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Lung diffusing capacities for nitric oxide and carbon monoxide at rest and post-walking in long COVID

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Abstract

Background About one third of long COVID patients reports breathlessness and fatigue even during activities of daily living. We hypothesized that abnormalities of combined lung diffusing capacity for nitric oxide (DL_{NO}) and carbon monoxide (DL_{CO}) at rest or after mild exercise are associated with breathlessness in patients with long COVID.

Methods Single-breath combined DL_{NO} and DL_{CO} were measured at rest and immediately after a short bout of treadmill exercise simulating ordinary walking in 32 Caucasian patients with long COVID and dyspnea at rest. Twenty subjects served as a control group.

Results At rest, combined DL_{NO}, DL_{CO}, and alveolar volume (V_A) were significantly lower in long COVID than in controls, with DL_{NO} and DL_{CO} being below the limits of normal in 69% and 41% of cases, respectively. Mean values of DL_{NO}/V_A and DL_{CO}/V_A in long COVID patients were less than controls yet, in only 22% and 12% of long COVID patients the values of DL_{NO}/V_A and DL_{CO}/V_A were below the limits of normal. After treadmill, DL_{NO}, DL_{NO}/DL_{CO}, V_A and heart rate increased significantly without differences between groups. DL_{NO} remained below the limit of normal in 47% of long COVID.

Conclusion These data suggest localized discrete loss of lung units in about half of long COVID patients, not completely explained by loss of V_A or of alveolar-capillary recruitment during exercise.

Trial registry ClinicalTrials.gov Identifier: NCT05430503, Protocol ID: Long COVID Exer DLNO DLCO.

Keywords Lung diffusing capacities for nitric oxide and carbon monoxide, treadmill exercise, alveolar-capillary recruitment, alveolar volume.

Introduction

Although the novel coronavirus disease 2019 (COVID-19) is often associated with relatively self-limiting upper airway syndrome, a substantial proportion of patients may develop interstitial pneumonia, which may ultimately progress to a severe hypoxemic respiratory failure [1]. Besides the clinical burden of acute disease, it has been recognized that ~30% of hospitalized patients and outpatients may experience various persisting symptoms, including breathlessness and poor exercise tolerance, for 3 or more months after recovery from the acute phase, a condition also referred to as long COVID [1]. Exercise studies showed reduced aerobic capacity after COVID-19 variably explained by ventilatory inefficiency [2], inappropriate hyperventilation [3], chrono- and/or inotropic incompetence [4], reduced O_2 extraction by peripheral muscles [5], loss of mechanical efficiency [6], and deconditioning [7]. Moreover, about one third of patients with long COVID complain of breathlessness and fatigue even during activities of daily living [1]. Although a decreased lung diffusing capacity for carbon monoxide (DL_{CO}) has been found at various time intervals ranging from zero [8] to six months [9, 10] after hospital discharge, only one recent study reported decreased DL_{CO} associated with fatigue and dyspnea in highly symptomatic long COVID patients [11]. Whether abnormalities of DL_{CO} are mechanistically involved in poor tolerance to ordinary physical activities in long COVID is unclear. In a previous study of patients recovering from the acute phase of COVID-19, the lung diffusing capacity for nitric oxide (DL_{NO}) was reduced more than DL_{CO}, which was interpreted as an impairment of alveolar membrane diffusive conductance (DM) with relatively preserved pulmonary capillary blood volume (V_C) [12].

Both DL_{NO} and DL_{CO} are expected to increase from rest to exercise because of alveolar and microvascular recruitment [13]. Thus, we hypothesized that abnormalities of DL_{NO} and DL_{CO} at rest or after exercise might be associated with breathlessness in patients with long COVID. To test

this hypothesis, we measured combined DL_{NO} and DL_{CO} at rest and immediately after a short bout of mild treadmill exercise in patients with long COVID referred to our pulmonary function laboratory because of dyspnea during activities of daily living.

Methods

Study subjects

Thirty-two Caucasian patients, three of whom had participated in a previous investigation [12], with a history of SARS CoV-2 infection, confirmed by nasopharyngeal swab with real-time polymerase-chain reaction, were included in the study. They were referred to our pulmonary function laboratory, between 98 and 686 days after being tested negative for SARS-CoV-2, because of dyspnea, fatigue and exercise intolerance persisting or occurring at least 3 months after the COVID-19 acute phase and lasting ≥2 months [14]. None of them had history of diseases potentially causing dyspnea or affecting pulmonary gas transport, *i.e.*, bronchial asthma, chronic obstructive pulmonary disease, pulmonary interstitial fibrosis or vasculitis, hematological diseases, systemic collagen diseases, congestive heart failure, and liver or renal diseases. The group included 6 patients who had mild COVID-19 treated at home with antipyretics (paracetamol or ibuprofen) and 26 patients who had been hospitalized with moderate-to-severe COVID-19 pneumonia and arterial hypoxemia treated with oxygen supplementation only (n=8), or helmet continuous positive airway pressure support (n=10), or invasive mechanical ventilation via tracheal intubation (n=8). During hospitalization, they had received corticosteroids (n=26), antibiotics (n=22), enoxaparin (n=21), oral hydroxychloroquine (n=4), tocilizumab or anakinra (n=4), and various antiviral drugs. As a control group, we selected 20 healthy volunteers among health professionals and their relatives without history of COVID-19 and vaccinated against SARS-CoV-2 infection who best matched our long COVID patients for anthropometric characteristics.

Standard lung function measurements at rest

The modified Medical Research Council (mMRC) dyspnea scale was used to score (from 0 to 4) breathlessness before starting lung function measurements. Digital pulse oximetry (Oxy-3 Pulse oximeter, GIMA, Gessate (MI), Italy) was measured after a resting period of at least 5 min.

Lung volumes [15], spirometry [16], and standard single-breath DL_{CO}, with actual breath-hold time of 11±0.5 s [17], were sequentially measured with subjects sitting in a whole-body plethysmograph (Vyaire Vyntus Body, Vyaire Medical GmbH; Höchberg, Germany). Smokers were asked to refrain from smoking for 24 h prior to the study. Results were compared with the predicted values from Hall *et al.* [18] for lung volumes, Quanjer *et al.* [19] for spirometry, and Stanojevic *et al.* [20] for DL_{CO} after adjustment for effective Hb concentration measured from available arterial or venous blood samples ($[Hb_{meas}]$) [21].

DL_{NO}-DL_{CO} measurements at rest and post-walk

At least 5-10 min after standard DL_{CO}, combined single-breath DL_{NO} and DL_{CO}, with actual breath-hold time of 5.3±0.3 s, were simultaneously measured (MasterScreen PFT System, Jaeger, Vyaire Medical GmbH; Höchberg, Germany) twice at 5-min interval *at rest* with subjects in a sitting posture and wearing a nose clip, as detailed elsewhere [22]. The values retained for analysis were the average of two repeatable measurements, *i.e.*, within 17 and 3.2 $mL \cdot min^{-1} \cdot mmHg^{-1}$ for DL_{NO} and DL_{CO}, respectively, obtained during the same testing session [23]. Five min later, subjects wearing a heart rate thoracic belt (Polar T31, Kempele, Finland) started walking on a treadmill (MTC climb e motion, Runner S.r.l., Cavezzo, MO, Italy) at a speed of 4 $km \cdot h^{-1}$ with 5% incline, which were increased by 2 $km \cdot h^{-1}$ and 2%, respectively, every min until the achievement of a target exertional heart rate (Heart

$Rate_{exer}$), calculated from maximal predicted heart rate (Heart $Rate_{max}$=208-0.7·age) [24] and resting heart rate (Heart $Rate_{rest}$) as follows [25]:

$$\text{Heart Rate}_{exer} = (\text{Heart Rate}_{max} - \text{Heart Rate}_{rest})/3 + \text{Heart Rate}_{rest}$$

Then, within 5-10 s after stopping exercise, combined single-breath DL_{NO} and DL_{CO}, with actual 5.1±0.4 s breath hold time, were measured once in a sitting position. Predicted values for combined DL_{NO}-DL_{CO}, V_A, DL_{NO}/V_A, and DL_{CO}/V_A were from Zavorsky *et al.* [23].

Chest CT

In 10 long COVID patients who had been hospitalized during the acute phase, a thin-section CT scan obtained between -10 to 88 days after pulmonary function measurements was available. Scans of the entire chest were obtained at 1.25 slice thickness while supine, during breath-holding at full inspiration, by a multi-detector row-spiral scanner (SOMATOM Emotion 6, Siemens AG Medical, Forchheim, Germany) [22]. Only scans with lung volume determined by CT ≥80% of plethysmographic TLC (*n*=9) were retained for automatic quantitative 3D analysis to obtain mean lung attenuation, coefficient of variation (ITK-Snap 3.8.0, Philadelphia, PA, US) [26], kurtosis and leftward skewness of density histograms (Horos OsiriX 3.3.6, Pixmeo, Geneva, Switzerland).

Statistical analysis

For each lung function measure, the percentage of predicted and *z*-score were calculated. As lower limits of normal for combined DL_{NO}-DL_{CO} measures, both the 5^{th} (LLN_5, -1.645 *z*-score) and the 2.5^{th} ($LLN_{2.5}$, -1.96 *z*-score) percentiles of the reference population were considered. Unpaired Student's *t*-test and two-factor (between/within groups) repeated-measures ANOVA, with Holm-Sidak method for pairwise comparison testing, were used for significance testing of continuous variables, while Fisher's

exact or McNemar's tests were used for categorical variables (SigmaPlot 11, 2008 Systat Software, Inc., Germany). Associations between variables were tested for significance by the coefficient of determination (R^2) (GraphPad Prism 8.4.2, GraphPad Software, San Diego, CA 92108, US). Data are presented as mean±SD. In all analyses, the acceptable type I error was set at $p<0.05$.

Results

The mMRC dyspnea scale score was 0 in all control subjects, ≥2 and 1 in 24 and 8, respectively, long COVID patients. BMI was significantly higher in long COVID than control group (p=0.001), with 10 and 3 patients having obesity of class I and class II, respectively.

Standard lung function at rest

Pulse oximetry (SpO_2) values were within the normal range in all patients without significant difference between the control and long COVID groups (97.6±0.7 *vs.* 97.3±0.9, p=0.226). Total lung capacity (TLC), standard DL_{CO} and V_A, either as percentage of predicted or *z*-score, were significantly lower ($p<0.001$) in the long COVID than control group. Nine long COVID patients had a restrictive abnormality associated, in four of them, with decreased standard DL_{CO} while four showed an isolated reduction of the latter. None of the 6 long COVID patients who had been treated at home showed any standard lung function measures outside the normal range (table 1).

Combined DL_{NO}-DL_{CO} at rest and post-walk

At rest, both absolute values (table 2) and *z*-scores (figure 1) of combined DL_{NO}-DL_{CO}, V_A, DL_{NO}/V_A and DL_{CO}/V_A were significantly lower in the long COVID than in the control group ($p<0.001$ for all

comparisons). The DL_{NO}/DL_{CO} ratio did not differ significantly between groups (p=0.411) and heart rate was significantly higher (p=0.005) in long COVID than in control group. DL_{NO}, as opposed to combined DL_{CO}, was decreased in a greater number of long COVID using both LLN_5 (22 vs. 13, *i.e.*, ~69% vs. ~41%, patients; p=0.008) and $LLN_{2.5}$ (19 vs. 10, *i.e.*, ~59% vs. ~31%, patients; p=0.004) as a threshold. By contrast, DL_{NO}/V_A and DL_{CO}/V_A were $<LLN_5$ in 7 and 4 patients, respectively, and $<LLN_{2.5}$ in 4 patients and 1 patient, respectively, without significant differences (p=0.371 with LLN_5 and p=0.248 with $LLN_{2.5}$). The CT scans, obtained between -10 and 88 days from lung function studies in 9 patients who had been hospitalized during the acute phase of COVID-19, showed normal mean lung attenuation (-809±50 HU), coefficient of variation (18±2%), kurtosis (5.57±1.63) and leftward skewness (2.15±0.32) of CT histogram without high- (1±2%) or low-attenuation (<1% in all cases) areas. Yet, 7 of them had $DL_{NO} <LLN_{2.5}$. There was no significant relationship between DL_{NO} and time elapsed from the acute phase of COVID-19 (figure 2).

After walk, heart rate significantly increased within groups (p<0.001), without significant interactions between groups, while Borg scale ratings of breathlessness were 0 in controls and 1 to 4 in long COVID patients. There were significant increments in DL_{NO} (p=0.002), DL_{NO}/DL_{CO} (p<0.001), and V_A (p=0.020) within groups, with no significant interactions between groups. By contrast, there were no significant changes within groups in combined DL_{CO} (p=0.626), DL_{NO}/V_A (p=0.144) and DL_{CO}/V_A (p=0.097). In the long COVID group, the number of patients with $DL_{NO} <LLN_5$ was reduced from 22 at rest to 15 after walk (p=0.023) and those with $DL_{NO} <LLN_{2.5}$ from 19 to 13 (p=0.041). Of the 6 patients who had mild COVID-19 treated at home, one had DL_{NO} slightly $<LLN_5$ and one $<LLN_{2.5}$ at rest but both had it increased $>LLN_5$ after walk, without other lung function abnormalities (table 3). The mean rates of rise (slope) in DL_{NO} with heart rate were remarkably similar between controls and long COVID patients (0.439 *vs.* 0.387 $mL \cdot min^{-1} \cdot mmHg^{-1} \cdot beats \cdot min^{-1}$, respectively) whereas the mean *y*-intercept was lower in the latter (69 *vs.* 115, respectively) (figure 3).

Discussion

The main findings of this study are that patients with long COVID and dyspnea on activities of daily living had *1)* combined DL_{NO}-DL_{CO} and V_A significantly lower than anthropometrically-matched healthy controls, *2)* resting DL_{NO} below the normal ranges in about two thirds of cases but combined DL_{CO} only in a minority of them, *3)* DL_{NO}/V_A and DL_{CO}/V_A also significantly lower than control subjects, but within the ranges of normality in the vast majority of cases, and *4)* significant increments of DL_{NO} and V_A after walking like control subjects, though DL_{NO} normalized in a minority of cases only.

Technical considerations

Substantial differences in DL_{NO} and V_A have been reported between commercially available devices [27], and different predicting equations have been proposed [23, 28]. We estimated the suitability of the above predicting equations to our population by comparing the *z*-score standard deviations [29] of our database of 104 healthy subjects and found no substantial differences. Therefore, the choice of reference equations does not appear to be a major source of bias in our present study.

We did not derive DM and V_C subcomponents from combined DL_{NO}-DL_{CO} because the validity of their calculations is critically dependent on the values chosen for the rate of Hb uptake (θ) and the diffusivity ratio of NO and CO. Although the values of DM_{NO}/DM_{CO} (~tissue/plasma diffusivity) and θ_{NO}/θ_{CO} are deemed to be 1.97 and 8.1 in normoxia, respectively [30], controversies on these ratios remain and their values are currently being reassessed.

Comments on results

To our knowledge, this is the first study investigating combined DL_{NO}-DL_{CO} at rest and after a relatively short (~4-5 min) bout of treadmill exercise simulating ordinary walking, in patients with long COVID and dyspnea on activities of daily living. Previous studies have reported decrement of standard DL_{CO} [8-10] and DL_{NO} [12] after hospital discharge in ~20-60% and more than 50%, respectively. Previous incremental symptom-limited exercise studies have documented a reduced aerobic capacity after COVID-19, suggesting ventilatory inefficiency [2], inappropriate hyperventilation [3], chrono- and/or inotropic incompetence [4], reduced O_2 extraction by peripheral muscles [5], loss of mechanical efficiency [6], and muscle deconditioning [7] as possible responsible mechanisms. However, although the assessment of maximal aerobic capacity during an incremental test has a substantial clinical utility, its relevance to activities of daily living is limited. Moreover, none of the above studies considered a possible association between breathlessness and decreased pulmonary gas exchange in long COVID.

Consistent with our previous study over shorter time intervals after the acute phase of COVID-19 [12], we have found that most patients with long COVID had resting DL_{NO}, expressed as *z*-score values, below the limits of normal, while combined DL_{CO} was reduced in a significantly lower number of cases. Since DL_{NO} is deemed to be more sensitive to changes in DM than V_C, while the opposite is the case for DL_{CO} [30], the findings of this study suggest that a prevailing impairment of DM persists for 1-2 years in most patients with long COVID. A reduction of DM could be simply due to loss of V_A because of obesity, which was indeed present in 41% of our long COVID patients [31]. However, loss of V_A due to incomplete alveolar expansion is expected to cause large increments of DL_{CO}/V_A [32] and, to a lesser extent, DL_{NO}/V_A as alveolar dimensions reduce, with concomitant decrease of DL_{NO}/DL_{CO} ratio [33, 34]. Thus, the apparently normal DL_{NO}/V_A and DL_{CO}/V_A *z*-scores, with DL_{NO}/DL_{CO} ratio within the normal range, in the majority of our patients with long COVID suggest, *first*, that loss of V_A was not the only cause of reduced DL_{NO} and DL_{CO}, *second*, that reduced DL_{NO} and DL_{CO} are

compatible with “localized” discrete loss of lung units and *third*, that normal DL_{NO}/V_A and DL_{CO}/V_A may be due to diversion of capillary blood volume from the lost to remaining alveolar units [32]. The combined DL_{NO} and DL_{CO} measurements of patients with long COVID were similarly reduced both at rest and post-walk in comparison with control subjects, thus leaving DL_{NO}/DL_{CO} unchanged. This suggests that long COVID could affect DM and V_C to a similar extent [35]. Indeed, concomitant changes of alveolar surface area and capillary volume are likely to occur in a complex parenchymal disease such as COVID-19. In our previous study, a reduced DL_{NO} was observed even in patients with absent or minimal CT abnormalities, which suggests that mechanisms other than alveolar membrane thickening may contribute to diffusion abnormality after COVID-19 [12]. Another explanation might be that functional abnormalities of alveolar-to-capillary diffusion occurred, which were too small to be seen on CT. In the present study, none of the patients with available CT scans had fibrotic or ground-glass abnormalities, though the interpretation of this data in terms of structure-to-function is hindered be the time interval between pulmonary function tests and CT. But this was beyond the scope of the present study.

After walk, DL_{NO} significantly increased in both groups while combined DL_{CO} did not change, thus resulting in an increased DL_{NO}/DL_{CO} ratio. These changes were associated with a significant increase in heart rate and V_A, without significant differences between groups. We have no data to explain the increase in V_A after walk. Although studies on lung volume responses to exercise in healthy subjects have consistently reported no changes of TLC at high intensities of exercise [36, 37], a slight increase of TLC [36], and a compatible decrement of pleural pressure suggestive of a reduction of lung elastic recoil [37], were observed at low intensities of exercise. The increment in V_A in the present study was substantially higher than the increase in TLC observed by Hanson *et al.* [36] but the difference might have been related to methods and times of measurements. However, the increment of DL_{NO} with insignificant change in DL_{NO}/V_A in the present work can be explained not just by a post-exercise

unfolding of the alveolar membranes but also by capillary blood recruitment within the alveolar septa allowing more NO binding with red cell Hb. The similarity of rate of DL_{NO} rise with exercise between long COVID patients and control subjects with persistent reduction in the former suggest a residual decrease of DM and possibly V_C despite a preserved capacity for alveolar-capillary recruitment.

The lack of post-exercise increase of DL_{CO} of the combined maneuver in both groups is rather surprising, considering the expected V_C recruitment, and at odds with studies using rebreathing technique during exercise either in health [38, 39] or disease [40]. Physiological and methodological reasons may explain the inconsistent changes of combined DL_{NO} and DL_{CO} and the increased DL_{NO}/DL_{CO} ratio found 5-10 s after cessation of mild exercise. The DL_{NO}-DL_{CO} single-breath technique requires a breath-hold of 4-6 s duration at full lung inflation following a rapid (<2.5 s) inhalation from residual volume [23]. This imposes large pressure swings on the pulmonary capillary wall with the effects of surface forces being negative in the alveoli but strongly positive on the free edge of the alveolar septa [41]. Such squeezing of interalveolar vessels with erythrocyte deformation [42] could be accentuated by decreasing thoracic blood volume during an inadvertent Valsalva maneuver [43]. Thus, owing to the greater impact of V_C on CO than NO uptake [30], the single-breath maneuver may blunt the signal of enhanced CO uptake due to expected recruitment of V_C with increased cardiac output, depending on whether the subject actively maintains lung volume or relaxes against the closed airway during breath-holding [44]. Thus, unlike the rebreathing method, the breath-hold technique may underestimate the exercise-related increment of gas transfer relatively more for DL_{CO} than DL_{NO}. Thus, we cannot exclude that a microvascular impairment may go undetected by this method.

All participating patients had been referred to our laboratory because of dyspnea but in a number of them, particularly those who had mild COVID-19, we found no abnormalities in lung function either at

rest or after walk. Other factors not investigated in this study, *e.g.*, chronotropic incompetence, muscle deconditioning, obesity, anxiety, might have contributed to dyspnea in these subjects.

Study limitations

The present study has limitations. *First,* the long COVID and control groups were not perfectly matched for anthropometric characteristics. There was a tendency, though statistically insignificant, for female-to-male ratio, age and body weight to be higher in long COVID than control group. Although the DL_{CO} responses to exercise may be greater in men than women and decreases with age [45], these differences would have blunted the response to exercise in long COVID more than control group, which was not the case (insignificant between- within-group interaction terms). On the other hand, greater body weight might have caused tachycardia to occur earlier in long COVID patients than in controls. However, the heart rate difference between rest and post-walk was the same in the two groups. *Second,* we did not measure O_2 uptake, CO_2 output, exercise ventilation, and ventilation equivalents and this may, at least in part, limit the interpretation of our findings. *Third,* combined DL_{NO}-DL_{CO} were measured in duplicate at rest but only once after walk. This was necessary because the required 5-min interval between measurements would have allowed complete heart-rate recovery after walk. Moreover, we did not attempt to measure combined DL_{NO}-DL_{CO} during walking, because the inspiratory vital capacity maneuver necessary to inhale test gases would have been difficult during walking in most subjects and measurement in standing upright posture would have been not comparable with reference values obtained in sitting posture. Thus, it cannot be excluded that the relationships between variables might have been influenced by variability in recovery time after walk. *Fourth,* the study was cross sectional without a control group of patients with prior COVID-19 but no symptoms of long COVID.

Conclusions

The results of this study show that “localized” discrete loss of lung units, not completely explained by loss of V_A or of alveolar-capillary recruitment during exercise, may persist in about half of patients with long COVID. Moreover, even though abnormalities of DL_{NO} and DL_{CO} at rest or after exercise could be associated with breathlessness and poor tolerance to activities of daily living in patients with long COVID, no definitive causal inference between gas exchange abnormalities and respiratory symptoms can be made.

Conflict of Interest

G.B. and V.B have no financial/nonfinancial interests to disclose.

Authors’ contribution

G.B. conceived and designed research and performed the experiments; G.B. and V.B. analyzed data; G.B. and V.B. interpreted results of experiments; G.B. prepared figures; G.B. and V.B. drafted manuscript; G.B. and V.B. edited and revised manuscript; G.B. and V.B. approved final version of manuscript.

Ethical approval

The regional review board at the Ospedale Policlinico San Martino IRCCS approved the protocol (N. Registro CER Liguria: 200/2022 - DB id 12289) and each subject gave written informed consent to use his/her anonymized personal data.

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Table 1. Subjects' anthropometric characteristics and standard lung function data *at rest*

	Controls	**Long COVID**	**P-value**
Female/Male	1/19	7/25	0.132
Age (years)	50.4 ± 9.81	56.3 ± 11.2	0.058
Stature (cm)	175 ± 6	172 ± 7	0.060
Weight (kg)	81 ± 10	87 ± 13	0.078
BMI ($kg \cdot m^{-2}$)	26 ± 3	30 ± 4	0.001
Smokers (current-former/never)	10/10	16/16	1.000
[Hb] ($g \cdot dL^{-1}$)	14.6 ± 0.34	14.2 ± 1.44	0.219
SpO_2 (%)	97.6 ± 0.71	97.3 ± 0.86	0.226
FVC (L) (% predicted) (*z*-score)	4.96 ± 0.69 105 ± 14 0.29 ± 1.00	4.06 ± 0.79 97 ± 16 -0.26 ± 1.07	<0.001 0.057 0.052
FEV_1 (L) (% predicted) (*z*-score)	3.95 ± 0.46 106 ± 11 0.41 ± 0.83	3.29 ± 0.62 100 ± 16 -0.02 ± 1.09	<0.001 0.168 0.137
TLC (L) (% predicted) (*z*-score)	7.00 ± 0.93 101 ± 9 0.07 ± 0.77	5.63 ± 1.04 87 ± 13 -1.06 ± 1.08	<0.001 <0.001 <0.001
DL_{CO} ($mL \cdot min^{-1} \cdot mmHg^{-1}$) (% predicted) (*z*-score)	30.8 ± 3.82 110 ± 13 0.56 ± 0.77	22.5 ± 4.58 89 ± 16 -0.77 ± 1.05	<0.001 <0.001 <0.001
V_A (L) (% predicted) (*z*-score)	6.88 ± 0.91 108 ± 12 0.65 ± 0.93	5.61 ± 0.94 95 ± 12 -0.43 ± 1.01	<0.001 <0.001 <0.001
DL_{CO}/V_A ($mL \cdot min^{-1} \cdot mmHg^{-1} \cdot L^{-1}$) (% predicted) (*z*-score)	4.51 ± 0.49 101 ± 10 0.06 ± 0.64	4.04 ± 0.68 93 ± 14 -0.50 ± 0.95	0.009 0.027 0.025

Data are absolute numbers or mean ± SD. *Definitions of abbreviations*: BMI, body mass index; SpO_2, pulse oximetry (at room air); FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; TLC, total lung capacity; DL_{CO}, standard single-breath (11±0.5 breath-hold time) lung diffusing capacity for carbon monoxide; V_A, alveolar volume (V_A).

Table 2. Combined lung diffusing capacities for NO and CO *at rest* and *post-walk*

	Controls		**Long COVID**		**P-values (two-way ANOVA)**				
	Rest	*Post-walk*	*Rest*	*Post-walk*	*Within-group*		*Between-groups*		*Interaction*
					Unadjusted	Holm-Sidak	Unadjusted	Holm-Sidak	
DL_{NO} ($mL \cdot min^{-1} \cdot mmHg^{-1}$)	144.1 ± 16.2	159.9 ± 16.5	98.1 ± 22.0	110.5 ± 26.5	0.002	<0.050	<0.001	<0.050	0.689
DL_{CO} ($mL \cdot min^{-1} \cdot mmHg^{-1}$)	34.7 ± 4.76	35.2 ± 24.9	23.4 ± 5.33	23.9 ± 6.01	0.626	>0.050	<0.001	<0.050	0.980
DL_{NO}/DL_{CO}	4.18 ± 0.29	4.57 ± 0.36	4.22 ± 0.41	4.67 ± 0.46	<0.001	<0.050	0.411	>0.050	0.738
V_I (L)	5.01 ± 0.72	5.01 ± 0.76	3.99 ± 0.76	4.09 ± 0.85	0.714	>0.050	<0.001	<0.050	0.800
V_A (L)	6.88 ± 0.84	7.38 ± 1.01	5.35 ± 0.90	5.79 ± 1.11	0.020	<0.050	<0.001	<0.050	0.896
DL_{NO}/V_A ($mL \cdot min^{-1} \cdot mmHg^{-1} \cdot L^{-1}$)	21.1 ± 1.89	21.9 ± 2.33	18.3 ± 2.84	19.1 ± 2.98	0.144	>0.050	<0.001	<0.050	0.916
DL_{CO}/V_A ($mL \cdot min^{-1} \cdot mmHg^{-1} \cdot L^{-1}$)	5.06 ± 0.56	4.82 ± 0.64	4.38 ± 0.75	4.13 ± 0.82	0.097	>0.050	<0.001	<0.050	0.993
Heart rate ($beats \cdot min^{-1}$)	66 ± 11	102 ± 9	74 ± 12	106 ± 9	<0.001	<0.050	0.005	<0.050	0.335

Data are absolute numbers ± SD. *Definitions of abbreviations*: DL_{NO} and DL_{CO}, combined single-breath (with actual 5.3±0.3 s and 5.1±0.4 s breath-hold times at rest and post-walk, respectively) lung diffusing capacity for nitric oxide and carbon monoxide, respectively; V_I, inspired volume of test gas. Others as in table 1.

Table 3. Lung function *z*-scores in 6 long COVID patients recovering from mild COVID-19

Sex	**Age** (yrs)	**BMI** ($kg \cdot m^{-2}$)	**Smoker**	**mMRC**	**Heart rate** ($beats \cdot m^{-1}$) *Post-walk*	**FVC** (*z*-score) *Rest*	**FEV_1** (*z*-score) *Rest*	**FEV_1/VC** (*z*-score) *Rest*	**TLC** (*z*-score) *Rest*	**DL_{NO}** (*z*-score) *Rest*	*Post-walk*	**$DL_{CO\ (combined)}$** (*z*-score) *Rest*	*Post-walk*
M	61	30	Former	2	122	-0.17	0.00	0.30	-1.34	-2.30	-1.59	-0.72	-0.80
M	50	27	Never	1	112	0.17	0.00	-0.34	-0.13	-1.71	-0.15	-0.60	-0.83
M	52	29	Never	0	114	-0.93	-0.30	1.30	-1.19	-1.61	-0.95	0.13	0.39
F	50	27	Never	2	103	-0.52	-0.08	0.77	-1.34	-1.42	-1.21	-1.08	-1.21
F	33	20	Never	2	96	1.32	0.42	-1.31	0.55	-0.77	-0.34	1.16	0.82
F	54	29	Never	1	117	-0.80	-0.71	-0.23	0.59	-1.37	-1.13	-1.36	-0.96

Definitions of abbreviations: mMRC, modified Medical Research Council questionnaire score. Other abbreviations as in tables 1 and 2.

Figures' legends

Figure 1. *Z*-scores of combined single-breath lung diffusing capacity for nitric oxide (DL_{NO}) *(a)* and carbon monoxide (DL_{CO}) *(b)*, and their ratio to alveolar volume (V_A), *i.e.*, DL_{NO}/V_A *(c)* and DL_{CO}/V_A (*d*), respectively, at rest and 5-10 s after stopping a mild treadmill walk. *Open symbols* indicate healthy controls (circles) and long COVID treated at home (triangles), *grey triangles and asterisks* long COVID hospitalized without and with CT scans available, respectively. Horizontal dashed and dotted lines correspond to the 5^{th} (*z*-score -1.645) and 2.5^{th} (*z*-score -1.96) percentiles, respectively.

Figure 2. Relationships between DL_{NO} *z*-scores and time from the end of the acute phase of COVID-19. Symbols and lines are as in Figure 1.

Figure 3. Changes in combined DL_{NO} and DL_{CO} as a function of changes in heart rate from rest to 5-10 s after mild treadmill walk. Data are mean and vertical and horizontal error bars SDs.

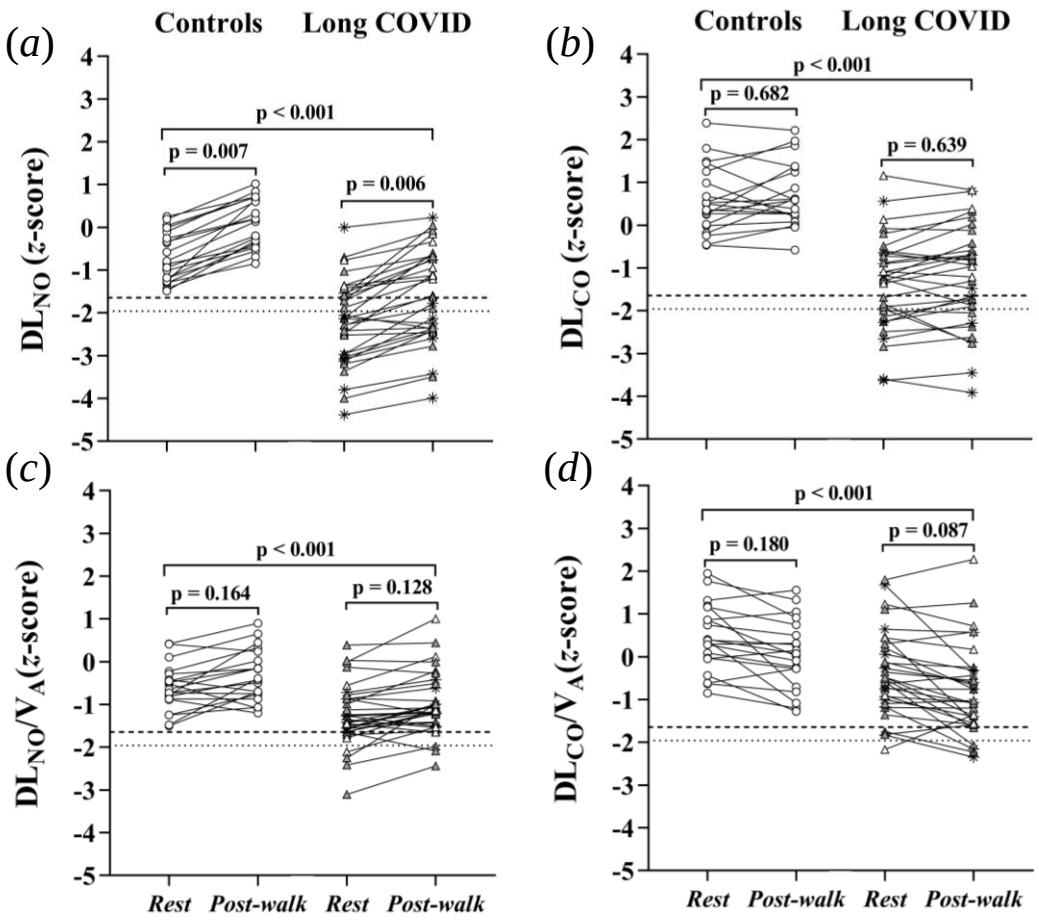
(a)
Controls
Long COVID
p < 0.001
p = 0.007
p = 0.006
DL_NO (z-score)
(b)
Controls
Long COVID
p < 0.001
p = 0.682
p = 0.639
DL_CO (z-score)
(c)
p < 0.001
p = 0.164
p = 0.128
DL_NO/V_A (z-score)
Rest
Post-walk
Rest
Post-walk
(d)
p < 0.001
p = 0.180
p = 0.087
DL_CO/V_A (z-score)
Rest
Post-walk
Rest
Post-walk

Figure 1

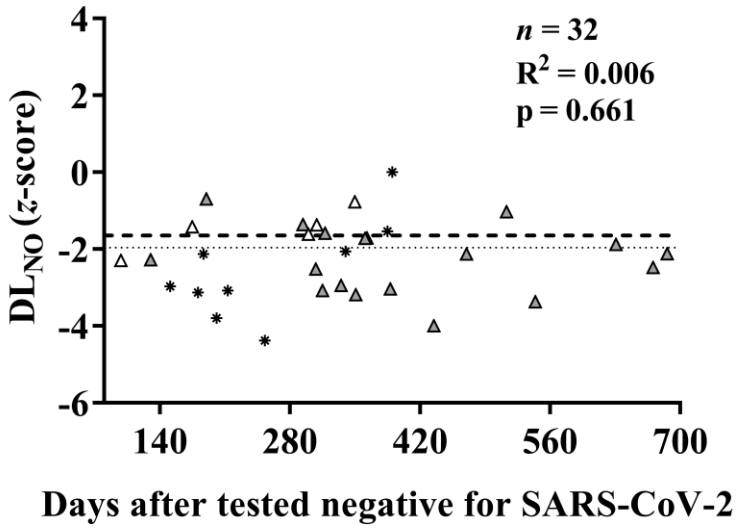
n = 32
R2 = 0.006
p = 0.661
DLNO (z-score)
4
2
0
-2
-4
-6
140
280
420
560
700
Days after tested negative for SARS-CoV-2

Figure 2

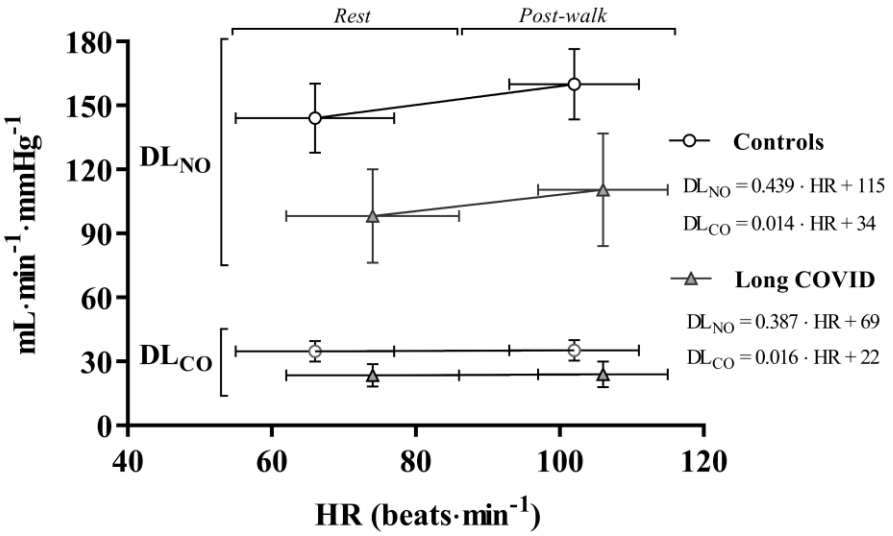
Rest
Post-walk
180
150
120
90
60
30
0
mL·min-1·mmHg-1
DLNO
DLCO
40
60
80
100
120
HR (beats·min-1)
Controls
DLNO = 0.439 · HR + 115
DLCO = 0.014 · HR + 34
Long COVID
DLNO = 0.387 · HR + 69
DLCO = 0.016 · HR + 22

Figure 3