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Title: Assessment of concordance between diffusion of carbon monoxide through the lung using the 10 s breath-hold method, and the simultaneous NO/CO technique, in healthy participants.

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Abbreviations and definitions:

CI: Confidence interval;

CO: Carbon monoxide;

DLCO: Pulmonary diffusing capacity for carbon monoxide;

DLCO₁₀: Pulmonary diffusing capacity for carbon monoxide measured by classical 10 s breath hold time;

DLCO₅: Pulmonary diffusing capacity for carbon monoxide measured using the simultaneous NO and CO method;

DLNO : Pulmonary diffusing capacity for nitric oxide;

DmCO: Alveolar-capillary membrane diffusing capacity for carbon monoxide;

FACO : Alveolar fraction of CO;

FACO : Alveolar fraction of NO;

FEV1: Forced expiratory flow in 1 s;

Hb: Haemoglobin;

HbCO: Carboxyhaemoglobin;

KCO: Carbon monoxide transfer coefficient;

NO: Nitric Oxide;

SD: Standard deviation;

Ta: Breath-hold time;

TLC: Total lung capacity;

θ_{CO} : Specific conductance of the blood for CO;

θ_{NO} : Specific conductance of the blood for NO;

VA: Alveolar volume;

Vc: Pulmonary capillary blood volume;

VC: Vital capacity;

Vi: Inhaled volume.

Introduction:

Pulmonary diffusing capacity for carbon monoxide (DLCO) represents the ability of the transfer gas to diffuse through the alveolar-capillary membrane and plasma and bind to the red cell.

The Roughton and Forster model is used to calculate DLCO (Roughton and Forster, 1957) and can be divided into two parts: gas diffusion from the air to erythrocytes and gas combination with haemoglobin. In order to make mathematical manipulations easier, these two variables are considered as resistors in series. Therefore, the diffusing capacity has a dimension of a conductance, where D_{mCO} represents alveolar-capillary membrane diffusing capacity for carbon monoxide, θ_{CO} specific conductance of the blood for CO, V_c pulmonary capillary blood volume (Eq. 1).

$$\frac{1}{DLCO} = \frac{1}{D_{mCO}} + \frac{1}{\theta_{CO} \times V_c} \quad (1)$$

The single breath-hold technique, using a 10 s breath-hold time is the most standardized one (American Thoracic Society, 1995; Graham et al., 2017) and thus the most commonly-used method in clinical laboratories. The 10 s apnoea duration, recommended by Forster in 1955 (Forster et al., 1955), is based on three criteria. The first one being the feasibility of sustaining this duration of apnoea in patients, the second one being the one in which 10 s allows time for a sufficient change in the concentration of alveolar CO for measurement and the last one being the Foster's justification regarding his choice by obtaining similar data to that reported in the literature.

American and European societies also recommend a 10 s breath hold time (American Thoracic Society, 1995; MacIntyre et al., 2005). The timing device should be accurate within 1% (100 ms over 10 s). Theoretically, the breath-hold time doesn't influence DLCO because it is a rate, but the time must be correctly measured. Blackmore (Blackmore et al., 1957) and Dressel (Dressel et al., 2008) measured lower DLCO values when breath-hold time increased. This observation may be explained by the uneven distribution of the lung ventilation-perfusion ratios.

The first technique (Nairn et al., 1965) was developed to specifically calculate the two components of lung diffusing capacity and requires measurements at two oxygen partial pressures. The specific uptake of CO for the two levels of oxygen

pressure is known and DLCO is calculated via a system of two equations and two unknowns. However, the need of two DLCO measurements is an inherent problem relating to this method.

Although measurements of DmCO and Vc are reimbursed by Health Insurance companies in France as well as in the United States (Crapo, 2008) their clinical utility has remained uncertain for years. This is due to the relative complexity of the measures and the lack of accuracy generated by the two tests repeated with two different oxygen pressures.

The DLCO-DLNO double diffusion test allows calculation of capillary volume and membrane conductance in a single measure (Guenard et al., 1987), limiting bias due to repeated measures. Specific NO uptake is considered an infinite value. Thus, DLNO is only affected by the membrane diffusing capacity, calculating Dm and Vc, the DmNO/DmCO ratio being a constant (Aguilaniu et al., 2008).

So:

$$\frac{1}{DLCO} = \frac{1}{DmCO} + \frac{1}{\theta CO \times Vc} \quad (1)$$

And:

$$\frac{1}{DLNO} = \frac{1}{DmNO} \quad (2)$$

With:

$$DmCO = k \times DmNO \quad (3)$$

Then:

$$\frac{1}{DLCO} = \frac{1}{(k \times DLNO)} + \frac{1}{\theta CO \times Vc} \quad (4)$$

Recently, challenges have arisen directed at θNO as an infinite value, in favour of a finite value of 4.5 mlNO.ml.blood⁻¹.min⁻¹.mmHg⁻¹ (Borland et al., 2010) and the 2017 ERS taskforce on DLNO now recommends the use of the θNO as being 4.5 (Zavorsky et al., 2017). This modification of θNO , from an infinite to finite value, changes the respective proportion of Dm and Vc in DLCO alteration. Corrections can be made accordingly (Martinot et al., 2013).

During simultaneous measurement of DLCO and DLNO, it is tempting to consider DLCO, measured during this test, as equivalent to DLCO using the classical technique. In fact, nothing distinguishes the two manoeuvres from a mechanical perspective, with the exception of the breath-hold time. Indeed, the decrease of NO in the alveolar gas during apnoea is much faster than CO. After ten seconds, it virtually disappeared from the alveolus. Therefore, DLNO becomes impossible to estimate, unless the measure is made with a chemiluminescence NO analyser, which has a better detection range than electrochemical cells. Unfortunately, this method is much more expensive. To keep a measurable NO level in the alveolar sample, a maximal breath-hold time of 4 to 5 s is used. Inhalation of higher concentration of NO is not applicable because of its physiological effects on the bronchi and blood vessels.

There is limited available literature comparing DLCO measured using the classical breath-hold time technique (DLCO₁₀) and DLCO obtained via the simultaneous NO/CO method (DLCO₅). In many studies involving the simultaneous NO/CO method, DLCO is checked using the classical method. If DLCO₁₀ and DLCO₅ are comparable, it could theoretically be unnecessary to check the measure using the classical 10 s breath hold method.

Objectives:

The primary objective of our study was to compare DLCO₅ and DLCO₁₀. The secondary objective was to test the reproducibility of DLCO₅.

Material and methods:

Between 2008 and 2011, 153 medical students were recruited at the Caen University Hospital and provided written informed consent. The students completed a questionnaire about their medical history (allergy, respiratory problem and smoking). Height and weight were recorded. Participants were randomly assigned to either DLCO₅ or DLCO₁₀ for the initial measurement. Arterialized earlobe blood gas was performed to obtain Hb and HbCO values.

Pulmonary function tests

Total lung capacity (TLC), forced expiratory flow in 1 s (FEV1) and forced vital capacity (FVC) were assessed by plethysmography and spirometry. The reference values used were those of Quanjer (Quanjer, 1983).

DLCO and DLNO measurements

All measures were performed between 12 p.m. and 3 p.m., before lunch, and without any prior physical activity. The participants were in a seated position and were wearing a nose-clip. A minimum of 4 min was allocated between the tests. The same system (MasterScreen-PFT, Jaeger, Graz, Austria®) with Lab5® software was used and calibrated each day.

We measured DLCO with 10-s breath-hold time, according to joint American and European recommendations (MacIntyre et al., 2005) and in agreement with the recent update including continuous and real-time gas analysis (Graham et al., 2017). Two measures of DLCO₁₀ were completed and the average of the two values determined the DLCO₁₀ rate. If the difference between the two values exceeded 10%, a third manoeuvre was completed and the closest values were averaged.

The DLCO-DLNO double diffusion test used the same respiratory manoeuvre and validity criteria, except for the breath-hold time (4-5 s). NO was analysed using electrochemical cells. A difference greater than 10% required another measure of DLCO₅. The membrane conductivity and the capillary volume were recalculated with a final value of θ_{NO} (4.5 mLNO.mLblood⁻¹.min⁻¹.mmHg⁻¹). The reference values used were those of the 2017 ERS statement (Zavorsky et al., 2017).

Inhaled gas

For the implementation of the conventional apnoea method, the gas tank contained a mixture of CO at 3000 ppm, CH₄ at 3000 ppm and a fraction of oxygen at 21%. For each measurement, the inspired and alveolar concentrations of CO and CH₄ were measured by a multi-gas analyzer. For the double diffusion test, the gas tank contained a mixture of 2800 ppm CO, a 21% oxygen fraction and an Helium fraction. The mixture of inspired gases was carried in a bag to allow the addition of NO at the last moment. It is indeed impossible to store it in a mixture containing oxygen (the concentration of the storage tank is 400 ppm NO). Oxygen is automatically added to the inspiratory bag to adjust the final concentration of O₂ to

21%. The NO concentration was measured using an electrochemical method. The dilution ratios were calculated from inspired and alveolar gas measurements.

Reproducibility of DLCO₅

For students who have passed the NO-CO transfer method, a second double diffusion test was completed one week later, under the same conditions and with a new earlobe blood gas measurement.

Corrections

The measurements were corrected for hemoglobin according to the ERS/ATS references (MacIntyre et al., 2005). To account for repeated measurements and CO accumulation, the correction of HbCO applied is that of Normand et. al (Normand et al., 2006). Since the mixture of the gases inspired for the double diffusion measurement is made extemporaneously, the value of DLCO₅ was recalculated a posteriori using the measured FiO₂, for a FiO₂ of 21%, identical to that of the gaseous mixture used for the measurement of DLCO₁₀.

Statistical analysis

Results are presented as means \pm SDs. The paired student t test was used to compare the means. Agreements were analysed using a Bland Altman plot (Journois, 2004). Log transformation was performed to correct heteroscedasticity, if necessary. The normal distribution was evaluated using Kolmogorov–Smirnov’s test. Correlations were analysed using Spearman’s rank test. P < 0.05 was considered statistically significant. Reproducibility was performed according to Bland and Altman graphical method and a measurement error method (Bland and Altman, 1996).

Results

Population characteristics

Characteristics of the population are presented in Table 1. Participants included 153 medical students, with an average age of 19.8 years \pm 2.8. Medical history included respiratory allergies (n = 9), childhood pneumonia (n = 2), asthma (n = 4), pertussis (n = 1), anaemia (n = 1) and occasional smoking (n = 6).

Inhaled gas

The measurements of inhaled gas concentrations were calculated as follows: CO 2994 ± 3 ppm, CH₄ 3006 ± 1 ppm, O₂ 21.03% for the measurement of DLCO₁₀. CO 1847 ± 1 ppm, He 6.1 ± 0.4 %, NO 48 ± 5 ppm, O₂ 22.4 ± 0.2 % for the measurement of DLCO₅.

Agreement of DLCO₅ and DLCO₁₀

The 153 participants underwent concordance test analysis between the two values of DLCO. The difference between the averages of DLCO₅ and DLCO₁₀ was not significant ($p > 0.05$). The intra-class correlation coefficient was 0.95; CI 95% [0.93; 0.96]. Figure 1 indicates both equality and regression lines between the two measurements. Figure 2 shows the Bland Altman plot. The mean difference was 0.09 and the limits of agreements were -1.47 and 1.65 . Discrepancies between the two measures of DLCO followed a normal distribution, but were significantly increased with DLCO value (« V » shaped in figure 2). Thus, the data log transformation was performed to correct heteroscedasticity. This showed that the limits of agreement were -0.14 and 0.16 with a mean difference of 0.01 . Back transformation showed that the ratio between DLCO values laid between 0.87 and 1.17 , for 95% of the subjects. In other words, for 95% of the subjects, the DLCO₅ values laid between -13% and $+17\%$ of their DLCO₁₀ values. The difference in value between DLCO₅ and DLCO₁₀ was ± 1.6 mmol.min⁻¹.kPa⁻¹ (4.6 mL.min⁻¹.mmHg⁻¹) (CI 95%).

DLCO₅ reproducibility

47 participants were included (Table 2). The 2 test sessions were carried out at 6 ± 2 day intervals. The mean difference between the two measurements of DLCO₅ was 0.24 . This result was significant ($p = 0.0078$). The intra-class correlation coefficient was 0.97; CI 95% [0.95 ; 0.98]. DLCO₅ measured during the second week was significantly lower than the one measured during the first week. Indeed, the vast majority of points in Figure 3 are under the first bisector.

The differences between the two measures of DLCO₅ significantly increased with DLCO values. Bland and Altman plot with log transformation was performed and homoscedasticity was verified. The exponential values of the superior and inferior limits of agreement were 0.88 and 1.09 respectively. Thereby, for 95% of the 47 participants, DLCO₅ measured during the second week of the study was 12% below or 9% above DLCO₅ measured during the first week of the study. The difference in

DLCO₅ values between the first and the second weeks was $\pm 1.2 \text{ mmol}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}$ ($3.6 \text{ mL}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$) (CI 95%). The reproducibility of measured values during simultaneous NO and CO diffusion were calculated using a measurement error method. The results thus obtained are presented in Table 3. Figure 3 and 5 illustrate the identity and regression lines between the 1st and the 2nd weeks for respectively DLCO₅ and DLNO by means of a scatter plot.

Discussion

There was no difference between the measurement carried out over 10 s and the measurement carried out over 5 s. This result obtained under the technical conditions of the equipment used (MasterScreen PFT) and for which the production of FiO₂ is not configurable, appear to contradict the previous observations (Dressel et al., 2008) and most of the historical publications on DLCO, where DLCO₅ measures are greater than DLCO₁₀. Our result could therefore be considered a happy coincidence because inspired volume, alveolar volume and especially FiO₂ measures differ from one method to the other. On the whole, DLCO could be decreased despite the increase in VA. Are the other variables combined in such a way that the final DLCO₅ and DLCO₁₀ are identical on the MasterScreen PFT? For the measurement of DLNO, the gas mixture is manufactured extemporaneously because NO is not stable in oxygen. On the other hand, during conventional measures, the concentration of inhaled oxygen is fixed and corresponds strictly to the gas concentration in the measuring tank. For the measurement of DLCO₁₀, the average of the FiO₂ for DLCO₅ is $22.4 \pm 0.2\%$, which is higher than the 21% oxygen of the gas cylinder for the measurement of DLCO₁₀. On the basis of 0.2% DLCO per torr of PiO₂ (Normand et al., 2004) this difference of 1.4% represents a 2% increase in DLCO for a barometric pressure of 760 torr. By taking the corrected individual measurements of this variation, we reach a DLCO₅ value of $10.5 \pm 2.3 \text{ mmol}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}$ and DLCO₅ is then significantly greater than DLCO₁₀ ($p < 0.001$). However, this calculation remains approximate because the gases delivered for the DLCO₁₀ measurement have a relative uncertainty of 2%. However, our results were consistent with the study led by Dressel et al. (Dressel et al., 2008) who observed a similar DLCO value for 6, 8 and 10 s of apnoea, but a 9% decrease in DLCO between the 4th and 10th seconds. The alveolar volume difference is coherent with

the apnoea time, the alveolar mix being all the more successful as the apnoea time is long, which leads to a reduction in the alveolar concentration of CH₄ and therefore to an increase in the measurement of the alveolar volume. The lower value of the inspired volume in the double diffusion method is more difficult to explain. The randomization of the running order makes it impossible to get an improvement effect for the inspiratory manoeuvre. On the other hand, the inspired gas, although measured on the same pneumotachograph, is not delivered under an identical pressure regime. In the double diffusion test, the gas is inhaled by the subject from a bag; whereas, in the conventional method, it is delivered by a valve system under positive pressure. Under these conditions, it may be possible to expect inspiratory improvement, and a potentially different response from the integrator of the pneumotachograph.

The corrections made for HbCO are empirical corrections and the increase in HbCO after NO/CO double diffusion is not known. The duration of apnoea is shorter (4-5 s) so the elevation of the HbCO level is probably lower than the levels obtained via the DLCO manoeuvre using the classical apnoea method. However, the same results were obtained for our participants when using the Hb and HbCO uncorrected data. The lack of impact following correction is partly explained by the randomized order of the tests and the fact that the participants' Hb values were normal, except for one.

We found a strong correlation between DLCO₅ and DLCO₁₀, as in a study led on a large sample of children in Europe (Thomas et al., 2014). Our results are different since in our study, DLCO₅ > DLCO₁₀ unlike Thomas et al., whose study achieved opposite results, but in healthy children. If we take a look at the literature, another study, performed using the same technique and consisting in keeping the other factors constant, except for the breath-hold time, also found DLCO₅ > DLCO₁₀. Our study on healthy adults does not support the hypothesis that other factors than breath-hold time have a greater impact on DLCO. But the most interesting thing is Bland and Altman analysis, since it provides an assessment of concordance between the two methods of measurements rather than a correlation which studies the relationship between the two variables but does not include the differences. This analysis shows that DLCO₅ deviates from 1,6 mmol.min⁻¹.kPa⁻¹ (4,6 mL.min⁻¹.mmHg⁻¹) or 15% of gold standard DLCO₁₀ (17% above and 13% below, for 95% of the subjects). This result is not clinically meaningful, since it is based on the 2017

ERS/ATS standards which recommend two acceptable DLCO measurements within $0,7 \text{ mmol}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}$ ($2 \text{ mL}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$). Furthermore, the Bland–Altman method shows that the difference between these two values increases with the DLCO value. The concordance tends to decrease for higher DLCO values. This observation is not related to a gap between the two methods, but to the extent of DLCO itself. Indeed, if we only focus on the classical 10s-apnoea method, the difference between two measurements expressed as a percentage does not increase with the average of DLCO. This result justifies the expression of the repeatability and reproducibility values of DLCO as a percentage, and not as an absolute value.

The reproducibilities of DLCO and DLNO are slightly better than those reported in twelve healthy subjects (Murias and Zavorsky, 2007) but this can be explained by the fact that we repeated fewer measurements. Murias and Zavorsky repeated the measurements 8 times over a period of 2 months, which explains the considerable variability of all parameters. We only measured DLNO and DLCO twice about one week apart. In comparison with the data from Ratdke et al., which included fewer measurements (three visits over a period of about 8 days), reproducibility values are better than the results observed in our study. But this study involved patients with cystic fibrosis, who therefore have lower average values on these parameters (Radtke et al., 2017a). Nevertheless, when assessed as a percentage, the reproducibility measures were close to our results (respectively 15% and 14% for DLCO and DLNO). The absence of a correction for CO uptake does not explain the difference because it induces a minimal correction and it does not modify our reproducibility results.

The reproducibility of DLNO calculated in our study is worse than that of DLCO measured during the double diffusion. These results are discordant with those of the literature which showed a better reproducibility of DLNO against DLCO. DLNO is considered relatively independent of haemoglobin, FiO_2 and alveolar volume (Zavorsky et al., 2017). Unlike Zavorsky et. al for which DLNO is considered less variable than DLCO for the apnoea time used, we observe a close reproducibility between DLCO and DLNO among our 47 participants (Table 4). However, the reproducibility of DLNO is similar to that seen in healthy subjects: $20.3 \text{ mL}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$ (13%) in our study, $19 \text{ mL}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$ (12%) in triathletes (Lavin et al., 2015), $19.5 \text{ mL}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$ (13%) in healthy subjects (Murias and Zavorsky, 2007),

and $20 \text{ mL}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$ recommended by the 2017 ERS statement (Zavorsky et al., 2017). Radtke et al. achieved a reproducibility of DLNO of $13.3 \text{ mL}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$ or 14% since the study included patients with cystic fibrosis for whom the average value of DLNO was lower (Radtke et al., 2017b).

In our study, the reproducibility of DLCO measured in double diffusion is different from the measures recorded in the literature. The reproducibility of DLCO measured in double diffusion is slightly worse than that found in healthy subjects with 10 seconds apnoea, with a value of 9.6% (Hathaway et al., 1989). Unfortunately, there was no DLCO measurement with a 10-second apnoea during the second week of the study. It is hence impossible to compare it with the DLCO₁₀ value of reproducibility. We chose to only focus on subjects who underwent double diffusion tests during the first week of the study in order to limit the risk of bias related to CO uptake in subjects who had previously undergone a DLCO₁₀ measurement before double diffusion. The reproducibility value we obtained was of 11% ($3.6 \text{ mL}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$), which is better than the percentage achieved by Murias et al.: 16% ($4.7 \text{ mL}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$). This difference is not related to the corrections of CO uptake since the reproducibility result remains the same without this correction. However, in our study, the main bias is the difference obtained between weeks 1 and 2 in the average value of DLCO measured in double diffusion. This is one of the limitations of our study when it comes to the calculation of DLCO reproducibility. Indeed, the comparison between DLCO₅ averages obtained at one week intervals using a double diffusion test on 47 healthy participants, shows a trivial (2%) but significant difference. The participants were recorded throughout the year and the results were not affected by calibration standards. One hypothesis would be a respiratory manoeuvre learning effect. The only element in our measurements that could account for this discreet decrease in DLCO would be the difference between FAO₂ (DLCO decreases and FAO₂ increases). Neither the inspired mixture (there was no difference in FiO₂) nor cooperation (no inspired volume difference, apnoea time or alveolar volume) can account for this difference. It is not possible to eliminate any difference in relaxation on the shutter (Normand et al., 2006), but it seems unlikely that participants who performed the same inspired volume and duration of apnoea will have a different mechanical behaviour from one week to the next. Concentrations of Hb and HbCO were identical and the measurements were corrected for Hb and HbCO. There was a higher FAO₂ in alveolar sampling at week 2, with unchanged

alveolar and inspired volumes. This translates to either a lower residual volume (RV) or less oxygen extraction during the time of apnoea. We did not identify RV during the two manoeuvres and thus, this assumption cannot be eliminated. However, even if the apnoea time was a little shorter ($p = 0.07$), a rapid calculation based on VO_2 of $300 \text{ mL}\cdot\text{min}^{-1}$, alveolar volume of 6 L, 5 s apnoea and FAO_2 of 19% showed that an increase in 100 ms apnoea time induced a FAO_2 change of less than 0.01%.

Our study contains several limitations. First, the results only apply to healthy young subjects (average age 20). The calculations of the differences between DLCO measured during double diffusion test and DLCO measured during 10 s single-breath carbon monoxide uptake method need to be performed with older subjects and in respiratory pathology conditions such as emphysema, obstructive disease, pulmonary fibrosis or pulmonary arterial hypertension. Another limit, as discussed above, is the FiO_2 that we measured during the double diffusion test (at 22,4% instead of 21%) and that represents a bias for the comparison of the two methods. Furthermore, the correction for FiO_2 depends of the carbon monoxide-Hb chemical reaction rate. This linkage is highly debated (Borland et al., 2017). In our study, the significant difference between the mean values of $DLCO_5$ and $DLCO_{10}$ appear with a correction for FiO_2 . This underlines the limitations of the Roughton and Forster interpretation of CO capture in the lungs (Kang et al., 2017a, 2017b).

In conclusion, mean value of DLCO measured during the double diffusion test in healthy participants is close and strongly correlated to mean value of DLCO measured with the classical method (10 s breath hold time). However, on the basis of agreement evaluation, DLCO measured during the double diffusion test is between -13% and +17% of the DLCO measured by classical 10 s breath hold time (for 95% of the participants). The discrepancies between the DLCO measurement methods during the double diffusion and the DLCO measurements carried out during a 10-second apnoea are quite large. It may be an indication that the Roughton and Forster interpretation to describe this type of measurements is inadequate.

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Tables

Table 1

Characteristics of the 153 participants. Hb: Haemoglobin; HbCO: Carboxyhaemoglobin; TLC: Total lung capacity; FVC: Forced Vital capacity; FEV1: Force expiratory volume in 1 s; DLCO: Pulmonary diffusing capacity for carbon monoxide; KCO: carbon monoxide transfer coefficient; Tbh: Breath-hold time; VA: Alveolar volume; Vi: Inhaled volume. TLC, FVC and FEV1 % predicted according to Quanjer (Quanjer, 1983). DLCO and DLNO % predicted according to the 2017 ERS standards (Graham et al., 2017).

	Classical method	Double diffusion	Double diffusion (corrected FiO ₂)
age	19.8 ± 2.8		
sex (M/F)	78/75		
Height (cm)	172 ± 9		
Hb (g/dL)	15 ± 2		
HbCO(%)	1.0 ± 0.2		
TLC (L)	6.25 ± 1.28		
TLC (%predicted)	101 ± 10%		
FVC (L)	4.98 ± 1.11		
FVC (%predicted)	106 ± 12%		
FEV1 (L)	4.3 ± 0.9		
FEV1 (%predicted)	110 ± 13%		
FEV1/FVC	0.87 ± 0.13		
FEV1/FVC (%predicted)	105 ± 16 %		
DLCO			
(mL.min ⁻¹ .mmHg ⁻¹)	30.4 ± 6.7	30.7 ± 6.7 ^{ns}	31.3 ± 6.8 ^{***}
(mmol.min ⁻¹ .kPa ⁻¹)	10.2 ± 2.2	10.3 ± 2.2 ^{ns}	10.5 ± 2.3 ^{***}
DLCO (%predicted)	92 ± 12 %	93 ± 12 %	95 ± 12 %
KCO			
(mL.min ⁻¹ .mmHg ⁻¹ .L ⁻¹)	5.0 ± 0.69	5.4 ± 0.6 ^{***}	
(mmol.min ⁻¹ .kPa ⁻¹ .L ⁻¹)	1.7 ± 0.23	1.8 ± 0.2 ^{***}	
Tbh (s)	10.0 ± 0.5	5.1 ± 0.4 ^{***}	
VA (L)	6.1 ± 1.3	5.7 ± 1.2 ^{***}	
Vi (L)	4.7 ± 1.1	4.7 ± 1.1 ^{***}	
FiCO (%)	0.30 ± 0.03	0.18 ± 0.01	
FACO (%)	0.10 ± 0.01	0.10 ± 0.01	
FiO ₂ (%)		22.4 ± 0.2	
F _A O ₂ (%)		19.7 ± 0.5	
FiNO (ppm)		48.2 ± 5.0	
FANO (ppm)		19.5 ± 0.5	

^{ns} not significant; * significant difference p < 0.05; *** significant difference p < 0.001

Table 2

Demographic and functional characteristics of the 47 students. Hb: Haemoglobin; HbCO: Carboxyhaemoglobin; TLC: Total lung capacity; FVC: Forced Vital capacity; FEV1: Force expiratory volume in 1 s; DLCO: Pulmonary diffusing capacity for carbon monoxide; KCO: carbon monoxide transfer coefficient; Tbh: Breath-hold time; VA: Alveolar volume; Vi: Inhaled volume. DmCO and Capillary volume calculated with tétaNO 4,5. TLC, VC and FEV1 % predicted according to Quanjer (Quanjer, 1983). DLCO and DLNO % predicted according to the 2017 ERS standards reference. ^{ns} not significant ; * significant difference $p < 0.05$; *** significant difference $p < 0.001$.

	Week 1	Week 2	Mean difference	SD difference
age	20.5 ± 1.8			
sex (M/F)	26/21			
Height (cm)	172 ± 10			
Hb (g/dL)	14 ± 2	14 ± 2	2x10 ⁻³	0.5
HbCO (%)	1.0 ± 0.3	1.0 ± 0.2	10 ⁻³	0.2
TLC (L)	6.38 ± 1.41			
TLC (%predicted)	102 ± 10%			
FVC (L)	5.14 ± 1.25			
FVC (%predicted)	108 ± 12%			
FEV1 (L)	4.4 ± 1.0			
FEV1 (%predicted)	112 ± 12%			
FEV1/FVC	0.86 ± 0.07			
FEV1/FVC (%predicted)	104 ± 8%			
DLCO (mL.min ⁻¹ .mmHg ⁻¹) (mmol.min ⁻¹ .kPa ⁻¹)	31.6 ± 6.9 10.6 ± 2.3	30.9 ± 6.6 *** 10.3 ± 2.2 ***	0.72 0.24	1.73 0.58
DLCO (% predicted)	93 ± 11 %	91 ± 10 %		
DLNO (mL.min ⁻¹ .mmHg ⁻¹) (mmol.min ⁻¹ .kPa ⁻¹)	153.7 ± 36.8 51.4 ± 12.3	151.6 ± 35.6 ^{ns} 50.7 ± 11.9 ^{ns}	1.91 0.64	10.3 3.44
DLNO (% predicted)	93 ± 12 %	92 ± 12 %		
KCO (mL.min ⁻¹ .mmHg ⁻¹ .L ⁻¹) (mmol.min ⁻¹ .kPa ⁻¹ .L ⁻¹)	5.4 ± 0.5 1.8 ± 0.2	5.3 ± 0.5 ^{ns} 1.8 ± 0.2 ^{ns}	0.11 0.03	0.3 0.1
Tbh (s)	5.0 ± 0.3	5.0 ± 0.3 ^{ns}		
VA (L)	5.8 ± 1.3	5.8 ± 1.3 ^{ns}	1.5x10 ⁻²	0.2
Vi (L)	4.8 ± 1.1	4.8 ± 1.1 ^{ns}	4x10 ⁻²	0.2
FiO ₂ (%)	22.4 ± 0.1	22.4 ± 0.2 ^{ns}	6x10 ⁻³	0.19
F _A O ₂ (%)	19.4 ± 0.5	19.5 ± 0.5*	0.13	0.37
DmCO (mL.min ⁻¹ .mmHg ⁻¹) (mmol.min ⁻¹ .kPa ⁻¹)	151 ± 51 50 ± 17	151 ± 50 ^{ns} 50 ± 17 ^{ns}	7x10 ⁻²	3.1
Capillary volume (mL)	75 ± 15	72 ± 14*	2.2	6.1

Table 3

Reproducibility of DLNO, DLCO, Dm and Capillary volume with a 5 s time apnoea.

	Test-to-test measurement error (within the same testing session)	Repeatability (within the same testing session)	Reproducibility (week-to-week change)	Smallest measurable change (week-to-week change)
DLCO				
mmol.min ⁻¹ .kPa ⁻¹	0.3 (3%)	0.8 (7%)	1.2 (11%)	0.6 (6%)
mL.min ⁻¹ .mmHg ⁻¹	0.8 (3%)	2.3 (7%)	3.6 (11%)	1.8 (6%)
DLNO				
mmol.min ⁻¹ .kPa ⁻¹	1.3 (3%)	3.5 (7%)	6.8 (13%)	3.4 (7%)
mL.min ⁻¹ .mmHg ⁻¹	3.8 (3%)	10.5 (7%)	20.3 (13%)	10.1 (7%)
DmCO				
<i>θ</i> _{NO} = 4.5				
mmol.min ⁻¹ .kPa ⁻¹	2.8 (6%)	7.6 (15%)	16.5 (32%)	8.2 (16%)
mL.min ⁻¹ .mmHg ⁻¹	8.2 (6%)	22.8 (15%)	49.2 (32%)	24.5 (16%)
Capillary volume				
<i>θ</i> _{NO} = 4.5				
mL	2.6 (4%)	7.2 (10%)	12.5 (17%)	6.3 (9%)

Test-to-test measurement error and repeatability was calculated for the 153 subjects of the study. Measurement error is the within-subject standard deviation for each variable and was calculated as the square root of the mean squares error. Repeatability was obtained multiplying the within-subject standard deviation by 2.77. Reproducibility and smallest measurable change was calculated with data of week 1 and week 2 sessions for 47 subjects. Reproducibility was calculated multiplying the week-to-week within subject standard deviation by 2.77. The smallest measurable change is half the reproducibility.

Figure legends

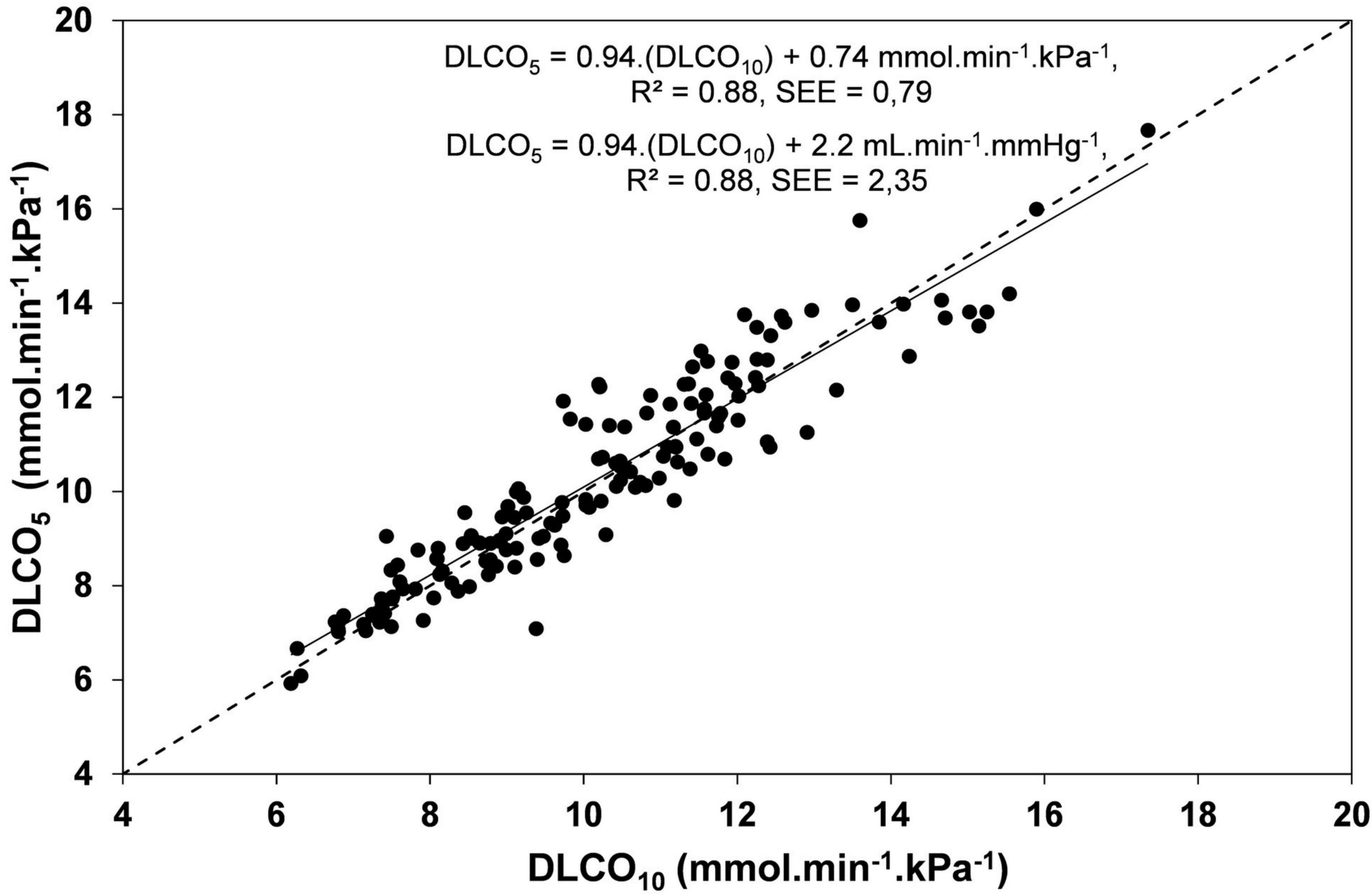
Figure 1. Scatter plot with identity and regression lines between DLCO₅ and DLCO₁₀ measurements.

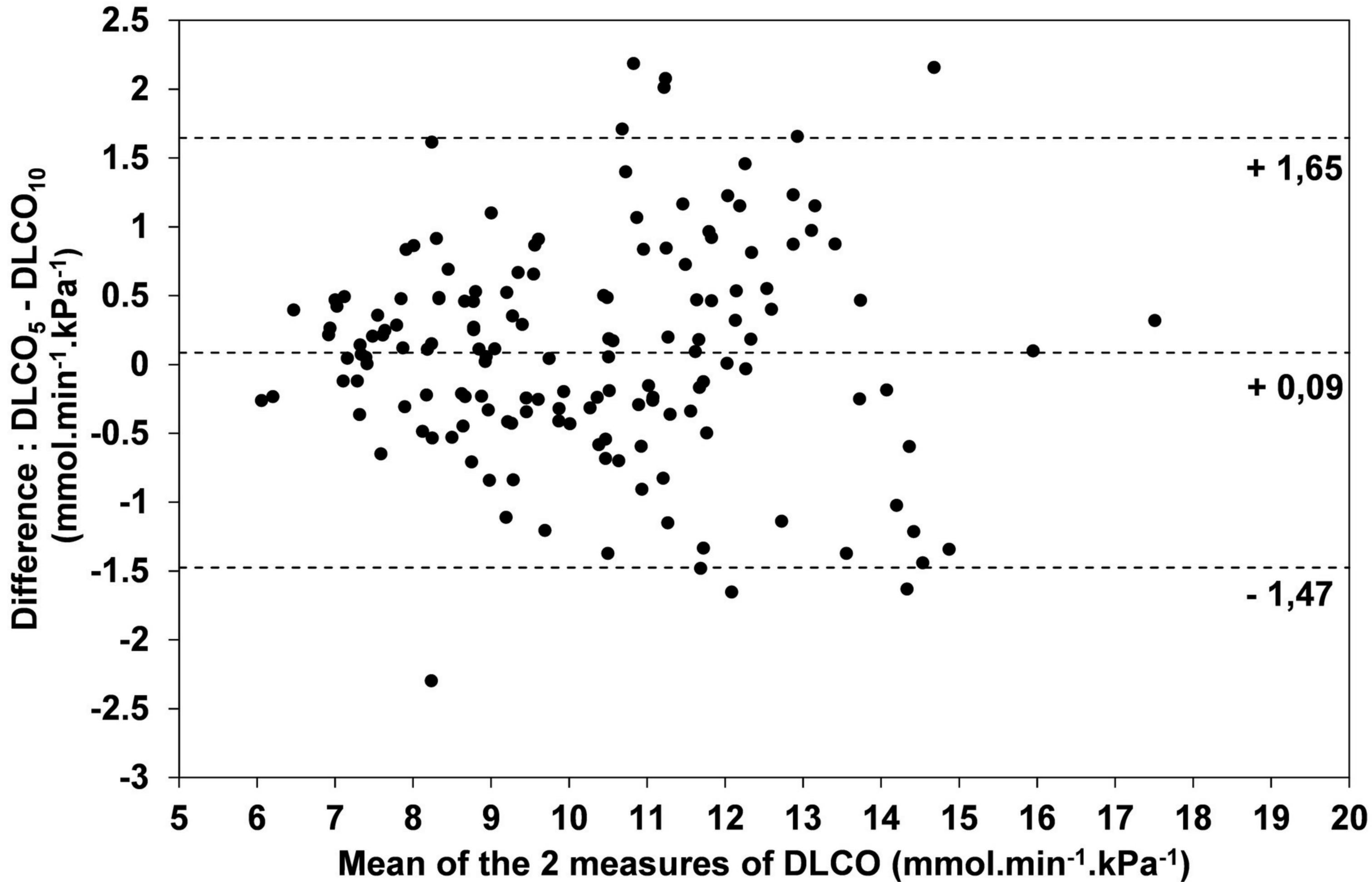
Figure 2. Plot of differences between DLCO₅ and DLCO₁₀ vs. mean of the two measurements.

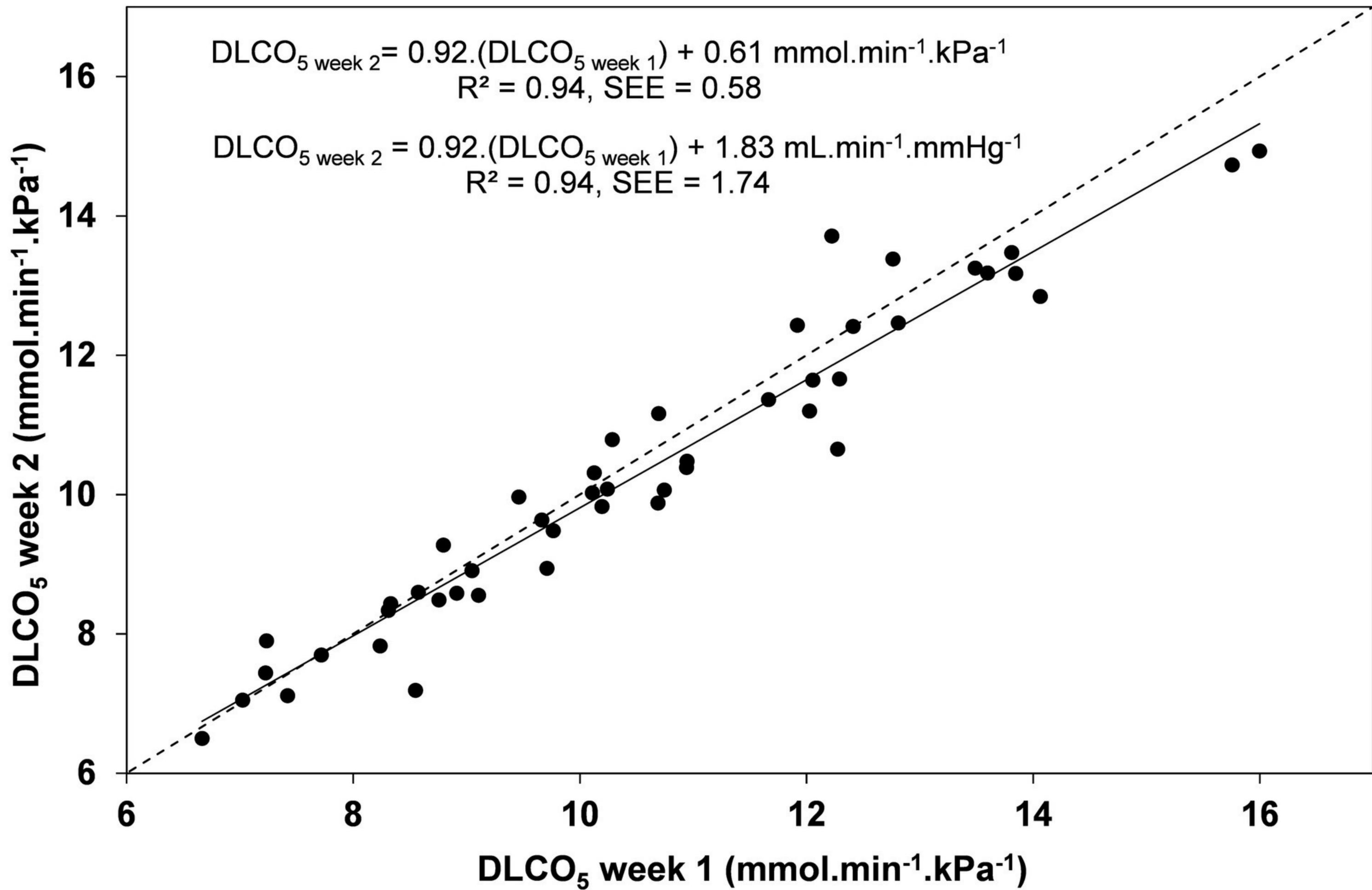
Figure 3. Scatter plot with identity and regression lines between DLCO₅ measured during week 1 and week 2.

Figure 4. Plot of differences between DLCO₅ week 2 and DLCO₅ week 1 vs. mean of the two measurements.

Figure 5. Scatter plot with identity and regression lines between DLNO measured during week 1 and week 2.







Difference : DLCO week 2 - DLCO week 1

(mmol.min⁻¹.kPa⁻¹)

