

Membrane conductance D_m

Although the overall results (Fig. 6.7) indicated a significant fall in D_m at alveolar volumes of 80% and higher when changing from sitting to supine, the individual responses were not uniform. This non-uniformity contributed to the large standard deviation of the average values.

Nevertheless, in both positions the changes in D_m with changing alveolar volume might be described by the relationship $D_m = kV_A^x$ [9], where x characterizes the changes in extension of the diffusion membrane when alveolar volume is changed. The relationship between x and the membrane conductance is based on two assumptions:

1) a proportional relationship between D_m and the area, A, of the alveolar-capillary membrane, and
2) an inversely proportional relationship between D_m and the thickness, d, of the membrane.

We compared the results with the following models:

I. When the alveolar volume increases as a bellows the membrane area and membrane thickness do not change with an increase in volume and the exponent x will be equal to 0.

II. When an increase in volume of the alveoli is isotropic, i.e. equal expansion in all directions without a change in membrane thickness, x should be 2/3.

III. A proportional increase of alveolar area with increasing alveolar volume and a constant membrane thickness, as in recruitment, will imply $x = 1$.

IV. The same enlargement of membrane area as in model II but with a proportional decrease of membrane thickness will result in $x = 4/3$.

Each of the models mentioned predicts a characteristic value of x. However, a value of x will only predict one of the models when the expansion of the alveoli is homogeneous throughout the whole lung. When inhomogeneities are present an observed x value of e.g. 1, found experimentally, does not necessarily indicate a proportional recruitment of alveolar area and constant membrane thickness with increasing alveolar volume (model III). There might also be a mixture of areas which enlarge partly with constant membrane thickness (model II), as shown by Weibel et al. [24] in morphological studies, and, for another part, with a decreasing membrane thickness (model IV). It even does not exclude a contribution of model I.

In our previous study of healthy sitting subjects [9]) we observed a change in D_m with V_A according to $D_m=kV_A^{2/3}$. This model is in agreement with morphometric studies [24] where the volume increase of the lung coincides with an area increase accomplished by unfolding of microfolds in the membrane.

The large variability in the responses of x, when changing body position, excludes general conclusions based on the models mentioned above. However, the majority of the x-values in supine position are more clustered around the 2/3 value than the x-values in sitting position. In the supine position a more isotropic expansion with constant barrier thickness seems to occur.

Gas is exchanged only through that part of the alveolar membrane which contacts capillaries filled with blood. Therefore, we assume that the responses of D_m to the change in body position also depend on changes in the blood volume of the capillaries. These changes might be a reason for the non-uniform changes in x between sitting and supine positions.

Pulmonary capillary blood volume, Q_c

The individual relationships between Q_c and V_A could be best described by a second order polynomial in both body positions. The maximum Q_c ($Q_{c,max}$) was found between FRC and TLC in the majority of the volunteers. The decrease in Q_c when alveolar volume decreases below its level of the maximum has been explained by collapse and convolution of capillaries [25] and by airway closure [26]. The decrease in Q_c at higher alveolar volumes was attributed to external compression of the capillary bed [25].

At rest $Q_{c,max}$ is not the potential maximum. In morphometric studies Weibel [27] described a maximal capillary blood volume much higher than the values we found in a resting situation. Crapo et al. [28] compared physiologically and morphometrically estimated Q_c values in dogs and concluded that Q_c measured by morphometry was more than twice the physiologically estimated Q_c in the same dogs. Brashear et al. [29] illustrated in dogs that Q_c was doubled when they were exercising. From all these data it may be concluded that the lung is an overdimensioned gas exchanger with a large reserve of capillary blood volume.

In the supine position the Q_c vs V_A relationship of our healthy subjects was shifted upwards compared with the sitting position (Fig. 6.8). This might be due to a shift of

blood from the systemic circulation into the pulmonary circulation when changing from upright to recumbent posture.

According to Lewis et al. [6], capillaries are simple endothelial tubes which should open fully if transmural pressure exceeds a critical opening pressure, implying a lack of volume changes in patent vessels. As a consequence capillaries in the basal parts of the lungs at lower alveolar volumes in the sitting position will be fully open, whereas in the apex the majority of the capillaries are closed. Thus, an increase in Q_c with increasing alveolar volume should be attributed to an upward extension of the zone with patent vessels. In the supine position gravitational effects have less effect, resulting in a more uniform perfusion and therefore in an increasing Q_c [30].

To simplify the analysis of individual responses we used Q_{cmax} as an indicator of the capillary blood volume. An advantage of this variable is its smaller random variation compared with the Q_c values at both sides of the maximum.

$Q_{c,max}$/BSA was significantly higher in the supine position at younger ages (Fig. 6.9). The position related difference decreased with age. Moreover, $Q_{c,max}$/BSA decreased with age in both positions, probably due to either a smaller capillary compliance or an underestimation of $Q_{c,max}$ if closing volume is present, or a combination of both mechanisms. Also the fact that $V_{AQc,max}$ was found at a higher alveolar volume in the older subjects is probably connected with changes in closing volume [26].

Perrault et al. [31] observed a more uniform distribution of pulmonary blood flow in older sitting normal subjects compared to young normals. They concluded that the improved uniformity is due to a higher pulmonary arterial pressure in the elderly. When filling of the capillaries [6] is related to flow, the slight posture-dependent changes in $Q_{c,max}$/BSA at older ages could depend on a more uniform lung perfusion [31]. This might explain the minor response in D_{LCO}/V_A and D_{LCO} to changes in posture in the older normal subjects. We derived similar results from the data of Ettinger et al. [3]. When at older ages the capillaries are more evenly perfused and filled with blood, the question remains why in both positions $Q_{c,max}$/BSA decreases with age (Fig. 6.9). Experimental data to answer this question are lacking. Brody et al. suggested [32] that the capillary component of the alveolar wall might diminish with age.

In conclusion:

- A response in D_{LCO}/V_A to a change in body position from sitting to supine at one level of V_A yields insufficient information to predict responses at other levels of V_A.
- The negative slope in the D_{LCO}/V_A vs V_A relationship implies an increased D_{LCO}/V_A value at FRC in supine position because of the smaller FRC in this body position. However, the increase in D_{LCO}/V_A did not compensate for the decrease in FRC in the majority of volunteers, leading to a decrease in D_{LCO}.
- The response of $Q_{c,max}$/BSA and D_{LCO}/V_A on change in body position from sitting to supine decreases with age. A reason for it seems to be a more even perfusion at older ages.
- The responses of D_m and Q_c on change in body position are highly significant at younger ages. These responses decrease with ageing and become non-significant above 50 years.
- In smoking volunteers the responses on change in body position were not different from those in non-smoking subjects.
- The exponent x in the relationship $D_m = kV_A^x$ was more clustered around 2/3 in supine than sitting subjects, indicating a more isotropic expansion of the diffusion membrane with increasing alveolar volume in the supine position. But no general conclusions as to any model are possible due to the large variability in the responses in x.

APPENDIX

θ (μmol.s^{-1}.kPa^{-1}.ml^{-1}) was calculated from the pH corrected formula recommended by Forster 1987 [14] $\frac{1}{\theta} = 0.23 + 0.0055\ P_{O_2}$, assuming a permeability ratio of the red cell membrane and the cell interior according to λ=2.5. The use of this formula resulted in negative values for $1/D_m$. The assumption of an infinite permeability of the red cell membrane with respect to the cell interior ($\lambda=\infty$) [15-17] resulted, after pH correction, in realistic D_m values. Therefore, we corrected the formula:

$$\frac{1}{\theta} = 0.059 + 0.0077\ P_{O_2}$$

with $\lambda=\infty$ and estimated at pH=8.0 [13] to pH=7.4, assuming that this change in pH

had no effect on $\frac{1}{\theta}$ at zero P_{O_2} and that θ decreases by about 8.8% per pH unit [14, 33]. This correction resulted in the formula:

$$\frac{1}{\theta} = 0.059 + 0.0073\ P_{O_2}$$

Borland and Higenbottam [34] estimated the diffusing capacity for CO and NO. Because in vitro NO combines 400 times faster than CO with dissolved reduced hemoglobin, θ_{NO} will greatly exceed θ_{CO} and $1/(\theta_{NO}Q_c)$ will tend towards zero. Thus, D_{LNO} should approximate D_{mNO} the more so as D_{LNO} is independent of P_{O_2}. Assuming the "extra-erythrocytic" resistance to be an aqueous layer, NO diffusivity (water solubility/square root of molecular weight) is a constant factor 1.8 times CO diffusivity. Recently Moinard and Guenard [35] compared the clàssical way of estimating D_m and Q_c (CO diffusion measurement at two different oxygen pressures) with a method in which D_{mNO} is measured directly and D_{mCO} is calculated via the difference in diffusivity of NO and CO. The D_m and Q_c values calculated with both methods appeared to be comparable. The use of the pH corrected formula above for $\frac{1}{\theta}$ with $\lambda = \infty$ resulted in D_m and Q_c values comparable with the results using the NO technique.

REFERENCES

1. O'Brodovich HM, Mellings RB, and Mansell AL. Effects of growth on the diffusion constant for carbon monoxide. *Am. Rev. Resp. Dis.* 1982; 125: 670-673.
2. Daly WJ, Giammona ST, Ross JC, and Feigenbaum H. Effects of pulmonary vascular congestion on postural changes in the perfusion and filling of the pulmonary vascular bed. *J. of Clin. Invest.* 1964; 43 (1): 68-76.
3. Ettinger WH, Wise RA, Stevens MB, and Wigley FM. Absence of positional change in pulmonary diffusing capacity in systemic sclerosis. *Am. J. of Med.* 1983; 75: 305-312.
4. Giammona ST, and Daly WJ. Gravitational effects on pulmonary diffusing capacity and on pulmonary capillary blood volume. *Amer. Rev. Resp. Dis.* 1968; 97: 63-68.
5. Zelkowitz PS, and Giammona ST. Effects of gravity and exercise on the pulmonary diffusing capacity in children with Cystic Fibrosis. *J. of Pediatrics.* 1969; 74 (3): 393-398.
6. Lewis BM, McElroy EJ, Hayford-Welsing EJ, and Samberg LC. The effects of body position, ganglionic blockade and norepinephrine on the pulmonary capillary bed. *J. Clin. Invest.* 1960; 39: 1345-1352.
7. Préfaut C, Ramonatxa M, Mouliade B, and Chardon G. Effets de la posture sur les échanges respiratoires du sujet normal. Pathologie Biologie 1977; 375-382 (juin).
8. Sundstrom G. Influence of body position on pulmonary diffusing capacity in young and old men. *J. Appl. Physiol.* 1975; 38 (3): 418-423.
9. Stam H, Versprille A, and Bogaard JM. The components of the carbon monoxide diffusing capacity in man dependent on alveolar volume. *Bull. Europ. Physiopath. Resp.* 1983; 19: 17-22.
10. Quanjer PhH. Standardized lung function testing. *Bull. Europ. Physiopath. Resp.* 1983; 19: Suppl 5.
11. Jones RS, and Meade F. A theoretical and experimental analysis of anomalies in the estimation of pulmonary diffusing capacity by the single breath method. *Q. J. Exp. Physiol.* 1961; 46: 131-143.
12. Cotes JE. Lung function: Assessment and application in medicine. 3rd Ed. Blackwell, Oxford, 1975.

13. Roughton FJW, and Forster RE. Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. *J. Appl. Physiol.* 1957; 11: 290-302.
14. Forster RE. Diffusion of gases across the alveolar membrane. In Fishman A. P. (Section Editor), Farhi L. E., Tenney S. M. (Volume Editors), Geiger S. R. (Executive Editor), Handbook of Physiology, Section 3: The Respiratory System, Volume IV Gas Exchange, American Physiological Society, Bethesda, MD, 71-88, 1987.
15. Kreuzer FJA, and Yahr WZ. Influence of red cell membrane on diffusion of oxygen. *J. Appl. Physiol.* 1960; 15(6): 1117-1121.
16. Thews G, and Niesel W. Zur Theorie der Sauerstoffdiffusion im Erythrocyten. *Pflügers Arch.* 1959; 268: 318-333.
17. Yahr WZ, and Kreuzer FJA. Influence of red cell membrane on O_2 diffusion. *Fed. Proc.* 1960; 19: 382.
18. Lebecque P, Mwepu A, Veriter C, Rodenstein D, Nemery B, and Frans A. Hysteresis of the alveolar capillary membrane in normal subjects. *J. Appl. Physiol.* 1986; 60(4): 1442-1445.
19. Michaelson ED, Sackner MA, and Johnson RL. Vertical distributions of pulmonary diffusing capacity and capillary blood flow in man. *J. Clin. Invest.* 1973; 52: 359-369.
20. Newth CJL, Cotton DJ, and Nadel JA. Pulmonary diffusing capacity measured at multiple intervals during a single exhalation in man. *J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.* 1977; 43(4): 617-625.
21. Billiet L. De bepaling van de pulmonaire diffusie capaciteit. Thesis, Leuven, 1965.
22. Marks A, Cugell DW, Cadigan JB, and Gaensler EA. Clinical determination of the diffusing capacity of the lungs. *Amer. J. Med.* 1957; 22: 51-73.
23. McGrath MW, and Thomson ML. The effect of age, body size and lung volume change on alveolar-capillary permeability and diffusing capacity in man. *J. Physiol., Lond.* 1959; 146: 572-582.

24. Weibel ER, Untersee P, Gill J, and Zulauf M. Morphometric estimation of pulmonary diffusion capacity VI. Effect of varying positive pressure inflation of airspaces. *Resp. Physiol.* 1973; 18: 285-308.

25. Assimacopoulos A, Guggenheim R, and Kapanci Y. Changes in alveolar capillary configuration at different levels of lung inflation in the rat. *Lab. Invest.* 1976; 34: 10-22.

26. Le Blanc P, Ruff F, and Milic-Emili J. Effects of age and body position on "airway closure" in man. *J. Appl. Physiol.* 1970; 28: 448-451.

27. Weibel ER. How does lung structure affect gas exchange? *Chest* 1983; 83(4): 657-665.

28. Crapo JD, and Crapo RO. Comparison of total lung diffusion capacity and the membrane component of diffusion capacity as determined by physiologic and morphometric techniques. *Resp. Physiol.* 1983; 51: 183-194.

29. Brashear RE, Ross JC, and Daly WJ. Pulmonary diffusion and capillary blood volume in dogs at rest and with exercise. *J. Appl. Physiol.* 1966; 21(2): 516-520.

30. Bryan AC, Bentivoglio LG, Beerel F, MacLeish H, Zidulka A, and Bates DV. Factors affecting regional distribution of ventilation and perfusion in the lung. *J. Appl. Physiol.* 1964; 19(3): 395-402.

31. Perrault JL, Morinet J, Nader FA, and Lockhart A. Influence de l'age et de l'exercice physique sur les pressions dans la circulation pulmonaire de subjects normaux ages de plus de quarante ans. *Bull. Physio-Pathol. Respir.* 1969; 5: 505-522.

32. Brody JS, and Thurlbeck WM. Development, growth and aging of the lung. In: Handbook of Physiology. The Respiratory System III, edit by A.P. Fishman. *Am. Physiol. Soc.* Section 3, vol. III, part 1, chapter 22, p. 378, 1986.

33. Krawiec J A, Forster RE, Gottliebsen TW, and Fish D. Rate of CO uptake by human red blood cells. *Fed. Proc.* 1983; 42: 993.

34. Borland CDR, and Higenbottam TW. A simultaneous single breath measurement of pulmonary diffusing capacity with nitric oxide and carbon monoxide. *Eur. Respir. J.* 1989; 2: 56-63.

35. Moinard J, and Guenard H. Determination of lung capillary blood volume and membrane diffusing capacity in patients with COLD using the NO-CO method. *Eur. Respir. J.* 1990; 3: 318-322.

CHAPTER 7

FINAL CONSIDERATIONS

In their report "Standardization of the measurement of the transfer factor" the ECCS [1] states that 1) D_{LCO} and D_{LCO}/V_A, determined with the single breath method at TLC, are reduced in almost all disorders of the lung parenchyma and both variables can contribute to diagnosis and follow up, and 2) that the association between D_{LCO}/V_A and V_A can lead to difficulty in interpretation, particularly during childhood and adolescence, in non-Caucasians and in patients in whom the total lung capacity is reduced. The chapters 2 and 3 provide an answer to these problems.

D_{LCO} reflects the effective diffusion area, the thickness of the blood gas barrier and the amount of Hb available in the lung capillaries for the uptake of CO. The proportionality between D_{LCO} and diffusion area and the increase in diffusion area with alveolar volume implies an increase in D_{LCO} with alveolar volume. If TLC is decreased by a disease D_{LCO} will be decreased by the volume decrease. If also the diffusing membrane is affected and lung volume is decreased, D_{LCO} is additionally decreased by volume loss. The decrease in D_{LCO} due to the diffusion disturbance is estimated with use of the D_{LCO} per liter alveolar volume (D_{LCO}/V_A). However, as we found in our studies, D_{LCO}/V_A should not be compared with D_{LCO}/V_A at the patient's reference TLC but at the patient's actual TLC to estimate the diffusion disturbance properly. An example of the lung function of a patient is given in Tables 7.1. and 7.2. D_{LCO}/V_A has a value of 96% of the patient's D_{LCO}/V_A reference value at reference TLC and 75% of the reference value at the patient's actual TLC, which is a decrease of almost 2 SD. We collected evidence which supports such an evaluation from patients in whom a restrictive lung disease developed in short time. After a volume restriction was developed D_{LCO}/V_A changed in a similar way during voluntary alveolar volume changes as in healthy conditions before the treatment. Therefore, we concluded that volume restriction caused a similar change in D_{LCO}/V_A as in voluntary volume decrease. As a consequence D_{LCO}/V_A at a limited TLC should be compared with reference D_{LCO}/V_A values at the lung volume at which measurements are performed.

Although the comparison of D_{LCO}/V_A with the reference value at a patient's disease limited TLC was only verified in one type of restrictive disease (after bleomycin treatment, chapter 4), we apply this type of evaluation to more patients with a restrictive lung disease. The results in chapter 3 from a child with another type of restriction encouraged us to do so. However, studies will have to be performed to verify whether the dependence of D_{LCO}/V_A on V_A is similar to that found in the patients treated with bleomycin.

Table 7.1. Example of the spirometric data of a 52 yr old female patient with a height of 1.52 m and a weight of 57 kg.

		Actual value	% Ref.		Reference	SD Ref.
TLC	(l)	3.40	80	*	4.24	0.60
FRC	(l)	2.01	82		2.46	0.50
(FRC/TLC)*100	(%)	59	111		53	6
RV	(l)	1.19	75	*	1.58	0.35
(RV/TLC)*100	(%)	35	96		36	6
VC insp.	(l)	2.21	87		2.56	0.42
(VC/TLC)*100	(%)	65				
FEV_1	(l)	1.52	72	*	2.10	0.38
(FEV_1/VC)*100	(%)	69	87	*	79	7

** Between 1 and 2 SD from reference. ** More than 2 SD from predicted mean.*

Table 7.2. Example of the data for the diffusing capacity of the patient of table 7.1.

		Actual value	% Ref.		Reference	SD Ref.
TLC(sb)	(l)	3.17	75	*	4.24	0.60
TLC(sb)/TLC(spir)		0.93				
VC(sb)	(l)	2.18				
VC(sb)/VC(spir)		0.99				
D_{LCO}	(μmol.s^{-1}.kPa^{-1})	62.1	52	**	119.4 ♧	19.0
D_{LCO}(+Hb cor)	(μmol.s^{-1}.kPa^{-1})	66.3	56	**	119.4 ♧	19.0
D_{LCO}/V_A(+Hb cor)	(μmol.s^{-1}.kPa^{-1}.l^{-1})	21.8	96		22.66 ♧	3.4
D_{LCO}/V_A(+Hb cor)	(μmol.s^{-1}.kPa^{-1}.l^{-1})	21.8	75	*	28.96 ♡	3.63
Hb	(mmol.l^{-1})	7.1	86		8.3	

** Between 1 and 2 SD from reference; ** More than 2 SD from predicted mean; ♧ Reference value at the patient's reference TLC; ♡ Reference value at the patient's actual TLC.*

We developed the rebreathing method at normal breathing to estimate diffusing capacity in patients who are not able to perform hyperventilation or a satisfactory vital capacity. The ECCS [1] recommended not to use the rebreathing method during hyperventilation for routine use, because it can be tedious and the results are not interchangeable with those of the breathholding procedure. Although the absolute values of single breath and rebreathing diffusing capacity are different, the results relative to the corresponding reference values appeared to be comparable, even in patients with unequal ventilation. Our results (Chapter 5) imply that the single breath method is not superior to the rebreathing procedure in this respect.

Our procedure based on normal breathing is not tedious, and can even be performed by severely ill patients and very young children. In many patients single breath diffusing capacity is measured several times a year for many years to follow up the progress of their disease or to study effects of medication. An example is given in Fig. 7.1. The changes in D_{LCO} relative to D_{LCO} predicted at reference TLC result both from restriction and impairment of diffusion at the level of the alveolar capillary membrane (solid line). The course in D_{LCO}/V_A relative to D_{LCO}/V_A reference values at a volume level equal to the disease limited TLC indicates the impairment at alveolar capillary level (solid line). If in this example single breath D_{LCO}/V_A had been compared to the predicted values at reference TLC, D_{LCO}/V_A would have been normal (about 90 % of reference, dotted line), but comparison with the reference value at the patient's actual TLC obviously indicates a decreased gas transfer. If severely ill patients cannot perform the single breath procedure anymore, follow up can be continued by using the rebreathing procedure. In our example the last two measurements have been performed with the rebreathing method (dashed line). Rebreathing D_{LCO}/V_A was compared with reference values (chapter 5), which contain V_A as a parameter. The single breath values represent the percentage of the volume dependent references (solid line). The relative values of both methods are about the same. A reference value of rebreathing D_{LCO} would contain actual V_A as a parameter leading to an underestimation of decrease in total D_{LCO}.

Relevance to paediatric pulmonology.

The past 2 to 3 years the estimation of the diffusion indexes became increasingly important in the paediatric pulmonology department of our hospital to diagnose interstitial auto-

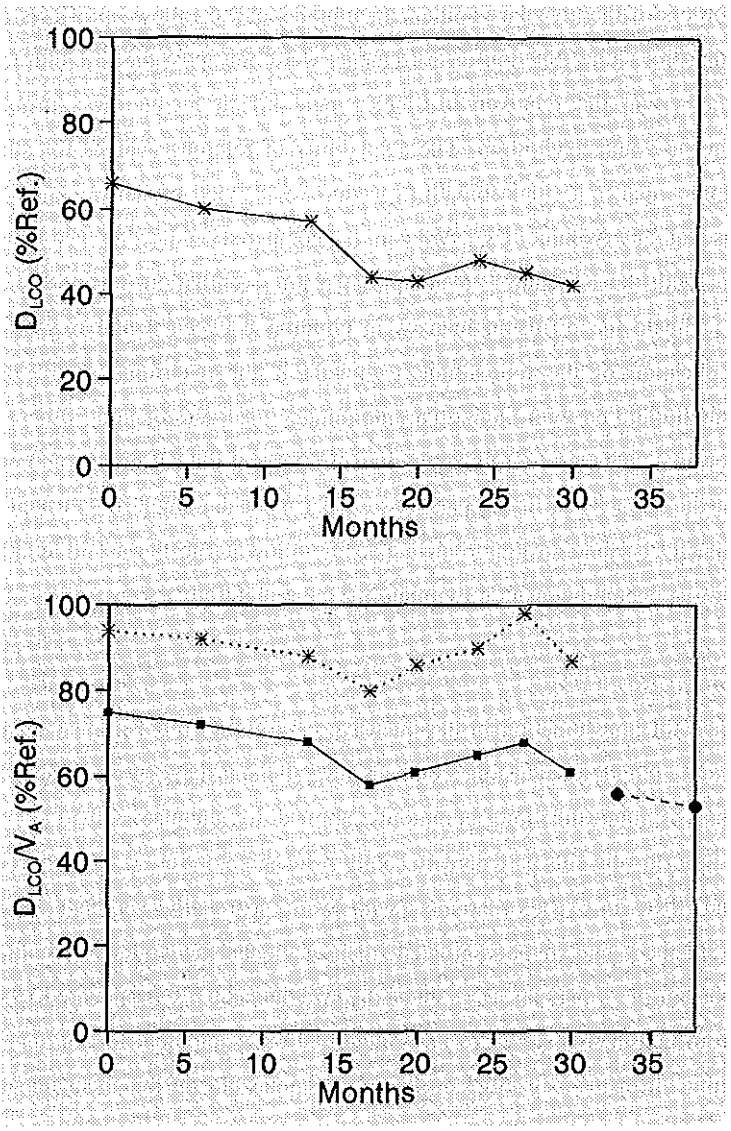

Fig. 7.1. *An example of a follow-up of the single breath and rebreathing diffusion indexes.*

D_{LCO}/V_A: ■ *and solid line percentage of reference at symptom limited TLC.*
* *and dotted line percentage of reference at reference TLC.*
● *and dashed line rebreathing values.*

D_{LCO} : * *and solid line percentage of reference at reference TLC.*

immune disorders of the alveolar capillary membrane and to follow up effects of medication. Before that time lung function measurements were only possible in children older than 6 years of age, and the relatively difficult single breath method was problematic or impossible in the younger children. The availability of reference values for children at all alveolar volumes makes a single breath procedure, with vital capacity maneuver unnecessary, because at any volume D_{LCO}/V_A can be compared with reference values [2, 3]. Furthermore, with the rebreathing technique at resting ventilation (chapter 5) it is proved possible to estimate the diffusion indexes in children between 2 and 6 years of age. A potential application of the rebreathing technique is the determination of the diffusion indexes in small children with interstitial autoimmune disorders or lung disease induced by treatment for malignancies to follow up the progress of the disorder, and to study the effects of medication. Thus in the paediatric department the single breath and rebreathing diffusing capacity became an important index for following up the negative side effects of bleomycin in the chemotherapeutic regimen of young children.

Relevance to Intensive Care.

The assessment of lung function in the intensive care unit is often limited to the measurement of arterial blood gas tensions. Only a few attempts have been described in which measurements of lung function, commonly applied to ambulatory patients, have been performed in critically ill patients. Recently McNaughton et al. [4] measured D_{LCO}, D_{LCO}/V_A and FRC in ARDS patients, who were artificially ventilated. They used a rebreathing method and hyperventilated their patients moderately (15 $lmin^{-1}$). They concluded that in these patients D_{LCO} and D_{LCO}/V_A, but not FRC, appeared to differentiate survivors from nonsurvivors. We recently modified our equipment for the measurement of the rebreathing diffusing capacity so, that it is applicable during mechanical ventilation without changing the ventilatory mode. In a pilot study we performed measurements of the rebreathing diffusing capacity in normal volunteers during mechanical ventilation. The preliminary results confirm that this technique can be used during mechanical ventilation. Studies in critically ill patients will be needed to answer the question whether D_{LCO} and D_{LCO}/V_A obtained in this way will be useful indicators of treatment and prognosis.

REFERENCES

1. Cotes JE, Chinn DJ, Quanjer PhH, Roca J, and Yernault JC. Standardization of the measurement of transfer factor (diffusing capacity). *Eur. Respir. J.* 1993; 6 Suppl 16: 41-52.
2. Tiddens HAWM, v.d. Laag JJ, Stam H, and de Jongste JC. Pulmonary involvement in 16 patients with juvenile-onset mixed connective tissue disease (MCTD). *Eur. Respir. J.* 1992; 5 Suppl 15: 231s.
3. Tiddens HAWM, v.d. Laag JJ, Stam H, and de Jongste JC. Pulmonary involvement in 14 patients with juvenile-onset mixed connective tissue disease (MCTD). Joint Meeting Eur. Paediatric Resp. Soc. and Paediatric Ass. of Eur. Resp. Soc., 1993; Oslo.
4. McNaughton PD, and Evans TW. Measurement of lung volume and D_{LCO} in acute respiratory failure. *Am. J. Respir. Crit. Care Med.* 1994; 150: 770-775.

SUMMARY

Chapter 1

The diffusing capacity for CO, D_{LCO}, gives information on changes in effective surface area and barrier thickness of the pulmonary gas-blood barrier and capillary blood volume. Because surface area is directly related to volume, diffusing capacity will depend on alveolar volume. As a consequence the single breath diffusing capacity, which is usually determined at TLC, is not comparable with a diffusing capacity determined at a lower volume level. The effect of alveolar volume on D_{LCO} and D_{LCO} per liter lung volume and its consequences for the evaluation of patients is addressed in the chapters 2-4. The single breath, the steady state and the rebreathing methods to estimate D_{LCO} are discussed as an introduction to chapter 5, in which the development of a rebreathing method during resting ventilation is presented. Because in severely ill patients measurements in the sitting position are not always possible, we studied the effect of the supine position on D_{LCO} and D_{LCO} per liter lung volume. This is described in chapter 6, where we also separated the diffusing capacity in its components: the membrane conductance D_m and the effective capillary blood volume Q_c.

Chapter 2

In this study we determined reference values of D_{LCO}, i.e. total diffusing capacity for carbon monoxide, and D_{LCO} per liter alveolar volume (D_{LCO}/V_A) at TLC and at lung volumes below TLC in sitting position. In 55 healthy nonsmoking volunteers (20-85 yr old), we determined reference values at TLC level in which age was the only parameter. In a subgroup (n=16) these predictions did not change by correcting for normal variability in hemoglobin concentration. In all volunteers D_{LCO} decreased and D_{LCO}/V_A increased with decreasing V_A. The increase in D_{LCO}/V_A was linear, and smallest in older subjects. We derived equations to calculate reference values of D_{LCO}/V_A for lung volumes at and below TLC with two methods: 1) the "Random coefficients linear" model (RCL), which predicts the reference values directly and 2) a conversion method which predicts D_{LCO}/V_A for lower V_A levels from reference values at TLC. An advantage of the conversion method is the suitability of D_{LCO}/V_A reference values at TLC of other populations. A disadvantage is the greater standard deviation of these reference values compared with those obtained with the RCL method. D_{LCO} can be found by multiplying D_{LCO}/V_A with V_A.

Chapter 3

In normal adults the diffusing capacity normalized per liter of alveolar volume (D_{LCO}/V_A), decreases, whereas total diffusing capacity (D_{LCO}) increases if alveolar volume (V_A) increases. We studied these relationships in a group of normal children below 20 years of age. Diffusion variables were determined using the single breath technique. The effects of sex, age and height on these relationships were estimated. D_{LCO} was higher and D_{LCO}/V_A was lower at larger alveolar volume. D_{LCO} and D_{LCO}/VA reference values at TLC appeared to be comparable with reference values at TLC published in the literature. Reference values of D_{LCO} and D_{LCO}/V_A derived from measurements at various alveolar volumes also predict similar values at TLC. The advantage of our reference equations is the applicability to patients with a restrictive lung disease. Actual D_{LCO}/V_A can be compared with reference D_{LCO}/V_A at actual (restrictive) TLC instead of reference D_{LCO}/V_A at reference TLC. This comparison extends the evaluation of a diffusion disorder.

Chapter 4

In patients with a restrictive lung disease we determined D_{LCO} and D_{LCO}/V_A for comparison with the reference values determined at their disease limited TLC as well as at their predicted TLC. We hypothesized that a volume restriction due to disease has a similar effect on the diffusion indexes as a voluntary volume reduction in normal volunteers, implying an increase in D_{LCO}/V_A at the decreased TLC. As a consequence a decreased D_{LCO}/V_A in such patient should be compared with a reference D_{LCO}/V_A at the disease limited TLC. To test this hypothesis, we studied the volume dependence of the diffusion indexes in a group of thirteen patients, who developed a diffusion disorder in combination with a volume restriction in a short period of time due to treatment with bleomycin. In the majority of these patients the D_{LCO}/V_A vs. V_A relationship shifted downwards during therapy, while the negative slope was not changed. This decrease in level illustrated the development of a decreased CO transfer at the level of the alveolar to capillary membrane. Seven of these patients also developed a volume restriction. We found that in these patients voluntary changes in lung volume caused the same changes in D_{LCO}/V_A as in normal volunteers. The D_{LCO} vs. V_A relationship, which can be described by a second order polynomial, is also decreased due to the bleomycin regimen. In patients, who developed a volume restriction, D_{LCO} decreased due to the restriction as well as due to a

diffusion disorder at alveolar capillary level. The volume decrease by the restrictive disease had a similar effect as voluntary volume decrease. Consequently, comparing D_{LCO}/V_A at a patient's lower TLC to reference D_{LCO}/V_A at his reference TLC will imply an underestimation of a diffusion disorder. The change in D_{LCO} reflects both an effect by restriction and an effect by alveolar capillary disorder, when compared with reference D_{LCO} at reference or pretreatment TLC.

Chapter 5

The diffusing capacity of carbon monoxide (D_{LCO}) and its value normalized to alveolar volume (D_{LCO}/V_A) are usually estimated with the single breath method at TLC. Severely ill patients and small children are not able to deliver a satisfactory vital capacity (VC) or to hold their breath for 10 seconds at TLC. The aim of this study was to develope a rebreathing procedure, in which diffusing capacity can be determined during spontaneous breathing. The conventional rebreathing method during hyperventilation was modified in such a way, that rebreathing volume and gas concentrations were kept constant by CO_2 absorption and O_2 supplementation.In healthy volunteers and in patients the diffusion indexes obtained with this rebreathing method during rest ventilation, were compared with those of the single breath method. D_{LCO}/V_A decreased with alveolar volume (V_A) and increased with alveolar ventilation (V_A') in children and adults. At V_A' above 35 $lmin^{-1}$ rebreathing D_{LCO}/V_A was similar to single breath D_{LCO}/V_A at a similar alveolar volume. Reference values of rebreathing D_{LCO}/V_A for both children and adults were determined. The D_{LCO}/V_A relative to their corresponding reference values were the same for both methods in patients irrespective of ventilation distribution disturbances. The diffusing capacity obtained with the rebreathing method at rest ventilation can serve as a valuable index to evaluate a diffusion disorder.

Chapter 6

Normal subjects have a larger diffusing capacity normalized per liter alveolar volume (D_{LCO}/V_A) in the supine than in the sitting position. Body position changes total lung diffusing capacity (D_{LCO}), D_{LCO}/V_A, membrane conductance (D_m), and effective pulmonary capillary blood volume (Q_c) as a function of alveolar volume (V_A). These functions were studied in 37 healthy volunteers.

D_{LCO}/V_A vs. V_A yields a linear relationship in sitting as well as in supine position. Both have a negative slope, but usually do not run parallel. In normal subjects up to 50 years D_{LCO}/V_A and D_{LCO} increased significantly when subjects moved from a sitting to a supine posture at volumes between 50% and 100% of total lung capacity (TLC). In subjects > 50 yr old the responses of D_{LCO}/V_A and D_{LCO} to change in body position were not significant at TLC. Functional residual capacity (FRC) decreases and D_{LCO}/V_A increases in all normal subjects when they change position from sitting to supine. When D_{LCO}/V_A increases more than predicted from the D_{LCO}/V_A vs. V_A relationship in a sitting position we may infer an increase in effective Q_c in the supine position. In 56% of volunteers, supine D_{LCO} was smaller than sitting D_{LCO} despite a higher D_{LCO}/V_A at FRC in the supine position because of the relatively larger decrease in FRC.

When the positional response at TLC is studied, an estimation obtained accidentally at a volume lower than TLC may influence results. Above 80% of TLC, D_m decreased significantly from sitting to supine. Below this lung volume the decrease was not significant.

The relationship between capillary blood volume Q_c and V_A was best described by a second-order polynomial characterized by a maximum Q_c at a V_A > 60% of TLC. Q_c was significantly higher in the supine position than in the sitting position, but the difference became smaller with increasing age. In observing the sitting and supine positions we saw a decrease in maximum Q_c normalized per m^2 body surface area with age.

Chapter 7

In this chapter the conclusions, clinical applications and consequences for our pulmonary function laboratory are considered.

SAMENVATTING

Hoofdstuk 1

De diffusiecapaciteit van de long voor koolmonoxide, D_{LCO}, geeft informatie over het transport van koolmonoxide gas (CO) uit de lucht in de longblaasjes naar het bloed in de longcapillairen. Dit transport is afhankelijk van het oppervlak en de dikte van de scheidingswand tussen longenlucht en het bloed, en de hoeveelheid bloed in de longcapillairen. Daar het oppervlak direct gerelateerd is aan het longvolume, hangt de diffusiecapaciteit af van het longvolume waarbij gemeten wordt. Dit betekent, dat bepalingen van de D_{LCO}, die uitgevoerd worden bij een longvolume kleiner dan TLC niet vergelijkbaar zijn met bepalingen die wel bij TLC worden uitgevoerd. TLC is de totale longcapaciteit, dit is het longvolume na maximale inademing. Een in de kliniek veel gebruikte methode om de D_{LCO} te bepalen is de "single breath" methode. Dit is een methode, waarbij men één maximale teug lucht inademt, waaraan een geringe hoeveelheid CO is toegevoegd. Na 10 seconden adem inhouden blaast men vervolgens ver uit. Deze "single breath" methode geeft de best reproduceerbare resultaten. De patiënt moet wel in staat zijn om 10 seconden de adem in te houden en minimaal een volume van 1,5 liter uit te ademen. Bij een deel van de patiënten met een longafwijking verkleint het longvolume ten gevolge van hun ziekte (volumerestrictie). Daar deze groep niet meer op hun normale TLC niveau onderzocht kan worden, is een belangrijke vraag welke invloed de longvolumevermindering op de gasoverdracht heeft. De onderzoekingen, die hierop betrekking hebben, worden in de hoofdstukken 2-4 beschreven.

Voor ernstig zieke patiënten en kinderen bestaan andere methoden: [1]) de "steady state" methode, waarbij de patiënt rustig inademt uit een reservoir en uitademt naar een mengvat en [2]) een "rebreathing" methode, waarbij de patiënt moet hyperventileren in een gesloten systeem. De resultaten van beide methoden zijn niet identiek en niet gelijk aan die van de "single breath" methode. Wij hebben een "rebreathing" techniek ontwikkeld, waarbij tijdens rust ademhaling gemeten wordt in een gesloten systeem. In hoofdstuk 5 wordt deze methode vergeleken met de "single breath" methode.

Omdat ernstig zieke patiënten niet altijd in de zittende houding gemeten kunnen worden, is in hoofdstuk 6 de diffusiecapaciteit in liggende houding vergeleken met die in zittende positie.

Hoofdstuk 2

Bij 55 normale vrijwilligers tussen 20 en 85 jaar werden in zittende positie referentiewaarden voor D_{LCO} en D_{LCO} per liter alveolair volume (V_A), D_{LCO}/V_A, bepaald zowel op TLC niveau als bij longvolumes kleiner dan TLC. Bij de referentiewaarden op TLC niveau bleek de leeftijd de enige voorspellende grootheid te zijn. In een groep van 16 vrijwilligers werd het effect van een correctie voor de normale variabiliteit van het hemoglobinegehalte op de referentiewaarden voor de diffusiecapaciteit onderzocht. De referentievergelijkingen van de diffusievariabelen bleken niet significant te veranderen, indien de Hb-correctie werd doorgevoerd, waarschijnlijk doordat de normale verdeling in de Hb-concentratie gemiddeld tot evenveel positieve als negatieve correcties van de diffusiecapaciteit aanleiding gaf.

In alle vrijwilligers daalde D_{LCO} en steeg D_{LCO}/V_A als het alveolaire volume afnam. D_{LCO}/V_A bleek lineair toe te nemen bij afname van V_A. De helling van deze lineaire relatie verminderde met de leeftijd. Er werden vergelijkingen opgesteld ter berekening van referentiewaarden voor D_{LCO}/V_A, zowel op TLC niveau als voor longvolumes kleiner dan TLC. Er zijn twee methoden gevolgd:

1) de methode met het "Random coëfficiënten lineaire" model (RCL), waarbij de referentiewaarden op alle longvolumes direct berekend werden en 2) de "Conversie" methode, waarbij de referentiewaarden van D_{LCO}/V_A voor een lager longvolume berekend werden uit reeds bekende referentiewaarden op TLC niveau. In het "RCL" model daalt D_{LCO}/V_A niet alleen lineair met de leeftijd en V_A, maar ook bevat dit model een interactieterm tussen de leeftijd en V_A. De "Conversie" methode is gebaseerd op een lineaire leeftijdsafhankelijke conversie, waarbij het longvolume als fractie van de referentie TLC en een leeftijdsafhankelijke D_{LCO}/V_A referentiewaarde bij referentie TLC gebruikt worden. Een voordeel van de "Conversie" methode is dat men D_{LCO}/V_A referentiewaarden bij TLC van andere populaties kan gebruiken. Een nadeel is de grotere standaard deviatie in vergelijking met de RCL methode. Referentiewaarden voor D_{LCO} kunnen bepaald worden door de D_{LCO}/V_A referentiewaarde op een bepaald V_A niveau te vermenigvuldigen met dit volume.

Hoofdstuk 3

Omdat onder de 20 jaar genoemde relaties tussen D_{LCO}/V_A en V_A beïnvloed kunnen worden door het groeiproces, zijn de resultaten voor volwassenen niet geëxtrapoleerd naar

jongere leeftijden. In een apart onderzoek werd de afhankelijkheid van leeftijd, geslacht en lengte voor de relaties D_{LCO} vs V_A en D_{LCO}/V_A vs V_A bepaald. De referentiewaarden bleken afhankelijk te zijn van V_A en de lengte. De leeftijd bleek geen invloed te hebben. Aan de hand van een voorbeeld werden de consequenties van het gebruik van de volume afhankelijke referentiewaarden voor de diffusievariabelen duidelijk gemaakt. In een Appendix werd toegelicht, waardoor de lengte een belangrijke factor is in de beschreven regressievergelijkingen.

Hoofdstuk 4

Tot nu werd het effect van longvolume vermindering bij normalen onderzocht door het vrijwillig ademinhouden bij een lager longvolume dan TLC. Het is echter de vraag in hoeverre een vrijwillige longvolume vermindering in normalen vergelijkbaar is met een vermindering ten gevolge van een longafwijking in patiënten. Bovendien stelden wij ons de vraag, of bij patiënten met een restrictieve longfunktie de D_{LCO}/V_A vergeleken moet worden met een referentiewaarde die geldt voor de door ziekte verminderde TLC dan wel met de referentiewaarde die geldt voor de referentiewaarde van TLC. Om deze vraag te beantwoorden werden bij 13 volwassen patiënten, die bleomycine als onderdeel van een chemotherapeutische behandeling van een carcinoom ontvingen, de relatie tussen D_{LCO}/V_A en V_A voor, tijdens en na deze kuur bepaald. De D_{LCO}/V_A vs V_A relaties bleken lineair te zijn en de hellingen veranderden niet ten gevolge van de chemotherapie. De daling van D_{LCO}/V_A werd geillustreerd door de niveauvermindering van de gehele D_{LCO}/V_A vs V_A relatie. Bij de patiënten die een volumerestrictie ontwikkelden was de niveauvermindering van de gehele relatie gelijk aan de vermindering van D_{LCO}/V_A ten opzichte van de uitgangswaarde bij een longvolume gelijk aan de verminderde TLC.

Wij concludeerden, dat bij patiënten met een restrictie van het longvolume door een behandeling met bleomycine, de vergelijking van de D_{LCO}/V_A bij de verlaagde TLC na chemotherapie met een uitgangswaarde van de D_{LCO}/V_A bij de TLC voor de behandeling, kan leiden tot een onderschatting van de D_{LCO}/V_A verandering. Om informatie te verkrijgen over een eventuele diffusieafwijking op alveolocapillair niveau adviseren wij dan ook om bij patiënten met een restrictieve longfunktie de D_{LCO}/V_A te vergelijken met D_{LCO}/V_A referentiewaarden, die bepaald zijn op een vergelijkbaar volumeniveau.

De totale D_{LCO} vergelijken wij echter met de referentiewaarden van de D_{LCO} op referentie

TLC, daar deze waarde zowel informatie geeft over de vermindering van de diffusie zowel tengevolge van de diffusiestoornis als van de volumevermindering.

Hoofdstuk 5

Ernstig zieke patiënten en kleine kinderen zijn vaak niet in staat om de "single breath" methode te volbrengen, omdat of de VC te klein is of omdat ze niet in staat zijn 10 s de adem op TLC niveau in te houden. Daarom is de "rebreathing" methode ontwikkeld, waarbij tijdens rustademhaling gemeten wordt, terwijl de geproduceerde CO_2 weggevangen en de geconsumeerde O_2 toegevoegd wordt. Wij hebben deze methode vergeleken met de "single breath" methode. In normalen bij een alveolaire ventilatie (V_A') die groter is dan 35 l.min^{-1} bleken de waarden van de "rebreathing" en "single breath" D_{LCO}/V_A identiek te zijn. Bij een kleinere alveolaire ventilatie bleek de waarde van de "rebreathing" D_{LCO}/V_A kleiner te zijn dan die gemeten werd met de "single breath" methode. Om de referentiewaarden van de rebreathing methode aan te passen aan het ademhalingspatroon van de patiënt werd de alveolaire ventilatie opgenomen als voorspeller van D_{LCO}/V_A. We hebben referentiewaarden voor zowel kinderen als volwassenen bepaald. Evenals bij de "single breath" methode bleken bij de volwassenen V_A en de leeftijd en bij de kinderen V_A en de lengte parameters in de regressievergelijkingen te zijn. De "rebreathing" en "single breath" methode bleken vergelijkbaar te zijn, wanneer ze uitgedrukt worden als percentage van de corresponderende referentiewaarde. De absolute uitkomsten van beide methoden kunnen echter verschillen door de invloed van V_A en V_A'.

Bij patiënten met en zonder ventilatie ongelijkmatigheid bleken relatieve veranderingen in de diffusie-indices bepaald met de "single breath" en bepaald met de "rebreathing" methode overeenkomstige waarden te geven.

Hoofdstuk 6

De longen zijn in verticale stand ongelijkmatig doorbloed tengevolge van hydrostatische drukverschillen tussen top en basis. In liggende positie zijn deze hydrostatische drukverschillen kleiner en worden de longen gelijkmatiger doorbloed. Dit is de reden dat gezonde proefpersonen in liggende positie een grotere D_{LCO}/V_A hebben in vergelijking met de zittende positie. Het ontbreken van een dergelijk verschil bij houdingsverandering werd vaak beschouwd als een indicatie voor pulmonale hypertensie of stuwing. Bij 37 gezonde

vrijwilligers is het effect van houdingsverandering op de relatie tussen V_A en respectievelijk D_{LCO}, D_{LCO}/V_A, de membraan conductantie D_m, en het effectieve capillaire bloed volume Q_c onderzocht. De relaties tussen D_{LCO}/V_A en V_A bleken zowel in zittende als liggende positie lineair te zijn met een negatieve helling, maar ze bleken meestal niet parallel te lopen. Bij de gezonde vrijwilligers tot 50 jaar bleken de relaties tussen achtereenvolgens D_{LCO} en D_{LCO}/V_A en V_A in de liggende positie op een hoger niveau te liggen vergeleken met de zittende positie. Bij de ouderen boven de 50 jaar bleek de reactie in deze variabelen op houdingsverandering bij TLC niet significant te zijn. Bij alle gezonden bleek de FRC af en D_{LCO}/V_A toe te nemen bij verandering van zittende naar liggende positie. Wij concludeerden, dat alleen wanneer D_{LCO}/V_A meer toeneemt dan de toename volgens de D_{LCO}/V_A versus V_A relatie in zittende positie gesproken kan worden van een toename van het effectieve capillaire bloed volume in liggende positie. Ondanks een grotere D_{LCO}/V_A op FRC niveau in de liggende positie bleek in 56% van de proefpersonen de D_{LCO} in liggende positie kleiner te zijn dan in de zittende positie, door de relatief grotere daling in FRC. Als men de verandering van D_{LCO}/V_A op houdingsverandering onderzoekt met de "single breath" methode op TLC niveau, zullen metingen, die ongewild op iets lager niveau dan TLC worden uitgevoerd, de mate van verandering aanzienlijk beïnvloeden. Boven 80% van de TLC bleek D_m significant te dalen bij verandering van zittende naar liggende positie, maar bij lagere longvolumes bleek de daling niet significant te zijn.

De relatie tussen Q_c en V_A bleek het best te beschrijven te zijn door een tweedegraads polynoom met een maximum bij een alveolair volume boven 60% van de TLC. In liggende positie bleek Q_c significant groter te zijn dan in zittende positie, maar het verschil werd kleiner met toenemende leeftijd. Zowel in zittende als in liggende positie bleek het maximale effectieve capillaire bloed volume, $Q_{c,max}$, per m^2 lichaamsoppervlak af te nemen met de leeftijd. Wij concludeerden, dat het bestuderen van de reactie van de diffusie-indices op houdingsverandering, indien op één longvolumeniveau bepaald, twijfelachtig is, omdat de D_{LCO}/V_A versus V_A relaties niet evenwijdig lopen en FRC ook verandert bij verandering in lichaamspositie. Bovendien bleek een reactie van de diffusie-indices op houdingsverandering afhankelijk te zijn van de leeftijd. Wij adviseren, bij de bestudering van de reactie van de diffusie-indices op houdingsverandering, de verandering van de totale relatie tussen D_{LCO}/V_A en V_A te bestuderen.

Hoofdstuk 7

In dit hoofdstuk worden de conclusies samengevat en enige klinische toepassingen en consequenties voor het longfunktie laboratorium besproken.

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CURRICULUM VITAE

De auteur van dit proefschrift werd op 30 september 1949 te Dordrecht geboren. In 1966 behaalde hij het diploma HBS-b aan het Gemeentelijk Lyceum te Dordrecht en in 1970 het diploma Chemische Technologie aan de HTS Dordrecht. Van 1970 tot 1974 vervulde hij vervangende dienstplicht bij het Technologisch Laboratorium RVO-TNO te Rijswijk. Vanaf 1974 was hij werkzaam op het Pathofysiologisch Laboratorium van de afdeling Longziekten van het Academisch Ziekenhuis Rotterdam-Dijkzigt. Gedurende meer dan 15 jaar participeert hij in de regionale opleidingen voor Longfunktie Assistenten en Intensive Care Verpleegkundigen en in het opleidingsprogramma voor junior co-assistenten van de Erasmus Universiteit. Sinds 1989 verzorgt hij seminars over longfysiologie voor longfunktie assistenten, longartsen, cardiologen, technici, etc. voor Jaeger Benelux BV te Breda. In 1994 werd de auteur gevraagd om als "reviewer" op te treden voor het wetenschappelijke tijdschrift "Thorax", voor de beoordeling van publicaties die betrekking hebben op het pulmonale gastransport.