EFFECT OF EXERCISE ON THREE-EQUATION DlCO IN SARCOIDOSIS AND IDIOPATHIC PULMONARY FIBROSIS

by

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ABSTRACT

Impairment in CO pulmonary diffusing capacity (D\textsubscript{L}CO) may be a factor limiting exercise in interstitial lung disease (ILD). We used the three-equation D\textsubscript{L}CO (D\textsubscript{L}CO\textsubscript{3EQ}) method to measure single breath D\textsubscript{L}CO during exercise to determine whether limited ability of D\textsubscript{L}CO to increase during exercise is related to exercise induced hypoxemia in two different ILD, idiopathic pulmonary fibrosis (IPF), and sarcoidosis. The D\textsubscript{L}CO\textsubscript{3EQ} technique involves continuous monitoring of exhaled CO and an inert tracer gas and accounts for CO uptake during the inhalation, breath holding, and exhalation phases of a breathing maneuver. This technique determines D\textsubscript{L}CO without requiring breath holding, allowing its use in patients with ILD during exercise. In 15 patients with ILD (8 sarcoidosis and 7 IPF) and 7 normal, healthy subjects, we determined D\textsubscript{L}CO\textsubscript{3EQ} at rest and during 2 levels of steady state exercise, 35% and 70% of the previously determined maximal power output. From rest to the 70% level of exercise, D\textsubscript{L}CO\textsubscript{3EQ} (units in mL min\textsuperscript{-1} mmHg\textsuperscript{-1} and in % predicted D\textsubscript{L}CO\textsubscript{SB} at rest) increased from 34.6±SE 2.7 (118%) to 48.7±SE 4.0 (164%) in control subjects, from 23.0±SE 2.8 (86%) to 33.8±SE 3.8 (126%) in sarcoidosis, and from 15.9±SE 0.7 (67%) to 20.5±SE 0.8 (87%) in the IPF patients. The % increase in D\textsubscript{L}CO\textsubscript{3EQ} for a given increase in VO\textsubscript{2} expressed as % of maximal predicted, was smaller in the IPF group when compared to both the sarcoidosis (p<0.0001) and the control groups (p<0.01). In the IPF patients, O\textsubscript{2} saturation by pulse oximetry decreased markedly from rest (96±SE 0.3%, n=7) to 88±SE 1.8% at the 70% level of exercise, and was associated with severe arterial hypoxemia (mean P\textsubscript{a}O\textsubscript{2} 52±SE 2 mmHg, n=4) and a widened A-a O\textsubscript{2} gradient (63±SE 2 mmHg, n=4), whereas the controls and sarcoidosis maintained O\textsubscript{2} saturation. We estimated that 37±SE 6% (n=6) of
the A-a $O_2$ gradient during exercise in IPF was due to diffusion limitation, assuming a constant venous admixture at rest and during exercise.
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CHAPTER ONE: INTRODUCTION

The main function of the lung is the uptake of $O_2$ and the elimination of $CO_2$. Exchange of $O_2$ and $CO_2$ across the alveolus between alveolar air and pulmonary capillary blood occurs by the process of passive diffusion. Diffusion of gas into a liquid phase is defined by the Fick (1) relationship:

$$V \propto \frac{A \times D \times P}{T}$$

where $V$ is the volume of gas diffused per unit time, $A$ is the surface area available for gas exchange, $D$ is the diffusivity of the gas in the liquid which depends on the diffusion coefficient and solubility of the gas in the liquid, $P$ is the partial pressure gradient of the gas, and $T$ is the thickness of the liquid interface through which the gas is diffusing. The alveolar capillary membrane has a $75m^2$ surface area with a thickness of only $1\mu m$ and is superbly adapted for allowing the passive exchange of the respiratory gases, $O_2$ and $CO_2$. Despite being a larger molecule, $CO_2$ diffuses 20 times more rapidly than $O_2$ from alveolar gas to capillary blood because its solubility in water is 25 times greater. The overall capacity of the lung to transfer gas from the alveolar gas to the hemoglobin in the pulmonary capillary blood is called the lung diffusing capacity ($D_L$), which is equal to gas uptake per minute divided by the mean alveolar to capillary driving pressure in mmHg.

Assessment of $D_L$ for $O_2$ by direct measurement of $O_2$ diffusion is difficult because $O_2$ uptake is limited by blood flow and is not entirely diffusion dependent, and because the
pulmonary capillary PO$_2$ is continuously increasing. The driving pressure for O$_2$ uptake is greatest at the start of the capillary where the capillary PO$_2$ is at mixed venous levels, and the driving pressure decreases as the blood passes along the pulmonary capillary till the end of the capillary where the capillary PO$_2$ approaches equilibrium with alveolar PO$_2$. For this reason, lung diffusing capacity is measured by using carbon monoxide as a test gas (D$_L$CO). CO has a diffusivity similar to O$_2$, but binds to hemoglobin with 200 times greater affinity making pulmonary capillary back pressure for CO negligible. The CO driving pressure along the capillary, therefore, is equal to alveolar PCO. In the normal lung, CO diffusion across the alveolar membrane is diffusion limited and not perfusion limited. In disease, D$_L$CO is not exclusively determined by diffusion, and may be affected by uneven distribution of ventilation, uneven ventilation to perfusion, and uneven distribution of alveolar surface and capillary blood volume to alveolar volume. Because of these limitations, D$_L$CO has also been termed CO transfer factor.

There are two components of the diffusion of CO from alveolar gas to capillary membrane: passive diffusion across the alveolar membrane, and the reaction of CO with the hemoglobin in the red blood cells in pulmonary capillaries. The two components are related with the following equation (1):

$$\frac{1}{D_L} = \frac{1}{D_M} + \frac{1}{\theta V_C}$$  \hspace{1cm} [2]

where $D_L$ is the diffusing capacity of the whole lung, $D_M$ is the diffusing capacity of the membrane, $\theta$ is the rate of reaction of CO and hemoglobin, and $V_C$ is the capillary blood volume.
MEASUREMENT OF $D_L CO$

$D_L CO$ can be measured using a variety of maneuvers: single breath holding, steady state breathing, and rebreathing techniques. $D_L CO$ can also be estimated from morphometric measurements of alveolo-capillary surface area and capillary volume. The most extensively used technique is the single breath method. The single-breath CO diffusing capacity ($D_L CO_{SB}$) was first determined by Marie Krogh in 1915 (2). Her technique involved subjects breathing inspiring an inhaled test gas containing about 0.1% CO to full lung inflation, then expiring rapidly to half of vital capacity (VC) at which point an alveolar gas sample was collected. The subjects held their breath for approximately 6 seconds before emptying the remaining air in their lungs. A second alveolar sample was collected from the second expiration. The concentrations of CO in the two alveolar samples were measured. The Krogh equation was used to calculate $D_L CO_{SB}$:

$$D_L CO_{SB} = V_A \times (STPD \text{ correction}) \times (60/t) \times (1/P) \times \ln\frac{F_A CO_o}{F_A CO_t}$$

where $D_L CO$ is the diffusing capacity for CO (mL of CO·min⁻¹·mmHg⁻¹), $V_A$ is the alveolar volume at ambient pressure and temperature in mL, STPD correction is to change the volume to standard pressure (760 mmHg) and temperature (0°C) dry, $F_A CO_o$ is the initial alveolar CO concentration determined from the first alveolar sample, $F_A CO_t$ is the final alveolar CO concentration at the end of breath hold, $P$ is the gas pressure in the alveolus (=barometric pressure- water vapour pressure), $t$ is the breath hold time in seconds, and 60 is to convert the seconds to minutes.

Ogilvie et al. (3) modified the Krogh technique with the introduction of helium, an inert gas, into the inhaled sample. The presence of an inert gas eliminated the need for two alveolar samples because initial CO alveolar concentration was determined from the inspired CO
concentration multiplied by the expired to inspired helium dilution ratio. Subjects rapidly breathed in a sample of test gas with known concentrations of CO and He to full lung inflation, held their breath for approximately 10 s, and expired rapidly. After the initial expired dead space volume was discarded, an alveolar sample was collected. Most of the CO uptake takes place during breath holding at full lung inflation, but some CO is taken up during the inspiratory and expiratory phases of the maneuver, which are not instantaneous. Inspiration takes up about 1-2 seconds and exhalation measured from the start of exhalation to completion of alveolar sampling can take up to 4 seconds. To help compensate for the lung volume changes, the breath hold time was set from the beginning of inspiration to the time just before the alveolar sample was collected (Fig. 1). The $D_{LCO_{SB}}$ was calculated from the volume of test gas inhaled, the gas concentrations in the exhaled alveolar and inspired gas samples, and the modified time of breath holding, according to equation [3].

To minimize errors in calculating $D_{LCO_{SB}}$ due to CO uptake during inspiration and exhalation, while the $D_{LCO_{SB}}$ equation assumes all CO uptake occurs during breath holding, Jones and Meade (4) proposed using as breath holding time the time from 0.3 of inspiration time to half of the sample collection time, and reducing the size of the alveolar gas sample collected immediately after dead space washout. Currently, the Jones and Meade method of determining breath hold timing is the most widely used and accepted, and is recommended by the American Thoracic Society (ATS). The ATS has thoroughly reviewed the single breath technique (5) and has made recommendations for a standardized technique which has been updated more recently (6). Despite rigid standardization of the 10 s breath hold time, volume of the dead space and washout samples, and rapid inhalation and exhalation, errors in $D_{LCO_{SB}}$ measurement can occur.
Figure 1. $D_2^1$CO single breath timing methods of Ogilvie et al. (3) and Jones and Meade et al.(4). VC is vital capacity, TLC is total lung capacity, RV is residual volume, Insp $t$ is time to inspire from RV to TLC, and Samp $t$ is expired gas sample collection time.
(7). This error is small in normal subjects who have little difficulty in maintaining high flow rates, breath holding, and adequate volumes; however, in obstructed patients, because of the greater time taken during the inspiratory and expiratory phases of the $D_L CO_{SB}$ maneuver; where CO uptake occurs at volumes lower than full inflation, the $D_L CO_{SB}$ is usually overestimated (8).

THREE-EQUATION METHOD OF $D_L CO$ MEASUREMENT

To avoid problems related to the changing lung volume and timing of the inspiratory and expiratory phases of the $D_L CO_{SB}$, Graham and Cotton (7) used separate equations to describe CO uptake during each phase of the breathing maneuver: inhalation, breath holding, and exhalation (Appendix I). This method makes a 10 sec breath holding maneuver unnecessary. Gas concentrations and lung volumes are monitored continuously from the mouth with rapidly responding CO and inert gas analyzers throughout the single breath maneuver. Using the three-equation algorithm, the mean exhaled carbon monoxide concentrations can be predicted for an assumed $D_L CO$. The predicted [CO] is then compared with the measured [CO], and if not matched, another value of $D_L CO$ is used to calculate predicted [CO]. The program then uses an iterative technique to determine the actual $D_L CO$ by matching the predicted [CO] and the measured [CO] to within 0.1%. Constant monitoring allows the entire exhaled alveolar gas to be used in the $D_L CO_{3EQ}$ calculation instead of a small alveolar gas sample, more accurately describing the mean CO uptake of the entire lung and correcting for any lung ventilation inhomogeneities. The three-equation method is more accurate than the $D_L CO_{SB}$ method in patients with air flow obstruction, small lung volumes, or who have difficulty holding their breath (7). Since breath holding is not necessary, this method is useful in evaluating $D_L CO_{3EQ}$.
during moderate to high intensity exercise where prolonged breath holding becomes difficult.

**D\textsubscript{L}CO DURING EXERCISE**

During exercise in normal subjects, desaturation of end capillary blood does not occur under normoxic physiological conditions and a low alveolar to arterial PO\textsubscript{2} difference is maintained (9) largely due to substantial increases in both ventilation and perfusion in the lung and to an increase in pulmonary capillary blood flow volume. Regional increases in perfusion are well matched with increases in ventilation. The increase in alveolar capillary membrane surface area and the increase in pulmonary capillary blood volume results in an increase the diffusing capacity (9).

Potts et al. (10) have implemented the three equation method to evaluate D\textsubscript{L}CO\textsubscript{3EQ} during exercise in normal, healthy adults. In their study, subjects performed a progressive exercise test on a cycle ergometer to determine maximal workload and maximum O\textsubscript{2} consumption (VO\textsubscript{2max}). D\textsubscript{L}CO\textsubscript{3EQ} was determined in each subject during steady state exercise at workloads of 25%, 50%, 75%, and 90% of the peak work load. D\textsubscript{L}CO\textsubscript{3EQ} was found to increase progressively with increasing workload. At 90% peak power output, subjects increased their D\textsubscript{L}CO\textsubscript{3EQ} 61%-75% from baseline. These observations are consistent with prior studies which have used conventional techniques to evaluate D\textsubscript{L}CO\textsubscript{SB} during exercise.

Billiet found that in 3 young, healthy untrained men exercising just below 20 Watts maximal exercise, 85% of the increase in D\textsubscript{L}CO\textsubscript{SB} occurred within the first 1.5 min of steady state exercise (11). A further 15% increase, amounting to a 2 to 3 mL\textperiodcentered min\textsuperscript{-1}\textperiodcentered mmHg\textsuperscript{-1} increase in D\textsubscript{L}CO\textsubscript{SB}, occurred within 5-7 min of additional exercise. On cessation of exercise, the D\textsubscript{L}CO\textsubscript{SB}
decreased sharply to a value 15% higher than the pre-exercise resting value within minutes, but remained slightly higher than the resting value 10 min after exercise.

MECHANISMS OF ARTERIAL HYPOXEMIA IN INTERSTITIAL LUNG DISEASE

In diffuse interstitial lung disease (ILD), where there is diffuse infiltration of lung parenchyma by disease, thickening of the alveolar interstitium and destruction of the surrounding alveolar capillary network could impair the adaptive mechanisms of the lung to increase diffusing capacity during exercise. Indeed, ILD patients frequently present with dyspnea on exertion, reduced lung volume, decreased resting $D_LCO$, and mild resting arterial hypoxemia which worsens during exercise (12). The exact contribution of diffusion impairment to an increased alveolar-arterial (A-a) $P_{O_2}$ gradient during exercise in ILD remains controversial.

Austrian et al. (13) were the first to suggest that resting and exercise induced hypoxemia in ILD are the result of an "alveolar-capillary block syndrome," or an impairment in the diffusion of $O_2$ from alveolar gas to capillary blood. Finley et al. (14) challenged this notion suggesting that ILD alters the alveolar-capillary membrane in a non-uniform manner throughout the lungs and that alveolar ventilation to perfusion ($V_A/Q$) mismatch is the mechanism of hypoxemia at rest. The development of the multiple inert gas elimination technique (MIGET) in the 1970s led to a better understanding of ventilation-perfusion relationships in ILD. The MIGET uses simultaneous venous infusion of six inert gases ($SF_6$, ethane, cyclopropane, enflurane, diethyl ether, acetone) with different solubilities in blood (i.e. different air to blood extraction coefficients) in trace concentrations to characterize the ventilation to perfusion ($V_A/Q$) ratios within the lung (15). Because inert gas transfer is limited by perfusion, but not by diffusion,
alveolar gas concentration is determined by alveolar ventilation to capillary blood flow ratio. Therefore, when alveolar to end capillary steady state equilibrium of the inert gases is reached after continuous infusion for about 30 min, the retention and excretion of inert gases are only dependent on the solubility of the gases and the $V_A/Q$ distribution. From the measurement of the relative concentrations of the 6 different gases in the alveolar air, the distribution of $V_A/Q$ can be determined. Based on the calculated distribution of $V_A/Q$ ratio across the whole lung, the arterial $PO_2$ ($P_{aO_2}$) can be predicted, assuming no $O_2$ diffusion limitation. Any discrepancy between observed and actual arterial A-a $PO_2$ gradient is due to a diffusion limitation.

Wagner (16) used the MIGET to investigate ventilation/perfusion inequality and gas exchange in advanced, chronic ILD. He measured arterial blood gases in 9 patients both at rest, during steady-state exercise, and 30 min post exercise while simultaneously measuring $V_A/Q$ distribution. The level of exercise was moderate so that the patients could maintain steady-state conditions for 10 min. Mixed venous $PO_2$ was sampled directly from the pulmonary artery. All patients had a decreased $PO_2$ at rest which fell an average of 10.6 mmHg during exercise. The $V_A/Q$ analysis predicted a fall in arterial $PO_2$ of only 6.4 mmHg with exercise. When the reduction in mixed venous $PO_2$ was taken into consideration, only 17% of the observed A-a $PO_2$ gradient was attributed to a diffusion limitation during exercise. Even though the contribution to the A-a $PO_2$ gradient is small, its presence indicates a substantial impairment in the diffusing properties of the lung, since a significant decrease in over all lung diffusion is required to increase the alveolar to end capillary $PO_2$ difference (17).

Jernudd-Wilhelmsson et al. (18) conducted a similar study investigating diffusion limitation at rest and during exercise in a heterogenous group of interstitial patients who
displayed a fall in arterial PO₂ with exercise. Vₘ/Q ratios were determined with the MIGET, and the O₂ and CO₂ exchange were measured using a mass spectrometer. The measured and predicted PₐO₂ at rest corresponded well and did not suggest any diffusion limitation. The moderate resting hypoxemia observed was attributed to small shunts and regions with low Vₘ/Q. During exercise levels averaging about 30 W, the patients showed marked impairment in oxygenation. Minute ventilation and cardiac output increased and the ventilation and perfusion distribution moved towards higher Vₘ/Q ratios, and areas of shunt and low Vₘ/Q ratios were unchanged. The measured A-a PO₂ gradient increased to 52.5 mmHg whereas the predicted A-a PO₂ gradient calculated from the Vₘ/Q distribution was 37.5 mmHg. Thus, 15 mmHg of about 30% of the measured A-a PO₂ gradient was explained by a limited diffusing capacity. The study suggested that the major contribution to exercise induced hypoxemia was from shunts and the perfusion of poorly ventilated areas.

DIFFERENT MECHANISMS OF EXERCISE HYPOXEMIA IN DIFFERENT ILD

These two studies (16, 18) evaluated patients with different forms of ILD, with possibly different pathophysiology. Mechanisms of exercise induced hypoxemia may be different in different types of ILD. Risk et al. (19) compared the exercise A-a PO₂ gradient in 168 interstitial patients stratified by disease severity as indicated by percent of predicted resting DlCOSB. The increase of A-a PO₂ gradient with exercise was greatest in IPF (mean 16 mmHg, n=32), least in sarcoidosis (mean 1 mmHg, n=61), and intermediate in desquamative interstitial pneumonia (mean 9 mmHg, n=28), berylliosis (mean 9 mmHg, n=29), and asbestosis (mean 7 mmHg, n=18). This ranking order existed for mild and moderate disease severities, but was lost among
patients with severe disease ($D_lCO_{SB} \leq 50\%$). From these results, the authors concluded that oxygen transfer seemed more dependent on the disease pathology. The greatest impairment was observed in IPF where the lung architecture was affected by honeycombing and fibrosis. Less impairment was seen in diseases which involved interstitial or intra alveolar cellularity, and the least impairment was seen in interspersed granulomatous lesions, such as in sarcoidosis.

In a subsequent study, Dunn et al. (20) compared gas exchange and diffusion at rest and during exercise in IPF and sarcoidosis. Both groups did not differ significantly in lung volumes, forced vital capacity (FVC), or forced expiratory volume in one second (FEV$_1$). $D_lCO_{SB}$ expressed as percent predicted was significantly smaller in the IPF ($43\pm SE 3\%, n=21$) group when compared to the sarcoidosis ($80\pm SE 5\%, n=20$) group. Consequently, the A-a PO$_2$ gradient which developed during exercise was larger in the IPF patients. The differences in gas exchange and $D_lCO_{SB}$ values could not be explained by differences in age, race, smoking history, or stage of disease. The authors concluded that differences in gas exchange in the two diseases existed because of their different histopathology.

Even with similar functional abnormalities at rest, fibrotic processes such as IPF and asbestosis exhibit different degrees of desaturation during exercise. Agusti et al. (21) compared the patterns of pulmonary gas exchange in IPF and asbestosis patients at rest and during exercise with similar resting ventilatory impairment, age, sex, and smoking history. Percent predicted $D_lCO_{SB}$ was significantly lower in the IPF patients ($45\pm SD 4\%, n=9$) than in the asbestosis patients ($70\pm SD 11\%, n=9$). Subjects were exercised progressively until dyspnea, weakness, leg pain, or marked desaturation ($<80\%$) developed. The $VO_{2\max}$ achieved during the maximum workload was similar in both groups when expressed in absolute values, or percent predicted. In
the IPF group, arterial PO\textsubscript{2} fell dramatically from 77±SD 11 to 51±SD 7 mmHg widening the A-a PO\textsubscript{2} gradient from 31±SD 13 to 60±SD 11 mmHg during exercise. The asbestosis group showed a more heterogenous response where the arterial PO\textsubscript{2} could increase, decrease, or remain unchanged during exercise. The authors explained that the heterogenous response seen in asbestosis was probably due to early peribronchiolar fibrosis which caused the development of airway disease and airflow obstruction, without the involvement of the pulmonary vasculature.

CONTRIBUTION OF DIFFUSION LIMITATION IN SARCOIDOSIS

Thoracic sarcoidosis may have differing clinical manifestations and is classified into stage I (bilateral hilar lymphadenopathy), stage II (bilateral hilar lymphadenopathy with diffuse parenchymal changes), stage III (diffuse pulmonary infiltrates without hilar lymphadenopathy), and stage IV (advanced fibrotic changes accompanied by cysts and bullae).

Eklund et al. (22) investigated the possible mechanisms of exercise induced arterial hypoxemia in stage II and stage III sarcoidosis patients. Eleven patients were progressively exercised on a cycle ergometer while their inspired and expired gases, ventilation, arterial blood gases, and pulmonary artery pressures were monitored. Perfusion scintigraphy of the lungs was performed with 99m\textsuperscript{Tc}-tagged microspheres administered in the supine position immediately after which radiospirometry was performed with \textsuperscript{133}Xe. The radiospirometry analysis showed matching ventilation and perfusion defects and prolonged washout. The distributions of V\textsubscript{A}/Q were determined using the inert gas technique earlier described by Wagner et al. (15). Patients had abnormally high pulmonary vascular resistance and a high mean pulmonary arterial pressure during exercise indicative of pulmonary vascular obstruction due to fibrotic changes and a
decrease in the capillary surface area. Five of the patients had regions with high $V_A/Q$ values suggesting disturbances in perfusion of the lung. These areas with high $V_A/Q$ ratios, however, disappeared during mild exercise indicating a more uniform distribution of perfusion in the lung. Resting arterial PO$_2$ was 73±SD 10 mmHg giving an A-a PO$_2$ gradient 31±SD 10 mmHg which was 11 mmHg less than predicted from the $V_A/Q$ distribution. This indicated that diffusion limitation contributed about 30% of the A-a PO$_2$ gradient at rest. During mild, supine exercise, the arterial PO$_2$ dropped to 68±SD 15 mmHg while the predicted arterial PO$_2$ remained about the same. The A-a PO$_2$ rose from 31 mmHg at rest to 38 mmHg during exercise. Fifty percent of the A-a PO$_2$ gradient during exercise was attributed to shunting and $V_A/Q$ disturbances, and the remaining 50% to a diffusion limitation. The study concluded that a significant diffusion limitation existed in patients with stage II and III sarcoidosis, both at rest and during exercise.

DIFFUSION LIMITATION IN IDIOPATHIC PULMONARY FIBROSIS

Generally, IPF patients have more fibrosis, pulmonary capillary destruction, and develop more hypoxemia during exercise when compared with sarcoidosis patients. Mild arterial hypoxemia which worsens during exercise is considered a common clinical feature of IPF (23). Agusti et al. (24) examined the mechanisms of gas exchange impairment during exercise in 15 patients with IPF not associated with collagen vascular disease or drug exposure. The patients were studied at rest (breathing room air or 100% O$_2$) and during steady state exercise at approximately 60% of their maximal predetermined workload while pulmonary hemodynamics and respiratory gas exchange variables were monitored. The MIGET was used to assess $V_A/Q$ distribution. At rest, $V_A/Q$ mismatch was moderate and approximately 2-4% of the cardiac output

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was perfusing poorly ventilated lung units. The A-a \( \text{PO}_2 \) gradient rose from 32+SD 3 mmHg at rest to 49+SD 4 mmHg during exercise. The \( \text{O}_2 \) diffusion limitation contributed 19% of the A-a \( \text{PO}_2 \) gradient during rest. During exercise, the \( V_A/Q \) mismatching did not change, but diffusion limitation increased to 40% of the A-a \( \text{PO}_2 \) gradient.

The relationship of pulmonary vascular tone to the regulation of gas exchange was also investigated because many IPF patients show some degree of pulmonary hypertension. Pulmonary hypertension reflects the destruction of the blood vessels and the available capillary surface area. Alveolar hypoxia induces pulmonary precapillary vessels to constrict to direct blood flow away from poorly ventilated areas; this vasoconstriction will be reversed by the administration of \( \text{O}_2 \). Agusti et al. (24) reported that while their patients breathed 100% \( \text{O}_2 \) at rest, the mean dispersion of the perfusion increased by 48% suggesting the release of hypoxic pulmonary vasoconstriction. Patients with more extensive fibrotic changes did not show redistribution of blood flow with \( \text{O}_2 \) suggesting anatomic vascular derangement rather than a reversible functional impairment. During exercise, patients who lacked a vasoconstrictor response to \( \text{O}_2 \) showed more \( V_A/Q \) mismatch, worsening gas exchange, and a greater degree of hypoxemia. Abnormal pulmonary vasculature induced a greater fall in mixed venous \( \text{PO}_2 \) and a shorter capillary transit time which increased any \( \text{O}_2 \) diffusion limitation. They found that resting \( D_L \text{CO}/V_A \) % predicted showed significant negative correlation with exercise A-a \( \text{PO}_2 \) gradient (\( r= -0.70 \)), exercise A-a \( \text{PO}_2 \) gradient due to diffusion limitation (\( r= -0.59 \)), and pulmonary vascular resistance (\( r= -0.80 \)) during exercise. They reported that the resting \( D_L \text{CO}/V_A \) was a good clinical indicator of the severity of gas exchange impairment during exercise.

The MIGET has consistently shown that most of the exercise A-a \( \text{PO}_2 \) difference is due to
ventilation and perfusion inequality in IPF (16, 18, 22, 24). Hughes et al. (25) used a rebreathing technique to determine $D_{LCO_{reb}}$ and pulmonary blood flow ($Q$) in 5 IPF patients at rest and after exercising at 60% of their maximal workload. The rebreathing maneuver consisted of 15 breaths of a 1 L mixture of 10% He, 10% SF$_6$, 10% Freon-22, 30% O$_2$, 40% Ar, and a trace amount of $^{11}$C-labelled carbon monoxide. The non-radioactive gas concentrations were measured using a mass spectrometer. $^{11}$CO is a positron emitting radioisotope and was measured using a positron detector. The inert gases He and SF$_6$, with widely different molecular weights, were used to correct Freon and $^{11}$CO for differences in gas diffusivity in the gas phase using Graham’s Law of Diffusion (diffusivity inversely proportional to square root of the molecular weight). The Freon-22 was used to determine pulmonary capillary blood flow. $D_{LCO_{reb}}$ was lower than conventional $D_{LCO_{sb}}$ by 17.2%, probably because $D_{LCO_{reb}}$ was done at a lower lung volume (tidal breathing) than $D_{LCO_{sb}}$ (maximal inspiration). Mean $D_{LCO_{reb}}$ ($\text{mL} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$) increased from 8.0 $\pm$ SE 0.8 at rest to 9.6 $\pm$ SE 1.3 during exercise (45-90 Watts), while in 5 normal subjects $D_{LCO_{reb}}$ increased from 25.6 $\pm$ SE 1.6 at rest to 32.2 $\pm$ SE 3.2 during exercise (60 Watts). In the IPF patients, O$_2$ saturation determined by pulse oximetry, decreased from 93 $\pm$ SE 1.9 at rest to 86 $\pm$ SE 3.8 during exercise. The A-a P$_{O_2}$ gradient (P$_{aO_2}$ estimated from O$_2$ saturation by oximetry) was 67 $\pm$ SE 5 mmHg during exercise. They used the pulmonary blood flow ($Q$) in L/min to calculate $D_LCO/Q$ ratios at rest and during exercise, which decreased in IPF patients from a mean of 1.88 at rest to 0.92 during exercise. In the control subjects, $D_LCO/Q$ was 3.86 at rest and 2.54 during exercise. Since the diffusion to perfusion ratio determines alveolar to end capillary P$_{O_2}$ equilibration, they used the $D_LCO/Q$ relationship to evaluate diffusion to blood flow limitation of
O₂ using the ratio $D_{L_{O_2}}/Q_{\beta_{O_2}}$, where $D_{L_{O_2}}$ is the diffusing capacity for O₂, Q is the pulmonary blood flow, and $\beta_{O_2}$ is the effective solubility of O₂ in blood, i.e. the tangent of the oxygen dissociation curve at a given PO₂. $D_{L_{O_2}}$ was considered equal to $D_{L_{CO}}$ multiplied by 1.2. They assumed a uniform lung and used a linear O₂ dissociation curve from mixed venous to arterial levels, and calculated that limitation of diffusion accounted for 99% of the A-a PO₂ gradient during exercise in their IPF patients. The main limitations to their study are that they assumed a uniform lung, linear oxyhemoglobin dissociation curve, and estimated $P_{a}O_2$ from pulse oximetry. These limitations were addressed in an accompanying paper by Hempleman and Hughes (26).

Hempleman and Hughes (26) examined the discrepancies in the results by Hughes et al. (25) from from their rebreathing technique when compared to MIGET (16, 18, 22, 24). In their technique, the $D_{L_{O_2}}$ was estimated from a multicompartmental $V_{A}/Q$ model and Bohr integration, and an actual O₂ dissociation curve to calculate O₂ uptake along the pulmonary capillaries (26). When the degree of $V_{A}/Q$ mismatch was assumed to cause all hypoxemia at rest and to remain unchanged during exercise, they calculated that diffusion limitation during exercise accounted for 36% of the total A-a PO₂ gradient difference, based on the expected arterial PO₂ caused by $V_{A}/Q$ inhomogeneity. The method of A-a PO₂ gradient partition into $V_{A}/Q$ and diffusion limitation components was dependent on whether $V_{A}/Q$ inequality was expressed either as a shunt or as multiple $V_{A}/Q$ compartments. Both of these methods produced different results, because in the first instance, the alveolar-capillary O₂ gradient due to diffusion occurred prior to the shunt, and thus would be on the flat portion of the O₂ dissociation curve, and would be greater than the case where the $V_{A}/Q$ abnormality was considered as multiple $V_{A}/Q$. 

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compartments. In the latter case, the $V_A/Q$ abnormality would cause a decrease in end-capillary PO$_2$ in regions with low $V_A/Q$ even with no diffusion limitation, resulting in a decreased $P_aO_2$. This diffusion limitation would result in a further lowering of $P_aO_2$ for the $V_A/Q$ abnormality, and the component of the A-a PO$_2$ gradient due to diffusion limitation would be on the steeper portion of the oxyhemoglobin dissociation curve and would be less than in the first curve. Partitioning of alveolar arterial difference based on blood O$_2$ content was not dependent on the model of $V_A/Q$ chosen, and they considered it a more realistic assessment of O$_2$ diffusion limitation than PO$_2$ differences. This method of analysis attributed 68% of the A-a equivalent blood O$_2$ content difference to a diffusion impairment. The authors concluded that previous MIGET studies using A-a PO$_2$ gradients underestimate the diffusion limitation to O$_2$ in exercising fibrotic patients.

USING THE THREE-EQUATION TECHNIQUE TO EVALUATE MECHANISMS OF EXERCISE INDUCED HYPOXEMIA IN ILD

The purpose of our present study was to evaluate impairment of diffusion during exercise as a factor contributing to exercise induced hypoxemia in IPF and sarcoidosis, using the $D_{LCO_{3EQ}}$ technique. The behavior of $D_{LCO_{3EQ}}$ during exercise was investigated to help understand the mechanisms which cause an increased A-a PO$_2$ difference during exercise. More specifically, the extent of increase in $D_{LCO_{3EQ}}$ with increasing O$_2$ uptake during exercise was determined and was correlated with the decrease in oxygen saturation and arterial PO$_2$ during exercise. Potential differences in the response of $D_{LCO_{3EQ}}$ to exercise was compared in IPF and sarcoidosis patients, and related to changes in O$_2$ saturation and PO$_2$ with exercise.
HYPOTHESES

1. Patients with ILD who do not increase their $D_L CO_{3EQ}$ adequately for a given increase in VO$_2$ during steady state will develop arterial hypoxemia during exercise.

2. Patients with IPF do not increase $D_L CO_{3EQ}$ with increasing VO$_2$ during steady state exercise, as much as patients with sarcoidosis and as a result, experience a greater decrease in arterial O$_2$ saturation for a given level of exercise, thus limiting exercise capacity.
CHAPTER TWO: METHODOLOGY

ETHICS APPROVAL

Ethical approval for the study was obtained from the University of British Columbia Clinical Screening Committee for Research and other Studies Involving Human Subjects, and from the Vancouver General Hospital Research Advisory Committee. A copy of the UBC Ethics approval is attached in Appendix II.

SUBJECT RECRUITMENT AND SELECTION

Fifteen subjects with ILD, 7 with idiopathic pulmonary fibrosis and 8 with sarcoidosis, were recruited for the study. All of the patients except one, were recruited from the Respiratory Clinic at VGH, or from the Pulmonary Function Laboratory at VGH; the exception was a patient who had a High Resolution Computed Tomography (HRCT) scan done at VGH and was recruited by contacting his treating internist. The diagnosis of idiopathic pulmonary fibrosis and sarcoidosis was based on histopathologic confirmation, or on clinical and radiological findings, including characteristic findings on a HRCT scan of the chest. Subjects were required to have stable disease and a recent HRCT within the last 6 months and a chest roentgenogram within the last 3 months. Specific inclusion criteria were bilateral interstitial lung involvement on the HRCT, clinical stability with no medication changes in the past 2 months, or if newly diagnosed, no immunosuppressive or corticosteroid treatment. Subjects with associated collagen vascular disease, exposure to agents known to cause fibrosis, disease of the pleura or chest wall, respiratory muscle weakness, or emphysema were excluded. We excluded from the study
subjects suffering from heart disease, airflow obstruction (FEV1,%FVC< 80% of predicted), resting hypoxemia (O2 saturation <90% at rest), severe restriction (FVC<1.2L), or any other disease that could impair exercise tolerance. Only one patient was a current smoker with a daily cigarette consumption of 2-3 cigarettes and a smoking history of 50 pack-years. All other subjects were non-smokers, or ex-smokers.

After getting permission from their treating respirologists, subjects who met the selection criteria were informed of the study either directly at the Respiratory Clinic or Lung Function Laboratory at VGH, or were sent a recruitment notice (Appendix III) requesting them to volunteer for the study. The general practitioners were also sent a letter describing the study (Appendix IV) and their approval was sought prior to the subjects being tested. Subjects were then contacted by telephone and an appointment was set up. All subjects read and signed an informed consent form (Appendix V) prior to any testing procedures.

CONTROL SUBJECTS

We sought to match each subject with interstitial disease with healthy control subjects of the same race, sex, and similar age (±6 years), but could only recruit 7 normal, healthy subjects with normal lung function as controls. Each control had a normal chest roentgenogram within the last 6 months and did not complain of any respiratory symptoms. We excluded as controls subjects who were currently smoking, or who had smoked a total of more than 30 pack years of cigarettes. Athletes, trained subjects, subjects with cardiovascular problems, or abnormal electrocardiograms, or any other significant health problems, were also excluded. Control subjects were also required to sign an informed consent and their physicians were informed of
the study.

INITIAL EVALUATION AND PHYSIOLOGIC TESTING

Clinical evaluation

Each subjects' clinical, lung function and radiological findings and history were reviewed by the supervising respirologist (Dr. Raja Abboud) and the patient was clinically examined prior to being included in the study. In addition to reviewing the respirologists chart and consultation letter, we administered a brief clinical questionnaire which documented current respiratory symptoms, allergies, medications, smoking history, and any other health problems (Appendix VI). Height was measured to the nearest centimeter with the subjects standing upright without shoes. Body weight was determined with subjects wearing light clothing with a medical scale to the nearest half kilo. Smoking history was converted to pack years where one pack year is the equivalent of smoking one pack of cigarettes daily for one year.

Spirometry

Spirometry was performed with a computerized dry rolling seal spirometer (Model no. 922; Sensormedics, Anaheim, CA). Subjects were required to inspire to full inflation, then forcibly "blast" the air out as fast and quickly as possible until the lungs were empty. The subjects then re-inspired to vital capacity to obtain a flow-volume loop. The FVC, FEV₁, FEV₁/FVC % ratio, and the peak expiratory flow rate (PEFR) were determined. Spirometry conformed to the current ATS criteria (27) and two maximal tests within 5% were considered acceptable. The forced expiratory flow from 25% to 75% of the expiration (FEF₂₅₋₇₅) was taken from the test with the highest sum of FVC and FEV₁. The prediction equations of Crapo et al.
(28) were used to calculate percent of predicted $FEV_1$, $FVC$, $FEF_{25-75}$, and $FEV_{1\%}FVC$. Predicted expiratory flow rates from the flow volume curves were determined using the equations developed by Knudson et al. (29).

Lung volume measurements

All the subjects had lung volumes and conventional $DLCO_{SB}$ measured as part of their clinical evaluation. These measurements were not generally repeated specifically for the study unless they had not been done in the previous 3 months. Functional residual capacity (FRC) was measured using helium dilution (Model "Transfer Test USA"; PK Morgan, Chatham, Kent, UK). The helium dilution technique involves the subject rebreathing a helium mixture in a closed circuit until equilibration is reached, while helium concentration is continuously monitored with a helium analyzer. Throughout the six or seven min duration of the test, $CO_2$ is chemically removed and $O_2$ is added. At the end of the helium dilution, the subject performs two slow vital capacity (SVC) maneuvers. Based on the degree of helium dilution, the volume of the lungs at the resting end-expiratory level is determined (FRC). TLC is calculated from FRC and the inspiratory capacity, while RV is calculated from FRC expiratory reserve volume. Equations used to calculate percent predicted TLC were those of Crapo (30).

Conventional $DLCO_{SB}$ measurement

Single breath $DLCO$ was determined according to standard technique (6) using a 10 s breath hold calculated by the Jones and Meade method (4). The equipment used was a PK Morgan lung function system (Model "Transfer Test USA"; PK Morgan, Chatham, Kent, UK). The washout and alveolar samples were set at 900 ml. The alveolar sample was passed through canisters containing anhydrous calcium sulphate and barium hydroxide to remove the water.
vapour and CO₂, respectively, before passing through the He analyzer and the infrared CO analyzer. Subjects were encouraged to relax and avoid exerting any inspiratory or expiratory effort during the breath holding. The mean of two DₐCO measurements within 5% were taken to be the determined DₐCOₛₐ. In order to satisfy this criteria, a maximum of four DₐCO maneuvers, separated by at least 4 minutes, may have been performed. The Krogh equation was used by the Morgan system to calculate DₐCOₛₐ:

\[
DₐCOₛₐ = V_A \times (\text{STPD correction}) \times (60/t) \times (1/P) \times \ln\left(\frac{(F_C CO-F_{E He})}{(F_{E CO}-F_{I He})}\right)
\]  [4]

where DₐCOₛₐ is the diffusing capacity for CO (mL of CO·min⁻¹·mmHg⁻¹), Vₐ is the alveolar volume at ambient pressure and temperature in mL, STPD correction is to change the volume to standard pressure (760 mmHg) and temperature (0°C) dry, F₁He and F₂He are the inspired and expired fraction concentrations of helium respectively, F₁CO and F₂CO are the inspired and expired CO fractional concentrations, P is the total alveolar gas pressure (=barometric pressure - water vapour pressure), t is the breath hold time in seconds, and 60 is to convert the seconds to minutes. Predicted values for DₐCO were calculated from the prediction equation of Miller et al. for non-smokers (31).

EXERCISE EQUIPMENT

All exercise was performed on a cycle ergometer (Model “Corival-400”; Lode, Groningen, the Netherlands) with subjects breathing through a mouthpiece attached to a two way Hans Rudolph valve (Model no. 2700; Hans Rudolph, Kansas City, MO) with a noseclip in place. Inspiratory flow was monitored using a turbine ventilometer (Model “Mark 2”; PK Morgan, Chatham, Kent UK). The expired gases were passed through a baffled mixing chamber.
and dried with anhydrous calcium sulphate before entering the gas analyzers. Oxygen concentration was measured using a zirconium electrochemical cell analyzer (Model “S-3A”; Applied Electrochemistry Inc., Sunnyvale, CA) and CO₂ concentration with an infrared analyzer (Model “CD-3A”; Applied Electrochemistry Inc., Sunnyvale, CA). The heart rate and electrical cardiac impulses were determined from a 12-lead EKG (Model no. 4000; Quinton, Seattle, WA) while the O₂ saturation was monitored continuously by pulse oximetry (Model “S-100e”; SiMed, North Bothell, WA). All the signals were sampled every 15 s and the output stored onto a PC type computer with a 486 microprocessor for later analysis. Metabolic and cardiopulmonary variables including the respiratory exchange ratio (RER), minute ventilation, respiratory rate, O₂ consumption, and CO₂ production were calculated from the flow, O₂, and CO₂ signals.

The perceived effort exertion for breathing and cycling was assessed using the modified Borg Scale (32). Before exercising, subjects were familiarised with the Borg scale and every minute during exercise were asked “how is your breathing?” and “how are your legs?.” Subjects would respond to each of the questions by pointing a finger to a number on the scale. Blood pressure was periodically taken during exercise and in the post exercise periods. All exercise equipment was calibrated and tested daily. For details of the calibration, refer to Appendix VII.

THREE-EQUATION D₁CO EQUIPMENT

Breathing apparatus

A schematic diagram of the breathing setup is provided in Fig. 2. The subjects breathed from a mouthpiece attached to a three way sliding Hans Rudolph valve (Model no. 2870; Hans Rudolph, Kansas City, MO). The inspiratory side could be switched either to a two way Hans
Figure 2. Breathing apparatus for the three-equation $D_LCO$ system.
Rudolph valve (Model no. 2700; Hans Rudolph, Kansas City, MO) for the measurement of gas exchange during exercise, or to the $DLCO_{35Q}$ equipment. The inspiratory ports of the two-way valve had the turbine ventilometer attached to it, while the expired port was connected to the mixing chamber of the exercise system previously described. The $DLCO_{35Q}$ circuit consisted of two one-way valve to separate inspired and expired circuits. The inspiring valve led to a switching valve connected to a bag in a box system, and allowed the subject to inspire either from the inspiratory bag, or room air from the box. The expiratory one-way valve emptied into the expired bag in the bag-box system. A #3 Fleisch pneumotach with a $+2 \text{ cmH}_2\text{O}$ differential pressure transducer (Model “MP45-14-871”; Validyne, Northridge, CA) was attached to the box to measure all flow in and out of the bag-box system. The pneumotach output was amplified in a carrier demodulator (Model “CD15”; Validyne, Northridge, CA) and the flow signal was integrated by the computer software to derive volume. A gas sampling port was located just distal to the mouthpiece from which gases were sampled from at a rate of 100 mL/s through the rapidly responding gas analyzers (see next section) by a vacuum pump (Model no. 8805; Sargeant-Welch, Skokie, IL). Water vapour was removed from the sampled gas using Permapure© tubing (Model “MD-110-72E”; Permapure Inc., Toms River, NJ) which is selectively permeable only to water, which was kept dry by flushing its exterior with dry $O_2$. This was done through the use of an external jacket of hard plastic tubing surrounding the Permapure© tubing through which dry $O_2$ was continuously flushed.

**Gas analyzers**

Implementation of the three-equation technique requires continuous measurements of $CO$ and inert tracer gas concentrations with rapid response gas analyzers. Traditionally, He has been
the tracer gas of choice determined by a mass spectrometer, but methane can be substituted as a tracer gas. The solubility of CH\textsubscript{4} in water at 37°C is 2.18 \times 10^{-5} as compared to a He water solubility of 6.987 \times 10^{-6}. The slightly greater solubility of CH\textsubscript{4} may lead to an overestimation of lung volume; however, the predicted effect is small (33). Infrared absorption analyzers (Model “BINOS® IR Gas Analyzer”; Leybold-Heraeus, Hanau, Germany) were used to monitor CO and CH\textsubscript{4} levels throughout the \textsubscript{DL}CO\textsubscript{3EQ} maneuver. The response time of the analysers was critical because a slow response time produces significant errors in the \textsubscript{DL}CO\textsubscript{3EQ} measurement (34). The response time of the analyzer is dependent on the chamber size, sampling rate, and the gas pressure in the sample cell (34). Increasing the sampling rate improves the response time of the analyzer, but reduces the density of gas in the chamber, hence decreasing the signal to noise ratio. An optimum signal is a balance between sampling rate and signal to noise ratio. A moderate sampling rate of 100 mL/s produces a 0-90% response time of 250 ms with less than a 1% error in \textsubscript{DL}CO\textsubscript{3EQ} (34). This sampling rate was considered acceptable for our experimental purposes. The lag time of the analyzer is the time elapsed from the sampling port on the mouthpiece to the sampling chamber in the analyzer. This value was measured and added to the response time of the analyzers in the processing of the data by the computer software. The lag and response times were checked regularly and the analyzer response times were verified to be under 250 ms (Appendix VIII).

\textit{Signal processing and data acquisition}

The CO analyzer, CH\textsubscript{4} analyzer, and flow analog signals were filtered with a 10Hz low pass filter and sampled at a rate of 50 Hz per channel. A 12 bit analog to digital converter (Model “STA08-PGA”; Keithley Metrabyte, Taunton, MA), with its full range adjusted to match the
signal amplitude, was used to digitize the signal before computer processing. The data acquisition system consisted of a PC with a 386 processor installed with a customized QUICKBASIC (Microsoft Corp., WA) software program containing the three-equation algorithm (kindly provided by Dr. Brian Graham, University of Saskatchewan, Saskatoon).

THREE-EQUATION EQUIPMENT CALIBRATION

Flow meter

The pneumotach was checked for linearity within 1% of full scale over a flow range of 0.5 L/s to 3 L/s for both inhalation and exhalation. Any nonlinearity was corrected for with digital signal processing. Before daily use, the flow signal was calibrated with a 3 L syringe without the gas analyzers aspirating any gas. The integrated volume during expiration and inspiration was verified to be within 1% of the actual syringe volume. The aspiration pump was turned on and with the gas analyzers aspirating, the syringe calibration was repeated. Again the inspiratory and expiratory volumes were verified to be within 1% of 3 L. This second calibration allowed for the measurement of the flow signal caused by the aspiration of the analyzers. This constant aspiration signal was used to offset the flow signal during actual breathing maneuvers effectively compensating for aspiration flow rate. Any detectable flow would then be attributed to the breathing maneuver itself. When the exhaled flow rate of the subject fell below the aspiration rate of the analyzers, the gas analyzer measurements were considered to be out of a meaningful range.

Gas analyzers

The presence of water vapour and of exhaled CO₂ can affect the gas analyzer
measurements, but this was not critical because the D$_L$CO$_{3EQ}$ technique is not dependent on absolute measurements of CO and CH$_4$ concentrations, but rather on the ratios of gas concentrations. Therefore, the linearity of the CO and CH$_4$ analyzers were of paramount importance. The gas analyzers were calibrated against direct sampling of tank gas (PRAXAIR Canada Inc., Mississauga, ON) containing known concentrations of CO (0.30%±0.02) and CH$_4$ (.30%±0.02). On a daily basis, the output of the CO and CH$_4$ analyzers were checked for linearity by both analyzers simultaneously the inspired gas mixture, which was then diluted at different proportions with room air. A graph of the CO and CH$_4$ concentrations at different dilutions was then plotted to make sure the gas concentrations remained linear at the different dilutions.

PERFORMANCE OF THREE-EQUATION D$_L$CO MANEUVER

Zeroing procedures

Before and after any breathing maneuvers, the zero levels of the CO analyzer, CH$_4$ analyzer, and the flow pneumotach were determined by averaging each signal over 2 s while the analyzers were aspirating room air. This value was taken to be the “dry zero.” If the gas analyzer zero drifted by more than 20 parts/million, or if the flow zero drifted more than 10 mL/s throughout the breathing maneuver then the D$_L$CO$_{3EQ}$ measurement was considered invalid.

Breathing maneuver

Subjects were seated upright for all D$_L$CO$_{3EQ}$ maneuvers. Before being switched into the breathing circuit, subjects tidally breathed room air at the mouthpiece with the analyzers sampling from the mouth in order to measure any residual CO back pressure. After being switched into the system, subjects were required to follow a template of a breathing maneuver on
a computer monitor (Fig. 3). The template was the same shape in all subjects, but the relative magnitudes of the inspiratory and expiratory segments were constructed on the basis of the subject’s FRC (functional residual capacity), inspiratory capacity (IC), and VC, which were entered in the computer program. The slope of the lines on the template allowed the subject to control inspiratory and expiratory flow rates, and breath hold time. Flow rates were set from 0.5 L/s to 2.5 L/s depending on the lung function of the subject. The first phase of the breathing maneuver consisted of a deep inspiration from FRC to TLC, a brief 1 s breath hold, and an expiration to FRC. The purpose of this first phase was to control for volume history and to determine the “wet zero” during the expiration. The wet zero is different from the dry zero in that it includes effects of residual CO, exhaled CO₂, and water vapour left after permatube drying.

The second phase of the breathing maneuver involved inhalation of a gas sample from the inspiratory bag containing 0.3% CO, 0.3% CH₄, 21% O₂, and the balance nitrogen. Subjects inhaled the gas from FRC to TLC, held their breath for 2-3 seconds, and exhaled to RV.

*Analysis*

The flow signal was integrated to obtain volume. The gas analyzer signals in the single breath inspiratory and expiratory phases were compared with the dry zero and the wet zero, respectively. The raw CO and CH₄ analyzer output and the volume signal were used to construct washout curves for CO and CH₄ concentrations versus volume. The CH₄ washout curve was used to measure RV. The RV was added to the vital capacity determined from the volume signal to obtain total lung volume (V₄₃). The anatomic dead space was determined using the Fowler method (35) except CH₄ washout was used instead of nitrogen washout. Alveolar volume was calculated by subtracting V₄₃ from the anatomic dead space. The dead space washout volume was
Figure 3. Template of breathing maneuver. The subject matches a real time display of volume to time to a template of the maneuver on the computer (shaded area). At the end of a normal breath, the subject inhales room air to TLC, pauses briefly then exhales to FRC to control for volume history. From $t_0$ to $t_1$ the subject inhales test gas containing CO and CH$_4$ to TLC, breath holds for 2-2.5 seconds then exhales to RV.
determined by first dividing the CO washout curve into three equal sections by volume (Fig. 4). A linear regression line passing through the middle third section was constructed. The point of intersection between the regression line and the first observed CO concentration was taken to be the point of dead space washout. The mean [CO] in each of the three samples was measured. The predicted [CO] calculated by the three-equation algorithm was compared with the measured [CO] using an iterative technique to determine $D_{LCO_{3EQ}}$.

Reproducibility of $D_{LCO_{3EQ}}$ at rest and during exercise

Prior to the study, the reproducibility of $D_{LCO_{3EQ}}$ measurements at rest and during different levels of steady state exercise were tested in 3 normal, healthy volunteers on 3 separate days, or in the case of one volunteer on 2 separate days. The mean coefficient of variation $D_{LCO_{3EQ}}$ was 4.3% at rest and 2.8% during steady state exercise.

EXPERIMENTAL PROTOCOL

On the first experiment day, subjects read and signed the informed consent form. The supervising physician clinically assessed the subject and filled in the clinical questionnaire. Spirometry was performed and compared with any prior results.

$D_{LCO_{3EQ}}$ measurements at rest

Subjects were made to rest in a seated position for 15 min prior to any diffusion measurements to normalize any prior activity. To familiarize the subjects with the breathing template, practice runs of the breathing maneuver were performed without the inhalation of test gas. Flow rates were adjusted to the breathing capabilities of the subject. This process was repeated until the subject’s breathing pattern sufficiently matched the flow rates and breath hold
Figure 4. CH$_4$ washout curve. The point of dead space washout is measured from the intersection of the CH$_4$ concentration washout curve with the phase III slope (dotted line).
times on the monitor template. A sample of test gas was then introduced into the inspiratory bag and the DLCO_{3EQ} measurements were made. Three DLCO_{3EQ} measurements were repeated at least 5 min apart.

Figure 5 shows that the resting D_LCO_{3EQ} (86+SD 7%) were significantly (p<0.01) greater than the conventional D_LCO_{SB} (72+SD 7%). This discrepancy was attributed to the differences of breathing maneuvers of the two techniques. In the D_LCO_{3EQ} method, subjects breathe out to FRC prior to the inhalation of test gas whereas in the D_LCO_{SB} technique, subjects breathe out to RV. Inhalation of test gas from FRC will increase D_LCO_{3EQ} values when compared to inhalation from RV (36).

**Progressive exercise test**

Maximum exercise capacity was determined by an incremental exercise test using a step protocol. A physician was present during all exercise testing. After being fitted with a 12-lead EKG, subjects were seated on a cycle ergometer and a vasodilator cream, Finalgon®, was applied sparingly to the ear lobe to facilitate blood flow. An oximeter probe was clipped to the ear and a good pulse correlation between the EKG and oximeter was confirmed. If the ear oximeter probe did not provide an adequate reading, a finger probe on the index finger was used. The subjects were asked to breathe quietly on the rubber mouthpiece of the exercise system while resting measurements were made for 3 min. The subject’s feet were strapped to the ergometer pedals after which they were asked to start pedaling at a workload of 30 W. The load was increased at a rate of 10 W every minute in a step wise fashion. Every minute, the subjects were asked to indicate their perceived effort for breathing and legs on the Borg scale. The exercise test was stopped when the subject reached 90% of the maximal predicted heart rate, felt fatigued and
Figure 5. Comparison of $D_L^C O_{SB}$ determined by conventional and three-equation techniques. Mean $D_L^C O_{3EQ}$ (86+SD 7%) was significantly higher than $D_L^C O_{SB}$ (72+SD 7%) in the same subjects (p<0.01). The dotted line is the line of identity.
unable to continue, if an abnormal EKG developed, or if the \( \text{O}_2 \) saturation fell below 85%. After exercise, the mouthpiece and nose clip were promptly removed and the subjects pedaled freely for another 5 min to cool down. This cool down period allowed the heart rate to decrease and prevented the venous pooling of blood in the lower extremities. If the subject desaturated, \( \text{O}_2 \) was administered with a mask. When the heart rate decreased sufficiently, the subject stopped pedaling and was taken off the ergometer and allowed to sit or lie down to recover completely.

The peak \( \text{VO}_2 \) attained was taken to be the \( \text{O}_2 \) consumption at the highest workload when the exercise was stopped. The predicted peak \( \text{VO}_2 \) in absolute amounts and expressed per kg of body weight were determined by the equations of Jones et al. (37). The maximal predicted heart rate and was calculated by subtracting two-thirds the age of the subject from 210 (37).

\( DLCO_{3EQ} \) during steady state exercise

Our intent was to have the subjects perform steady state \( DLCO_{3EQ} \) measurements on a separate day; however, for subjects unable to return on another occasion, steady state measurements were made the same day. Subjects were given 45-60 min of rest after the progressive exercise test to recover before they proceeded to the second portion of the experiment.

Subjects were seated a cycle ergometer and were monitored by a 12-lead EKG and an ear oximeter. Subjects were asked to free pedal on a cycle ergometer for 1 min to warm-up. The ergometer was set to a workload corresponding to 35% of the predetermined maximal workload. A constant pedaling rate at this workload was maintained for 3 min at the end of which a steady state \( DLCO_{3EQ} \) measurement was made while the subject continued to pedal. The workload was increased to 70% of maximal capacity for another 3 min. At 3 min, the steady state \( DLCO_{3EQ} \)
measurement was made at the higher workload. After another 1 min of pedaling, the $D_{L}CO_{3EQ}$ measurement was repeated. After the diffusion measurements were made, subjects cooled down for 5 min while pedaling freely on the ergometer to allow their heart rate to recover. The subjects were taken off the ergometer and allowed to recover in a seated position.

Selected subjects had steady state gas exchange measured at each workload just prior to the steady state $D_{L}CO_{3EQ}$ measurements. These subjects were required to breathe through the mouthpiece 30 s prior to the $D_{L}CO_{3EQ}$ measurement while their ventilation and expired respiratory gases were monitored by the exercise system. The three-way valve was switched to the three-equation diffusion system when the diffusion measurements were made.

At the higher workload of 70% of maximal power output, subject F6 markedly desaturated, and subject C5 could not maintain pedaling for a sufficient duration to obtain any diffusion measurements. In these subjects, after a period of recovery, the workload was reduced from 70% to a more tolerable workload. The subjects started pedaling at this new workload for another 3 min, and we were able to obtain steady state $D_{L}CO_{3EQ}$ measurement.

In a few cases, after a single $D_{L}CO_{3EQ}$ measurement was made at the higher workload, subjects could no longer continue pedaling long enough to repeat the higher $D_{L}CO_{3EQ}$ measurement. These subjects were allowed to recover before another attempt was made. Once recovered the subjects were started pedaling directly at the higher workload. After 3 min at the same workload, the second $D_{L}CO_{3EQ}$ measurement was made.

Arterial blood gas sampling

In selected subjects who agreed, 1-2 mL blood samples from the radial artery were taken at rest and during steady state exercise at the highest workload. An Allen’s test was performed to
check for sufficient collateral circulation. The resting blood sample was taken with a 2 mL glass syringe coated with heparin with a 23 gauge needle. Glass syringes were used to reduce any gas diffusion out of the sample. The exercise blood sample was taken using a “Quik” arterial blood gas kit (Marquest, Englewood, CO) with the same size needle. All samples were stored on ice until they could be analyzed which was generally within 15 minutes of the second arterial sample. Arterial PO$_2$, PCO$_2$, and pH were measured in duplicate using a radiometer (Model “ABL 500”; Radiometer, Copenhagen, NV, Denmark). Arterial carboxyhemoglobin (COHb) was measured using a CO hemoximeter (Model “OSM3”; Radiometer, Copenhagen, NV, Denmark).

STATISTICAL ANALYSIS

Analysis of the data was done using Microsoft Excel™ 7.0 and a personal computer; we determined means and SEM for the different variables in each group. Comparisons between different groups for anthropometric, D$_L$CO$_{SB}$ and lung function characteristics were made using a two-tailed unpaired $t$ test. D$_L$CO$_{SB}$ and D$_L$CO$_{3EQ}$ in the same subjects were compared using the two-tailed Student’s paired $t$ test (38). The change in D$_L$CO$_{3EQ}$ from rest to the higher level of exercise expressed as a ratio to the change in VO$_2$ was compared between the groups using the unpaired Student’s $t$ test. For multiple comparisons between IPF, sarcoidosis, and control groups, the Bonferonni correction (39) was applied and a $p$ value of $(0.05^{÷3})$ i.e. 0.0167 was taken to indicate statistical significance.
CHAPTER THREE: RESULTS

SUBJECT RECRUITMENT

Twenty-five patients, 11 with sarcoidosis and 14 with IPF, met the study criteria and were requested to participate. Of these, 8 with sarcoidosis and 10 with IPF subjects consented to participate. All 8 sarcoidosis patients completed the study. Three of the 10 IPF subjects did not complete the study. One subject developed frequent premature ventricular complexes during the progressive exercise test and was withdrawn from the study. The other two subjects had progressive exercise testing, but were unable to perform the breathing maneuver required for the $D_L{CO}_{3eq}$ determination during exercise. Of these two IPF patients, one was a 72 year old male with a FEV$_1$ of 1.84 L (54% of predicted), a FVC of 2.23 L (50% of predicted), a $D_L{CO}_{SB}$ of 11.89 mL·min$^{-1}$·mmHg$^{-1}$ (47% of predicted), and peak VO$_2$ of 1.44 L/min (76% of maximal predicted). The other was a 77 year old female with a FEV$_1$ of 1.53 L (72% of predicted), a FVC of 1.87 L (72% of predicted), a $D_L{CO}_{SB}$ of 6.80 mL·min$^{-1}$·mmHg$^{-1}$ (35% of predicted), and a peak VO$_2$ of 0.90 L/min (56% of maximal predicted). Both these patients had a lower FVC and $D_L{CO}_{SB}$ when compared to the other IPF patients indicative of more advanced disease.

All sarcoidosis patients except one (S7) had received treatment or were currently on treatment at the time of the study. All patients with IPF were on corticosteroid and/or immunosuppressive treatment either at the time of the study or had been previously treated.

Five of the sarcoidosis and two of the IPF subjects were matched by age, race, and sex with 7 controls subjects; we had difficulty in recruiting healthy control subjects for the patients with IPF older than 60 years. The control subjects recruited for the study had normal lung
function, a normal chest roentgenogram within the past 6 months, no cardiovascular disease, and did not have any other significant health problems. Four of the control subjects were recruited from patients referred to the Pulmonary Function Laboratory at VGH, two were friends of VGH staff, and one was the father of one of the patients.

SUBJECT CHARACTERISTICS

All the ILD subjects showed bilateral lung parenchymal involvement on the chest roentgenograms and HRCT. Three of the 8 sarcoidosis subjects, none of the IPF subjects, and 1 of the 7 controls were women. The subject characteristics are given in Tables 1A, 1B, and 1C. The mean age of the IPF group was significantly greater than the mean age of the sarcoidosis patients (46±SE 5, p<0.001) and the control subjects (46±SE 7, p<0.001) groups. There were no significant differences in height or in weight between any of the groups.

Only one patient (F2) was a current smoker (total cigarette consumption of 50 pack-years). He was smoking 2-3 cigarettes per day, but did not smoke on the day when $D_LCO_{3EQ}$ measurements were made; this amount of smoking was considered to have a negligible effect on the $D_LCO_{3EQ}$ measurements. All the other subjects were non-smokers, except for 5 ex-smokers (subjects S4, F3, F4, F5, and F6 with a total cigarette consumption of 10, 40, 30, and 25 pack years, respectively). Only one control subject (C4) had a previous smoking history of 30 years; the others had never smoked.

LUNG FUNCTION CHARACTERISTICS

Spirometry was performed on all subjects prior to being tested (Tables 1A, 1B, and 1C).
### TABLE 1A: Lung function characteristics of sarcoidosis subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Race</th>
<th>Age (yr)</th>
<th>Ht (cm)</th>
<th>Wt (Kg)</th>
<th>FVC (L) (%P)</th>
<th>FEV₁ (%FVC)</th>
<th>D₁CO₅SB (mL/min/mmHg) (%P)</th>
<th>D₁CO₅SB/Vₐ (mL/min/mmHg/L) (%P)</th>
<th>TLC (L) (%P)</th>
<th>Sₒ₂ (mmHg)</th>
<th>Pₐ₂ (mmHg)</th>
<th>A-a O₂ (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>M</td>
<td>EI</td>
<td>48</td>
<td>167</td>
<td>61</td>
<td>2.47 (56%)</td>
<td>90% (111%)</td>
<td>13.5 (46%)</td>
<td>4.15 (85%)</td>
<td>3.36 (54%)</td>
<td>99%</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>S2</td>
<td>M</td>
<td>EI</td>
<td>29</td>
<td>167</td>
<td>65</td>
<td>5.10 (107%)</td>
<td>68% (80%)</td>
<td>24.75 (73%)</td>
<td>4.32 (79%)</td>
<td>6.65 (110%)</td>
<td>98%</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>S3</td>
<td>M</td>
<td>C</td>
<td>36</td>
<td>177</td>
<td>78</td>
<td>5.38 (103%)</td>
<td>82% (101%)</td>
<td>27.97 (83%)</td>
<td>4.53 (90%)</td>
<td>6.10 (90%)</td>
<td>99%</td>
<td>---</td>
<td>---</td>
</tr>
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<td>3.66 (109%)</td>
<td>76% (96%)</td>
<td>27.32 (82%)</td>
<td>4.81 (111%)</td>
<td>5.94 (109%)</td>
<td>97%</td>
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<td>---</td>
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<td>47</td>
<td>157</td>
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<td>3.09 (109%)</td>
<td>78% (94%)</td>
<td>23.17 (105%)</td>
<td>5.88 (124%)</td>
<td>4.31 (91%)</td>
<td>97%</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>S6</td>
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<td>157</td>
<td>66</td>
<td>2.67 (88%)</td>
<td>73% (88%)</td>
<td>12.25 (56%)</td>
<td>3.92 (83%)</td>
<td>3.26 (69%)</td>
<td>97%</td>
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<td>---</td>
</tr>
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<td>74% (89%)</td>
<td>18.18 (55%)</td>
<td>4.31 (80%)</td>
<td>4.56 (76%)</td>
<td>99%</td>
<td>86</td>
<td>17</td>
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<td>62</td>
<td>146</td>
<td>59</td>
<td>2.16 (99%)</td>
<td>83% (102%)</td>
<td>15.17 (81%)</td>
<td>5.32 (113%)</td>
<td>2.86 (72%)*</td>
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<td>100</td>
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<td>163</td>
<td>74</td>
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<td>78% (95%)</td>
<td>20.29 (73%)</td>
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<td>4.76 (84%)</td>
<td>98%</td>
<td>93</td>
<td>13</td>
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<td>7</td>
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<td>2.23 (7%)</td>
<td>0.23 (6%)</td>
<td>0.51 (8%)</td>
<td>0.3</td>
<td>7</td>
<td>4</td>
<td></td>
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</table>

Abbreviations used: EI=East Indian, C=Caucasian, B=Black, Ht=Height, Wt=Weight, FVC=Forced vital capacity, FEV₁=Volume expired in 1 second, D₁CO₅SB=Single breath lung diffusing capacity for CO, D₁CO₅SB/Vₐ=Ratio of D₁CO₅SB to single breath alveolar volume (Vₐ) at which D₁CO₅SB was determined, TLC=Total lung capacity, Sₒ₂=Oxygen saturation by pulse oximetry, Pₐ₂=Arterial partial pressure of O₂, A-a O₂=Alveolar-arterial O₂ partial pressure gradient, and (%P)=Percent of predicted.

*TLC not determined; substituted by Vₐ from D₁CO₅SB
## TABLE 1B: Lung function characteristics of IPF subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Race</th>
<th>Age (yr)</th>
<th>Ht (cm)</th>
<th>Wt (Kg)</th>
<th>FVC L (%P)</th>
<th>FEV₁/FVC (%)</th>
<th>D₁COSB mL/min/mmHg (%P)</th>
<th>D₁COSB/Vₐ mL/min/mmHg/L (%P)</th>
<th>TLC L (%P)</th>
<th>S₁O₂ %</th>
<th>P₁O₂ mmHg</th>
<th>A-a O₂ mmHg</th>
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<tbody>
<tr>
<td>F1</td>
<td>M</td>
<td>C</td>
<td>77</td>
<td>167</td>
<td>69</td>
<td>2.74 (74%)</td>
<td>83 (108%)</td>
<td>9.27 (41%)</td>
<td>2.90 (74%)</td>
<td>3.85 (62%)</td>
<td>96%</td>
<td>---</td>
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</tr>
<tr>
<td>F2</td>
<td>M</td>
<td>C</td>
<td>76</td>
<td>173</td>
<td>83</td>
<td>4.13 (104%)</td>
<td>75 (99%)</td>
<td>11.53 (48%)</td>
<td>2.34 (60%)</td>
<td>5.56 (83%)</td>
<td>96%</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>F3</td>
<td>M</td>
<td>C</td>
<td>72</td>
<td>178</td>
<td>83</td>
<td>2.03 (46%)</td>
<td>81 (106%)</td>
<td>14.40 (66%)</td>
<td>4.06 (110%)</td>
<td>4.00 (61%)</td>
<td>96%</td>
<td>59</td>
<td>54</td>
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<tr>
<td>F4</td>
<td>M</td>
<td>O</td>
<td>62</td>
<td>161</td>
<td>62</td>
<td>2.64 (92%)</td>
<td>84 (105%)</td>
<td>9.36 (37%)</td>
<td>2.76 (60%)</td>
<td>3.72 (66%)</td>
<td>98%</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>F5</td>
<td>M</td>
<td>C</td>
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<td>173</td>
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<td>3.81 (93%)</td>
<td>79 (106%)</td>
<td>17.00 (69%)</td>
<td>2.90 (76%)</td>
<td>5.70 (84%)</td>
<td>95%</td>
<td>79</td>
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<td>M</td>
<td>C</td>
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<td>166</td>
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<td>85 (108%)</td>
<td>10.89 (44%)</td>
<td>2.06 (48%)</td>
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<td>96%</td>
<td>69</td>
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</tr>
<tr>
<td>F7</td>
<td>M</td>
<td>C</td>
<td>81</td>
<td>174</td>
<td>77</td>
<td>2.70 (66%)</td>
<td>79 (104%)</td>
<td>10.45 (45%)</td>
<td>2.66 (72%)</td>
<td>4.07 (97%)</td>
<td>97%</td>
<td>91</td>
<td>18</td>
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<td>MEAN</td>
<td></td>
<td></td>
<td>73</td>
<td>170</td>
<td>75</td>
<td>3.08 (81%)</td>
<td>81 (105%)</td>
<td>11.84 (50%)</td>
<td>2.80 (71%)</td>
<td>4.60 (72%)</td>
<td>96%</td>
<td>77</td>
<td>26</td>
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<tr>
<td>SEM</td>
<td></td>
<td></td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>0.28 (8%)</td>
<td>1 (1%)</td>
<td>1.08 (5%)</td>
<td>0.24 (7%)</td>
<td>0.34 (5%)</td>
<td>0.3</td>
<td>7</td>
<td>5</td>
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</tbody>
</table>

**Abbreviations used:** O = Oriental; other abbreviations in Table 1A.
### TABLE 1C: Lung function characteristics of control subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Race</th>
<th>Age (yr)</th>
<th>Ht (cm)</th>
<th>Wt (Kg)</th>
<th>FVC (L (%P))</th>
<th>FEV₁ (%FVC)</th>
<th>D₁CO&lt;sub&gt;S&lt;/sub&gt;B (mL/min/mmHg (%P))</th>
<th>D₁CO&lt;sub&gt;S&lt;/sub&gt;B/V&lt;sub&gt;A&lt;/sub&gt; (mL/min/mmHg/L (%P))</th>
<th>TLC (L (%P))</th>
<th>Resting S&lt;sub&gt;p&lt;/sub&gt;O₂ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>M</td>
<td>EI</td>
<td>23</td>
<td>176</td>
<td>72</td>
<td>4.86 (90%)</td>
<td>85% (100%)</td>
<td>44.47 (133%)†</td>
<td>6.99 (128%)†</td>
<td>6.36 (95%)*</td>
<td>99%</td>
</tr>
<tr>
<td>C2</td>
<td>M</td>
<td>C</td>
<td>36</td>
<td>175</td>
<td>76</td>
<td>6.59 (130%)</td>
<td>81% (99%)</td>
<td>43.96 (131%)</td>
<td>5.38 (106%)</td>
<td>8.34 (106%)</td>
<td>97%</td>
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<tr>
<td>C3</td>
<td>M</td>
<td>C</td>
<td>75</td>
<td>176</td>
<td>84</td>
<td>3.75 (88%)</td>
<td>79% (104%)</td>
<td>27.73 (112%)</td>
<td>4.91 (128%)</td>
<td>6.06 (88%)</td>
<td>98%</td>
</tr>
<tr>
<td>C4</td>
<td>M</td>
<td>O</td>
<td>68</td>
<td>162</td>
<td>66</td>
<td>3.46 (95%)</td>
<td>80% (101%)</td>
<td>28.97 (121%)†</td>
<td>6.44 (148%)†</td>
<td>4.50 (78%)*</td>
<td>98%</td>
</tr>
<tr>
<td>C5</td>
<td>M</td>
<td>EI</td>
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<td>162</td>
<td>60</td>
<td>3.28 (82%)</td>
<td>81% (98%)</td>
<td>26.28 (93%)†</td>
<td>5.95 (121%)†</td>
<td>4.42 (77%)*</td>
<td>99%</td>
</tr>
<tr>
<td>C6</td>
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<td>175</td>
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<td>82% (101%)</td>
<td>25.58 (99%)</td>
<td>5.25 (116%)</td>
<td>5.05 (87%)</td>
<td>99%</td>
</tr>
<tr>
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<td>171</td>
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<td>84% (101%)</td>
<td>30.23 (91%)</td>
<td>4.95 (95%)</td>
<td>6.10 (95%)*</td>
<td>96%</td>
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<tr>
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<td></td>
<td>46</td>
<td>171</td>
<td>77</td>
<td>4.50 (99%)</td>
<td>82% (101%)</td>
<td>32.46 (110%)</td>
<td>5.70 (120%)</td>
<td>5.83 (89%)</td>
<td>98%</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td></td>
<td>7</td>
<td>2</td>
<td>5</td>
<td>0.46 (6%)</td>
<td>1% (1%)</td>
<td>3.09 (10%)</td>
<td>0.30 (7%)</td>
<td>0.51 (7%)</td>
<td>1%</td>
</tr>
</tbody>
</table>

**Abbreviations:** as in Table 1A and 1B.

* TLC not determined; substituted by V<sub>A</sub> from D<sub>L</sub>CO<sub>S</sub>B (or from D<sub>L</sub>CO<sub>3EQ</sub>)

† Conventional D<sub>L</sub>CO<sub>S</sub>B not determined; D<sub>L</sub>CO<sub>3EQ</sub> used instead
The mean FVC was 3.51±SE 0.42 L (92% of predicted) in the sarcoidosis group, 3.08±SE 0.28 L (81% of predicted) in the IPF group, and 4.50±SE 0.46 L (99%) in the control group. The FVC appeared to be lower in the IPF patients than the controls, but the difference expressed as % predicted did not reach statistical significance (p=0.04). There were no statistically significant differences between FVC expressed as percent predicted between the IPF and sarcoidosis patients (p=0.18), and between the sarcoidosis and control subjects (p=0.21). The FEV₁ to FVC ratio was close to 100% of predicted in all subjects, indicating no airflow obstruction.

Lung volumes were determined by helium dilution in all subjects except for subjects S8, C1, C4, C5, and C7 (Tables IA, IB, and IC). In subjects S8 and C7, lung volume at full inspiration (Vₐ) determined from DLCOₛₐ was considered equivalent to TLC. In subjects C1, C4, and C5, Vₐ from the resting DLCO₃EQ test was considered as TLC. The mean TLC for the sarcoidosis group was 4.76±0.51 L (84% of predicted), 4.60±0.34 L (72% of predicted) for the IPF group, and 5.83±0.30 L (89% of predicted) for the control group. TLC was only significantly different in the IPF and control groups (p<0.05).

DLCOₛₐ measurements using the Morgan equipment were already performed prior to the study in all ILD subjects and 4 control subjects (Tables IA, IB, and IC). The DLCOₛₐ values reported in Table IC for the remaining 3 control subjects were the DLCO₃EQ measurements. DLCOₛₐ for the sarcoidosis subjects (20.29 ± 2.23 mL·min⁻¹·mmHg⁻¹ or 73% of predicted) was statistically different (p<0.01) from the IPF subjects (11.84 ± 1.08 mL·min⁻¹·mmHg⁻¹ or 50% of predicted). When corrected for lung volume, the DLCOₛₐ/Vₐ ratio of the sarcoidosis group (4.66 mL·min⁻¹·mmHg⁻¹·L⁻¹ or 96% of predicted) was statistically different (p<0.05) from the IPF group (2.80 mL·min⁻¹·mmHg⁻¹·L⁻¹ or 71% of predicted). The mean resting DLCOₛₐ for the control
subjects was 11.84 ± 1.08 mL·min⁻¹·mmHg⁻¹ (110% of predicted) and the $D_L CO_{SB}/V_A$ ratio was 5.70 mL·min⁻¹·mmHg⁻¹·L⁻¹ (120% of predicted).

PROGRESSIVE EXERCISE TESTING

All subjects had progressive exercise testing on a cycle ergometer starting at a workload of 30 W with incremental increases of 10 W until the subjects reached their maximal predicted heart rate, or had a significant decrease in $O_2$ saturation by pulse oximetry, or were unable to continue. Tables 2A, 2B, and 2C show the mean peak VO₂ for the sarcoidosis, IPF, and control groups which were 2.18±0.29 L/min (94% of maximal predicted), 1.40±0.15 L/min (77% of maximal predicted), and 3.15±0.44 L/min (122% of maximal predicted), respectively. The peak VO₂ expressed as % of maximal predicted was not significantly higher in the sarcoidosis than the IPF patients probably because of small number of subjects. In subject S7, a progressive exercise test was performed and the maximal power output was determined; however, due to a mechanical error in the sampling of expired gases, the determination of peak VO₂ in this subject was not reliable and was not reported.

$D_L CO_{3EQ}$ DURING REST AND STEADY STATE EXERCISE

Three determinations of resting $D_L CO_{3EQ}$ that agreed within 10% of each other were obtained and averaged (Tables 2A, 2B, and 2C). To adjust for differences in sex, age, and height for different subjects, $D_L CO_{3EQ}$ was expressed as percent of predicted $D_L CO_{SB}$. The mean $D_L CO_{3EQ}$ in the sarcoidosis, IPF and control subjects were 23.00±2.78 mL·min⁻¹·mmHg⁻¹ (86% of predicted), 15.90±0.72 mL·min⁻¹·mmHg⁻¹ (67% of predicted), and 34.57±2.70 mL·min⁻¹
### TABLE 2A: Oxygen consumption (VO₂) and three-equation D\text{LCO} (D\text{LCO}_{3\text{EQ}}) at rest and during steady state exercise in sarcoidosis subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Peak VO₂*&lt;sub&gt;5&lt;/sub&gt; mL/min (%P)†</th>
<th>D\text{LCO}_{3\text{EQ}} mL/min/mmHg (%P)</th>
<th>VO₂ mL/min (%P)</th>
<th>D\text{LCO}_{3\text{EQ}} mL/min/mmHg (%P)</th>
<th>S\text{pO₂} %</th>
<th>VO₂ mL/min (%P)†</th>
<th>D\text{LCO}_{3\text{EQ}} mL/min/mmHg (%P)</th>
<th>S\text{pO₂} %</th>
<th>P\text{aO₂} mmHg</th>
<th>A-a O₂ mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>1605 (61%)</td>
<td>14.44 (49%)</td>
<td>924 (35%)</td>
<td>19.35 (66%)</td>
<td>99%</td>
<td>1146 (43%)</td>
<td>21.94 (75%)</td>
<td>98%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S2</td>
<td>2411 (74%)</td>
<td>33.83 (100%)</td>
<td>1055 (32%)</td>
<td>42.28 (125%)</td>
<td>98%</td>
<td>2084 (64%)</td>
<td>47.38 (140%)</td>
<td>98%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S3</td>
<td>3310 (109%)</td>
<td>32.80 (97%)</td>
<td>1479 (49%)</td>
<td>40.55 (120%)</td>
<td>99%</td>
<td>2966 (98%)</td>
<td>46.84 (139%)</td>
<td>99%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S4</td>
<td>2660 (129%)</td>
<td>29.15 (116%)</td>
<td>1563 (76%)</td>
<td>34.40 (137%)</td>
<td>97%</td>
<td>2181 (106%)</td>
<td>39.79 (159%)</td>
<td>97%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S5</td>
<td>2610 (135%)</td>
<td>22.86 (103%)</td>
<td>2109 (109%)*</td>
<td>35.52 (160%)</td>
<td>97%</td>
<td>2503 (130%)*</td>
<td>39.42 (178%)</td>
<td>97%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S6</td>
<td>1500 (79%)</td>
<td>16.02 (73%)</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>1568 (83%)</td>
<td>25.15 (115%)</td>
<td>96%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S7</td>
<td>---</td>
<td>17.80 (54%)</td>
<td>1150 (37%)*</td>
<td>22.02 (67%)</td>
<td>99%</td>
<td>1566 (50%)*</td>
<td>27.22 (83%)</td>
<td>94%</td>
<td>79</td>
<td>38</td>
</tr>
<tr>
<td>S8</td>
<td>1188 (69%)</td>
<td>17.36 (93%)</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>1100 (64%)*</td>
<td>22.79 (122%)</td>
<td>99%</td>
<td>104</td>
<td>15</td>
</tr>
<tr>
<td><strong>MEAN</strong></td>
<td>2183 (94%)</td>
<td>23.03 (86%)</td>
<td>1380 (57%)</td>
<td>32.35 (113%)</td>
<td>98%</td>
<td>1901 (80%)</td>
<td>33.81 (126%)</td>
<td>97%</td>
<td>92</td>
<td>27</td>
</tr>
<tr>
<td><strong>SEM</strong></td>
<td>290 (11%)</td>
<td>2.78 (9%)</td>
<td>177 (13%)</td>
<td>3.90 (16%)</td>
<td>0.4</td>
<td>233 (11%)</td>
<td>3.78 (13%)</td>
<td>0.6</td>
<td>13</td>
<td>12</td>
</tr>
</tbody>
</table>

*Level 1 and 2 are about 35% and 70% respectively of maximum exercise workload

*Peak VO₂ determined from the progressive exercise test.

†Percent predicted VO₂_max

*VO₂ in these subjects was actually determined using steady state exercise; in the other subjects VO₂ was estimated from the VO₂ during the progressive exercise test (details in text).
TABLE 2B: Oxygen consumption (VO\textsubscript{2}) and three-equation DLCO (DLCO\textsubscript{3EQ}) at rest and during steady state exercise in IPF subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Peak VO\textsubscript{2}* mL/min (%P)†</th>
<th>DLCO\textsubscript{3EQ} mL/min/mmHg (%P)</th>
<th>VO\textsubscript{2} mL/min (%P)</th>
<th>DLCO\textsubscript{3EQ} mL/min/mmHg (%P)</th>
<th>S\textsubscript{p}O\textsubscript{2} %</th>
<th>VO\textsubscript{2} mL/min (%P)†</th>
<th>DLCO\textsubscript{3EQ} mL/min/mmHg (%P)</th>
<th>S\textsubscript{p}O\textsubscript{2} %</th>
<th>P\textsubscript{s}O\textsubscript{2} mmHg</th>
<th>A-a O\textsubscript{2} mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1208 (70%)</td>
<td>14.96 (66%)</td>
<td>781 (46%)</td>
<td>18.38 (81%)</td>
<td>93%</td>
<td>1173 (69%)</td>
<td>18.64 (82%)</td>
<td>87%</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>F2</td>
<td>1605 (90%)</td>
<td>13.41 (56%)</td>
<td>864 (49%)</td>
<td>18.49 (77%)</td>
<td>94%</td>
<td>1264 (71%)</td>
<td>18.99 (79%)</td>
<td>92%</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>F3</td>
<td>10020 (53%)</td>
<td>17.46 (80%)</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>1000 (53%)</td>
<td>21.46 (98%)</td>
<td>84%</td>
<td>49</td>
<td>59</td>
</tr>
<tr>
<td>F4</td>
<td>1000 (45%)</td>
<td>18.23 (72%)</td>
<td>523 (24%)</td>
<td>21.03 (83%)</td>
<td>96%</td>
<td>904 (41%)</td>
<td>20.83 (82%)</td>
<td>96%</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>F5</td>
<td>2080 (120%)</td>
<td>14.99 (62%)</td>
<td>1240 (71%)</td>
<td>21.76 (91%)</td>
<td>93%</td>
<td>1742 (100%)</td>
<td>22.33 (93%)</td>
<td>88%</td>
<td>58</td>
<td>56</td>
</tr>
<tr>
<td>F6</td>
<td>1550 (76%)</td>
<td>14.36 (58%)</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>1400 (70%)</td>
<td>17.84 (72%)</td>
<td>82%</td>
<td>51</td>
<td>68</td>
</tr>
<tr>
<td>F7</td>
<td>1350 (85%)</td>
<td>17.86 (78%)</td>
<td>843 (53%)</td>
<td>20.65 (90%)</td>
<td>92%</td>
<td>1140 (72%)</td>
<td>23.11 (100%)</td>
<td>86%</td>
<td>51</td>
<td>68</td>
</tr>
<tr>
<td>Mean</td>
<td>1399 (77%)</td>
<td>15.90 (67%)</td>
<td>850 (48%)</td>
<td>20.06 (84%)</td>
<td>94%</td>
<td>1237 (68%)</td>
<td>20.46 (87%)</td>
<td>88%</td>
<td>52</td>
<td>63</td>
</tr>
<tr>
<td>SEM</td>
<td>145 (9%)</td>
<td>0.72 (4%)</td>
<td>105 (7%)</td>
<td>0.69 (3%)</td>
<td>0.7</td>
<td>107 (7%)</td>
<td>0.76 (4%)</td>
<td>1.8</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

*Level 1 and 2 are about 35% and 70% respectively of maximum exercise workload
*Peak VO\textsubscript{2} determined from the progressive exercise test.
†Percent predicted VO\textsubscript{2}max
*VO\textsubscript{2} in these subjects was actually determined using steady state exercise; in the other subjects VO\textsubscript{2} was estimated from the VO\textsubscript{2} during the progressive exercise test (details in text).
TABLE 2C: Oxygen consumption (VO\(_2\)) and three-equation D\(_L\)CO (D\(_L\)CO\(_{3EQ}\)) at rest and during steady state exercise in control subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Peak VO(_2)(^*) mL/min (%P)(\dagger)</th>
<th>D(<em>L)CO(</em>{3EQ}) mL/min/mmHg (%P)</th>
<th>VO(_2) mL/min (%P)(\dagger)</th>
<th>D(<em>L)CO(</em>{3EQ}) mL/min/mmHg (%P)</th>
<th>VO(_2) mL/min (%P)(\dagger)</th>
<th>D(<em>L)CO(</em>{3EQ}) mL/min/mmHg (%P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>4120 (120%)</td>
<td>44.47 (133%)</td>
<td>2150 (63%)(\ast)</td>
<td>53.12 (159%)</td>
<td>3800 (111%)(\ast)</td>
<td>59.75 (179%)</td>
</tr>
<tr>
<td>C2</td>
<td>4280 (141%)</td>
<td>43.48 (119%)</td>
<td>1721 (57%)</td>
<td>54.49 (149%)</td>
<td>2665 (88%)</td>
<td>59.97 (164%)</td>
</tr>
<tr>
<td>C3</td>
<td>2120 (120%)</td>
<td>32.25 (131%)</td>
<td>1100 (63%)(\ast)</td>
<td>44.00 (178%)</td>
<td>1600 (91%)</td>
<td>41.86 (169%)</td>
</tr>
<tr>
<td>C4</td>
<td>1800 (88%)</td>
<td>28.97 (121%)</td>
<td>1275 (63%)(\ast)</td>
<td>28.43 (118%)</td>
<td>1900 (93%)</td>
<td>36.42 (152%)</td>
</tr>
<tr>
<td>C5</td>
<td>2140 (83%)</td>
<td>26.28 (93%)</td>
<td>1500 (58%)</td>
<td>34.29 (122%)</td>
<td>Not Done</td>
<td>Not Done</td>
</tr>
<tr>
<td>C6</td>
<td>3000 (150%)</td>
<td>30.03 (117%)</td>
<td>1300 (65%)(\ast)</td>
<td>35.43 (138%)</td>
<td>2100 (105%)(\ast)</td>
<td>42.80 (166%)</td>
</tr>
<tr>
<td>C7</td>
<td>4600 (150%)</td>
<td>36.47 (110%)</td>
<td>1866 (61%)(\ast)</td>
<td>43.69 (131%)</td>
<td>2952 (96%)(\ast)</td>
<td>51.60 (155%)</td>
</tr>
<tr>
<td>MEAN</td>
<td>3151 (122%)</td>
<td>34.57 (118%)</td>
<td>1559 (61%)</td>
<td>41.92 (142%)</td>
<td>2502 (97%)</td>
<td>48.73 (164%)</td>
</tr>
<tr>
<td>SEM</td>
<td>443 (10%)</td>
<td>2.70 (5%)</td>
<td>141 (1%)</td>
<td>3.69 (8%)</td>
<td>329 (12%)</td>
<td>4.04 (4%)</td>
</tr>
</tbody>
</table>

\(\ast\)Level 1 and 2 are about 35% and 70% respectively of maximum exercise workload
\(\ast\)Peak VO\(_2\) determined from the progressive exercise test.
\(\dagger\)Percent predicted VO\(_{2\text{max}}\)
\(\dagger\)VO\(_2\) in these subjects was actually determined using steady state exercise; in the other subjects VO\(_2\) was estimated from the VO\(_2\) during the progressive exercise test (details in text).
\(\ddagger\)O\(_2\) saturation by pulse oximetry was \(\geq 97\%\) in all subjects.
mmHg⁻¹ (118% of predicted), respectively.

Our original intention was to do the progressive exercise test and \( D_L \text{CO}_3 \text{EQ} \) steady state measurements on separate days; however, several subjects were unwilling or were unable to come for the study on two different days. In these subjects, the steady state exercise \( D_L \text{CO}_3 \text{EQ} \) was determined after 45-60 min of rest following the progressive exercise test. As a result, the study was completed on the same day in 4 of the 8 sarcoidosis, 5 of the 7 IPF, and 5 of the 7 control subjects.

The ILD subjects were exercised at steady state workloads corresponding to approximately 35% and 70% of their maximal workload determined from the progressive exercise test. The \( D_L \text{CO}_3 \text{EQ} \) measurement was made as a single determination at the lower workload, but was done in duplicate at the higher workload. Three ILD subjects, F3, S6, and S8 had maximal workloads equal to or less than 60 W and were only tested at the higher 70% workload. The control subjects were exercised at workloads that covered the same workload range as their patients with ILD with whom they had been matched. The coefficients of variation between the two \( D_L \text{CO}_3 \text{EQ} \) measurements at the higher level were 5.4%, 5.4%, and 6.9% for the sarcoidosis, IPF, and control subjects, respectively.

Control subject C5 had difficulty performing the \( D_L \text{CO}_3 \text{EQ} \) breathing maneuver at the 70% workload; 2 \( D_L \text{CO}_3 \text{EQ} \) measurements were made at a single workload equivalent to 45% of his maximal workload. In subjects S5, S7, S8, F6, F7, C1, C3, C4, C5, C6, and C7, \( O_2 \) consumption at each level of steady state exercise was measured using the exercise system gas analyzers. In these subjects, the steady state \( VO_2 \) was compared to the \( VO_2 \) at the same workload during the progressive exercise test (Fig. 6). The ratio of the \( VO_2 \) during steady state exercise to the \( VO_2 \)
Figure 6. Comparison of VO$_2$ during steady state exercise with VO$_2$ at the same workload from the progressive exercise test. There were 8 subjects tested at 2 different levels of exercise, 35% and 70% of their maximal workload. The dotted line is the line of identity and the solid line is the regression line.

$y = 1.08x + 132.1$ ($R^2 = 0.85$)
during the progressive exercise test at the lower level of exercise was 0.82±SE 0.08 and at the higher workload was 0.88±SE 0.06. In the other subjects who did not have measurements of VO\textsubscript{2} during steady state exercise, these two ratios were used to convert the VO\textsubscript{2} of the progressive exercise test to estimate steady state VO\textsubscript{2} at the lower and higher workloads, respectively. The D\textsubscript{L}CO\textsubscript{3EQ} measurements at each level of exercise and the corresponding VO\textsubscript{2} for the sarcoidosis, IPF, and control subjects are summarised in Tables 2A, 2B, and 2C, respectively.

For each subject, D\textsubscript{L}CO\textsubscript{3EQ} (expressed as % of predicted D\textsubscript{L}CO\textsubscript{SB} at rest) was plotted against VO\textsubscript{2} (expressed as % of maximal predicted VO\textsubscript{2}) both at rest and during the higher level of steady state exercise. Figures 7, 8, and 9 show the results separately for the control, sarcoidosis, and IPF groups, respectively. With increasing VO\textsubscript{2}, all subjects increased their D\textsubscript{L}CO\textsubscript{3EQ}, but to varying degrees. Figure 10 compares the mean results of the relationship between the D\textsubscript{L}CO\textsubscript{3EQ} and VO\textsubscript{2} at rest and the higher workload in the three groups. The IPF group did not increase D\textsubscript{L}CO\textsubscript{3EQ} to the same extent as the sarcoidosis group (p<0.0001) or the controls (p<0.01) for a given increase in VO\textsubscript{2}. The sarcoidosis patients increased their D\textsubscript{L}CO\textsubscript{3EQ} significantly greater than the normal subjects (p<0.01) for the increase in VO\textsubscript{2}.

**O\textsubscript{2} SATURATION AND ARTERIAL BLOOD GAS SAMPLING**

In the sarcoidosis subjects, % O\textsubscript{2} saturation did not decrease significantly from rest to steady state exercise at the higher workload; however, in the IPF patients O\textsubscript{2} saturation decreased significantly (p<0.05) as they exercised from rest to steady state exercise at the higher workload (Fig. 11). Arterial blood gas samples were also taken from 2 sarcoidosis and 4 IPF subjects at rest and during the higher level of steady state exercise. The P\textsubscript{a}O\textsubscript{2}, P\textsubscript{a}CO\textsubscript{2}, pH, and COHb were
measured and the A-a PO₂ was calculated in each of the samples. The resting PₐO₂ and A-a PO₂ are reported in Tables 1A and 1B and the exercise PₐO₂ and A-a PO₂ are reported in Tables 2A and 2B. All IPF subjects experienced a fall in PₐO₂ and a widened A-a PO₂ from rest to steady state at the higher level. The two sarcoidosis subjects, who had arterial blood gases, increased their mean A-a PO₂ from 13 to 27 mmHg (n=2), while the IPF subjects increased their mean A-a PO₂ from 26 to 63 mmHg (n=4). The significance of differences between the two groups could not be determined due to the small number of subjects in each group. Mean % COHb during the second level of exercise prior to the last DₜCO₂EQ was 2.5±0.4 (n=5). This low residual amount of COHb would not effect the DₜCO₂EQ measurements significantly.
Figure 7. $D_{LCO_{3EQ}}$ at rest and the higher level of steady-state exercise in normal subjects (n=6). Subject C5 could not perform the $D_{LCO_{3EQ}}$ maneuver at the higher workload.
Figure 8. $D_LCO_{3EQ}$ at rest and the higher level of steady-state exercise in sarcoidosis patients (n=8).
Figure 9. $D_L$CO$_{3EQ}$ at rest and the higher level of steady-state exercise in IPF patients (n=7).
Figure 10. Mean $D_L CO_{3EQ}$ at rest and during the higher level of steady-state exercise in the three groups of subjects. Standard error bars are shown. The mean ratio of increase in $D_L CO_{3EQ}$ to the increase in $VO_2$ (both expressed as % predicted) was significantly less in the IPF group when compared to the sarcoidosis ($p<0.0001$) and normal ($p<0.01$) groups. Mean ratios were $0.53 \pm SE 0.03$, $0.68 \pm SE 0.04$, and $0.39 \pm SE 0.04$ for the normal, sarcoidosis, and IPF groups, respectively.
Figure 11. O₂ saturation by pulse oximetry at rest and during steady state exercise in IPF and sarcoidosis subjects.
CHAPTER FOUR: DISCUSSION

The main finding of this study is that IPF patients had only a limited increase in $D_L CO_{3EQ}$ with exercise, when compared with sarcoidosis patients and normal subjects. The slope of the increase in $D_L CO_{3EQ}$ with increasing VO$_2$ in patients with IPF was about half that of the sarcoidosis patients. The limited increase in $D_L CO_{3EQ}$ with exercise in the IPF patients may reflect the alveolar capillary membrane thickening and the reduction in the pulmonary capillary bed and surface area available for gas exchange. At the higher level of steady state exercise, all of the sarcoidosis and control subjects maintained arterial O$_2$ oxygenation close to resting values, while in the IPF patients, O$_2$ saturation fell dramatically during exercise suggesting a possible diffusion limitation. We will review and discuss our data, and a theoretical analysis, using a simple lung model with venous admixture (shunt-like effect), to estimate the contribution of diffusion limitation to exercise hypoxemia.

SUBJECT RECRUITMENT

A limitation of our study is the small number of subjects studied. We had expected to recruit about 10-12 patients with sarcoidosis with diffuse interstitial lung disease and a similar number of patients with IPF. However, we were able to recruit only 8 suitable patients with sarcoidosis, and 7 with IPF, despite the collaboration and review of the records of all of the 9 respirologists at VGH. We also sought to increase the base of our search by reviewing records of all patients having chest CT scans for interstitial lung disease at VGH. Many of the patients with sarcoidosis detected had very minimal or no lung involvement, or the disease had cleared after
corticosteroid therapy, while many of the patients with IPF had advanced disease and were not candidates for the study. A few IPF patients were reluctant to take part in the study. Of the patients considered suitable for the study, no selection bias seemed apparent because the lung function characteristics of the patients who refused to participate were not different from the patients who were recruited. Matching the ILD subjects with suitable controls also proved difficult in the sixty to seventy age range, because we had difficulty enrolling subjects in this age group who did not have cardiovascular or respiratory problems.

\[ D_{L,CO_{3eq}} \] INCREASES WITH EXERCISE

As expected with increasing levels of steady state exercise, \[ D_{L,CO_{3eq}} \] increased in all subjects to accommodate the greater \( O_2 \) needs of the exercising muscles. Potts et al. (10) using the same \[ D_{L,CO_{3eq}} \] equipment, measured \[ D_{L,CO_{3eq}} \] in 11 normal, healthy subjects at levels corresponding to 25%, 50%, 75%, and 90% of their respective maximal power output. They reported an increase in % predicted \[ D_{L,CO_{3eq}} \] from 129+SE 3% at rest to 187+SE 5% at 75% of their peak power output; his subjects were generally fit and young. In our normal subjects, % predicted \[ D_{L,CO_{3eq}} \] increased from resting values of 118+SE 5% to 158+SE 7% at the 70% workload. The subjects in the Potts study, however, had a higher % predicted \[ D_{L,CO_{3eq}} \] (129+SE 3%) at rest than our control subjects (118+SE 5%) which can be attributed to differences in mean age between the two groups (mean age in Potts study was 29+SE 2 years, while our control subjects were 46+SE 7 years old).

The sarcoidosis patients increased their \[ D_{L,CO_{3eq}} \] similar to our control subjects, allowing for adequate oxygenation of arterial blood, probably due to increases in both pulmonary blood
capillary volume and alveolar capillary membrane surface area available for gas exchange.

O₂ saturation in the sarcoidosis patients decreased minimally from rest to exercise (mean decrease, 0.9±SE 0.6%) demonstrating the absence of any significant diffusion limitation. Eklund et al. (22) in stage II and stage III sarcoidosis patients reported mild resting hypoxemia (PₐO₂ of 73±SD 10 mmHg) and a widened A-a O₂ gradient (31±SD 10 mmHg) at rest, which worsened during progressive exercise (PₐO₂ 68±SD 15 mmHg, A-a O₂ gradient 31±SD 10 mmHg). Using Vₐ/Q distributions obtained from MIGET at rest and during exercise, the authors calculated a 30% and 50% diffusion limitation at rest and during exercise, respectively. The sarcoidosis subjects in the Eklund study, however, had a substantial degree of airflow obstruction (mean FEV₁ 59.2±SD 23% of predicted) which would have caused greater disturbances in Vₐ/Q distributions and gas exchange. Since PₐO₂ was measured in only 2 of our sarcoidosis patients, the sarcoidosis patients in our study may have had a low resting PₐO₂ and developed a widened A-a O₂ gradient during exercise, which the oximeter measurements would not have detected because of the flat upper portion of the oxyhemoglobin dissociation curve.

DIFFUSION LIMITATION IN IPF PATIENTS

In the IPF patients (n=7) the increase in DₐCO₃EQ with increasing VO₂ was significantly lower than in the sarcoidosis patients (n=8) or controls (n=6), suggesting that diffusion limitation may occur in IPF during exercise. The IPF patient’s O₂ saturation decreased markedly (mean decrease, 8.4 ±SE 1.7%); they experienced severe hypoxemia (PₐO₂ was 52±SE 2 mmHg, n=4) and a widened A-a O₂ gradient (63±SE 3 mmHg, n=4) during exercise. Two mechanisms may have been responsible for the lower DₐCO₃EQ increases during exercise in the IPF patients,
namely alveolar-capillary membrane thickening and inadequate increase in the pulmonary capillary blood volume. Membrane thickening would increase the diffusion distance of O\(_2\) from alveolar gas to pulmonary capillary blood, while failure of pulmonary capillary volume to increase sufficiently with increased cardiac output would decrease the mean transit time of erythrocytes in the pulmonary capillaries. Both of these factors would lead to a diffusion limitation during exercise. To provide insight in the degree of contribution of a diffusion limitation to the A-a O\(_2\) gradient during exercise in our IPF patients, we partitioned the observed A-a O\(_2\) gradient in our patients into diffusion and V\(_A\)/Q components, using a simple lung model with venous admixture (shunt-like effect) due to non-uniform V\(_A\)/Q.

The theoretical analysis was based on the assumptions that 81% of the A-a O\(_2\) gradient at rest was caused by V\(_A\)/Q mismatch and 19% due to diffusion limitation, and that the venous admixture % of cardiac output was the same during exercise and rest. These two assumptions were based on the findings of Agusti et al. using MIGET (24). We also assumed that the difference between arterial and mixed venous blood O\(_2\) saturation was 22.5% at rest. The equilibrated end-capillary P\(_{O_2}\) (P\(_C\)O\(_2\)) assuming no shunt or diffusion limitation was considered equal to P\(_A\)O\(_2\). The P\(_C\)O\(_2\) and P\(_a\)O\(_2\) were converted to O\(_2\) saturation, using a standard oxyhemoglobin dissociation curve (40). For 2 of the IPF patients for which P\(_a\)O\(_2\) was not measured, O\(_2\) saturation was equivalent to the pulse oximeter readings. We calculated the expected arterial P\(_{O_2}\) (P\(_a\)O\(_2\)) and the expected arterial saturation (S\(_a\)O\(_2\)) by adding the 19% A-a O\(_2\) gradient due to diffusion to the arterial P\(_{O_2}\). At rest, V\(_A\)/Q mismatch was represented as a venous admixture acting as a shunt by passing an alveolar compartment with the remaining blood flow. The shunt fraction at rest was calculated using the shunt equation:
\[
\frac{Q_S}{Q_T} = \frac{S_{CO_2} - S_{eaO_2}}{S_{CO_2} - S_{VO_2}} \quad [5]
\]

where \(S_{CO_2}\) is the assumed end-capillary \(O_2\) saturation from the alveolar \(PO_2\), \(S_{eaO_2}\) is the expected arterial \(O_2\) saturation assuming no diffusion limitation, \(S_{VO_2}\) is \(O_2\) saturation of the mixed venous return \((S_{eaO_2} - 22.5)\), \(Q_S\) is the shunt flow, and \(Q_T\) is the cardiac output. The calculated shunt fraction \((Q_S/Q_T)\) is shown in Table 3.

The shunt fraction \((Q_S/Q_T)\) was assumed to remain unchanged from rest to steady state exercise, while the \(O_2\) saturation difference between mixed venous and arterial blood during exercise was estimated to be 35%. From the ratio of venous admixture \((Q_S)\) to total cardiac output \((Q_T)\), the expected arterial \(O_2\) saturation assuming no diffusion limitation was calculated using the shunt equation [5]. The expected arterial \(P_{eaO_2}\) was calculated from the \(S_{eaO_2}\) (Table 3). The expected \(P_{eaO_2}\), which assumed no diffusion limitation, was compared to the measured arterial \(PO_2\) \((PaO_2)\) and the difference between the two was attributed to a diffusion limitation (Table 3). The diffusion and shunt components of the A-a \(PO_2\) difference are represented as follows:

\[
(PA_2 - PaO_2) = (PA_2 - P_{eaO_2}) + (P_{eaO_2} - PaO_2) \quad [6]
\]

where \(PA_2\) is the alveolar \(O_2\), \(PaO_2\) is the arterial \(PO_2\), and \(P_{eaO_2}\) is the expected arterial \(PO_2\) with no diffusion limitation. During exercise in IPF patients, a diffusion limitation contributed to Table 3.
**TABLE 3: Analysis of the contribution of diffusion limitation to A-a O\textsubscript{2} gradient during steady state exercise**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Resting</th>
<th>Steady state exercise (level 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(P_{A}O_2) ((S_cO_2)) mmHg (%)</td>
<td>(P_{A}O_2) ((S_cO_2)) mmHg (%)</td>
</tr>
<tr>
<td>---------</td>
<td>---------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>F1</td>
<td>105 (97.7) 82 (96.1) 73.6 86 (96.5) 0.050</td>
<td>112 (98.1) 53* (87) 52 96 79 26</td>
</tr>
<tr>
<td>F2</td>
<td>105 (97.7) 82 (96.1) 73.6 86 (96.5) 0.050</td>
<td>112 (98.1) 63* (92) 57 96 83 20</td>
</tr>
<tr>
<td>F3</td>
<td>113 (98.2) 59 (90.0) 67.5 69 (93.9) 0.140</td>
<td>108 (97.9) 49 (84) 49 91 60 11</td>
</tr>
<tr>
<td>F5</td>
<td>103 (97.6) 79 (95.8) 73.3 84 (96.3) 0.054</td>
<td>114 (98.1) 58 (90) 55 96 79 21</td>
</tr>
<tr>
<td>F6</td>
<td>109 (97.9) 69 (93.0) 71.4 76 (95.2) 0.102</td>
<td>118 (98.2) 51 (85) 50 93 66 16</td>
</tr>
<tr>
<td>F7</td>
<td>109 (97.9) 91 (96.9) 74.4 94 (97.2) 0.030</td>
<td>119 (98.2) 51 (85) 50 97 92 41</td>
</tr>
</tbody>
</table>

*Abbreviations used: \(P_{A}O_2\)=alveolar O\(_2\) expressed as partial pressure, \(S_cO_2\)=capillary O\(_2\) saturation equilibrated with \(P_{A}O_2\), \(P_{a}O_2\)=arterial O\(_2\) expressed as partial pressure, \(S_aO_2\)=arterial O\(_2\) expressed as O\(_2\) saturation, \(S_vO_2\)=O\(_2\) saturation of mixed venous return, \(Q_s/Q_t\)=shunt fraction, \(S_{ea}O_2\)=expected arterial O\(_2\) saturation assuming no diffusion limitation, \(P_{ea}O_2\)=predicted arterial PO\(_2\) assuming no diffusion limitation, and A-a O\(_2\)=alveolar-arterial O\(_2\) gradient.

*\(P_{a}O_2\) not measured; estimated from oximeter readings
37±SE 6% (range 19-60%) of the A-a PO\textsubscript{2} gradient. Such a wide range could be explained by different stages of fibrosis and pulmonary vascular damage in each of the subjects.

The results from our analysis are consistent with those of Agusti et al. (24), who using MIGET and a multi-compartmental V\textsubscript{A}/Q model, found a 21 mmHg discrepancy between predicted P\textsubscript{a}O\textsubscript{2} (80±SE 4 mmHg) and measured P\textsubscript{a}O\textsubscript{2} (59±SE 5 mmHg) in IPF patients during exercise suggesting that 40±SE 5% of the A-a PO\textsubscript{2} gradient was due to diffusion limitation. From oximeter measurements, Hempleman and Hughes (26) estimated P\textsubscript{a}O\textsubscript{2} (54±SE 4 mmHg) and A-a O\textsubscript{2} gradient (67±SE 5 mmHg) during exercise in 5 IPF patients. Based on expected arterial PO\textsubscript{2} during exercise and a similar shunt/alveolar model, diffusion limitation accounted for 36%±SE 8% of the A-a O\textsubscript{2} gradient during exercise. Oximeter readings have an error of ±2% (41) and their calculations based on P\textsubscript{a}O\textsubscript{2} determined indirectly from pulse oximetry may not be precise. Another important consideration is that our analysis evaluated the overall contribution to diffusion limitation assuming a simple lung model with uniform distribution of diffusion to perfusion, and did not account for the distribution of diffusing properties throughout different areas of the lungs.

A recent study by Yamaguchi et al. (42) compared the D/Q and V\textsubscript{A}/Q distributions in IPF patients at rest using O\textsubscript{2}, CO\textsubscript{2}, and CO with six inert gases as indicator gases. Based on HRCT findings, patients were categorised into those with predominantly inflammatory alveolitis/acinitis with little fibrosis (Group I), those with predominantly fibrosis with little alveolitis/acinitis (Group II), and those who had both fibrosis and alveolitis/acinitis (Group III). Using MIGET analysis the measured and predicted A-a O\textsubscript{2} indicated that 34%, 15%, and 21% of the A-a O\textsubscript{2} gradients in Groups I, II, and III, respectively, were caused by a diffusion limitation. Analysis of
the distributions of D/Q and V_{A}/Q in Group I demonstrated the existence of only modest V_{A}/Q abnormalities with primarily low D/Q areas. Arterial hypoxemia and the perfusion of low V_{A}/Q areas were not different in Groups II and III, but the perfusion of regions with low D/Q was significantly greater in Group III. Arterial hypoxemia and a widened A-a O_{2} gradient was less in Group I when compared to Groups II and III, indicating that impairment in O_{2} exchange was more sensitive to V_{A}/Q mismatch rather than diffusion limitation. The study concluded that active alveolitis and acinitis were important in producing regions with low D/Q regions, whereas fibrotic alterations caused both regions of low D/Q and V_{A}/Q. Fibrosis has also been shown by Agusti et al. (24) to impair the vasoconstrictive mechanisms of the pulmonary vasculature leading to perfusion of areas with poor ventilation giving rise to areas of low V_{A}/Q.

In our study, the number of subjects is too small to make a meaningful relationship between CT findings and gas exchange at rest and during exercise in ILD. A study of a larger number of patients may allow for correlation of CT findings and the impairment in diffusing capacity at rest and during exercise.
CHAPTER FIVE: CONCLUSION

The main findings of the study are summarized below.

1. The three-equation method can be used to measure \(D_L\)CO during exercise in patients with diffuse interstitial lung disease and limited exercise tolerance.

2. \(D_L\)CO\(_{3EQ}\) increases with increasing levels of steady state exercise in sarcoidosis patients and normal subjects, but does not substantially increase in IPF patients.

3. The failure of \(D_L\)CO\(_{3EQ}\) to increase in IPF patients results in exercise induced hypoxemia suggesting that a substantial diffusion limitation exists.

4. Theoretical analysis using a simple shunt/alveolar lung model indicates that 37% of the A-a \(O_2\) gradient during steady state exercise in our IPF patients may be attributed to a limitation in diffusion.
WORKS CITED


ABBREVIATIONS

A-a O₂  alveolar arterial oxygen gradient
ATS    American Thoracic Society
COHb   arterial carboxyhemoglobin
Dₗ    lung diffusing capacity
DₗCO   CO pulmonary diffusing capacity
DₗCOₑ₃EQ three-equation CO lung diffusing capacity
DₗCOᵦₑ₅B DₗCO determined by rebreathing technique
DₗCOₛᵦ single breath CO pulmonary diffusing capacity
FEF₂₅₋₇₅ forced expiratory flow from 25% to 75% of expiration
FEV₁    forced expiratory volume in 1 second
FRC    functional residual capacity
FVC    forced vital capacity
HRCT   high resolution computed tomography scan
IC     inspiratory capacity
ILD    interstitial lung disease
IPF    idiopathic pulmonary fibrosis
MIGET  multiple inert gas elimination technique
PₐO₂   arterial PO₂
PₑO₂   equilibrated end-capillary PO₂
PₑₑO₂  expected arterial PO₂ for the assumed Vₐ/Q mismatch without diffusion limitation
PEFR  peak expiratory flow rate
PO₂  partial pressure of oxygen
Q    blood flow
Qₛ   shunt-flow
Qₜ   total cardiac output
Qₛ/Qₜ shunt expressed as fraction of cardiac output
RER  respiratory exchange ratio
SₑaO₂ expected arterial O₂ saturation for the assumed Vₐ/Q mismatch without diffusion limitation
SVC  slow vital capacity
TLC  total lung capacity
Vₐ   alveolar volume
Vₐ/Q ratio of ventilation to perfusion
VO₂ₘₐₓ maximum oxygen consumption
APPENDIX I: THREE-EQUATION ALGORITHM

The three-equation algorithm uses separate equations to describe CO uptake, i.e. $D_{LCO_{3EQ}}$, through each phase of the single breath breathing maneuver. The three equations are

for inhalation,
\[
\frac{d[V_A(t) \times F_{A CO}(t) \times F_{A CO}(t)]}{dt} = -D_{L CO}(t) \times (P_B - 47) \times F_{A CO}(t) + F_{i CO} \times dV_A(t)/dt \quad [7]
\]

for breath holding (the Krogh equation)
\[
V_A(bh) \times dF_{A CO}(t)/dt = -D_{L CO} \times (P_B - 47) \times F_{A CO}(t) \quad [8]
\]

and for exhalation
\[
V_A(t) \times dF_{A CO}(t)/dt = -D_{L CO} \times (P_B - 47) \times F_{A CO}(t) \quad [9]
\]

where $V_A(t)$ is the alveolar volume, $F_{A CO}(t)$ is the fractional alveolar concentration of CO at time $t$, $F_{i CO}$ is the fractional initial concentration of CO in the inhaled test gas, $P_B$ is the barometric pressure, and $V_A(bh)$ is the alveolar volume during breath holding (7).
APPENDIX II: UBC ETHICS APPROVAL
APPENDIX III: RECRUITMENT LETTER TO SUBJECTS
December 31, 1997

XXX
Patient Address

Dear Mr. XXX:

**RE:** Request for your participation in an exercise study to determine how oxygen diffuses in the lung

We are doing a study evaluating carbon monoxide diffusing capacity during exercise in patients with diffuse lung disease. We wish to request your participation in the study as a subject. The measurement of carbon monoxide diffusing capacity, is a standard test of lung function, which determines how gases diffuse in the lung from the air into the blood. You already have had this test as part of your detailed breathing tests. The concentration of carbon monoxide is very low and should not cause any side effects. The test will be done while you are resting and then while you are exercising on a bicycle at two different work loads, equivalent to 35% and 70% of your maximal exercise capacity. On a separate day, prior to the determination of carbon monoxide diffusion during exercise, you will have a progressive exercise test on the bicycle, to determine your maximum exercise capacity. This type of exercise testing is safe and is done regularly in evaluation of patients with breathing problems. Your heart rate, electrocardiogram, oxygen saturation, gas exchange, and ventilation, will be continuously monitored during the test.

The carbon monoxide diffusing capacity done during exercise uses a special analyzer with a rapid response, so that you do not need to hold your breath for more than 1-2 seconds after taking a deep inspiration. During exercise, you will be asked to take a deep breath, hold it for 1-2 seconds and then breathe out at controlled rate following the pattern shown on a video screen.

We have already checked with your specialist, Dr. XYZ, and have obtained his approval for your participation. We will also inform your family doctor of the study. If you are willing to participate, we will provide you with further details and a copy of the consent form. My research assistant, Sundeep Rai, will be calling you to answer any questions and to set up a convenient appointment time.

Thank you for your cooperation.

Sincerely yours,

R. T. Abboud, MD, FRCP(C)
APPENDIX IV: LETTER TO GENERAL PHYSICIANS
January 1, 1997

Dear Dr. XXX:

RE: Your patient:

DOB:

We would like your permission to enrol your patient in a study evaluating carbon monoxide diffusing capacity during exercise as a factor leading to exercise limitation and oxygen desaturation in patients with interstitial disease. Patients with sarcoidosis, will be compared with patients with interstitial pulmonary fibrosis, and compared to a control group of patients referred to the Lung Function Laboratory for evaluation of lung function, who have normal lung function tests. Your patient has sarcoidosis/idiopathic pulmonary fibrosis/or is being tested as a control subject with normal lung function.

In the study, patients will have a progressive exercise test on a cycle ergometer, to determine their maximum exercise capacity, and the degree of oxygen desaturation, if any, that occurs with exercise. On another day, the patients will come for evaluation of diffusing capacity during exercise. The diffusing capacity will be done using the three equation technique, which utilizes continuous monitoring of exhaled carbon monoxide and methane as an inert tracer gas, and calculates diffusing capacity, taking into account CO uptake during inspiration, breath holding and exhalation. This technique allows measurement of DLCO during exercise, without the requirement of a 8-10 seconds of breath holding, which is needed for the standard DLCO test. The patients will have measurement of diffusing capacity at rest, and during semi-steady state exercise, at 35% and 70% of their maximum exercise capacity as determined previously. The exercise will be done with the patient seated on a cycle ergometer, with continuous monitoring of ventilation, gas exchange, 0₂ saturation, and electrocardiogram. Exercise testing is very safe and is used regularly in the evaluation of patients with interstitial disease. In the present study, the additional requirement is to determine DlCO during exercise, and this should not pose any additional discomfort or risk to the patient. If the patient develops significant hypoxemia (0₂ saturation less than 85%) or the heart rate increases to 90% of the patient's predicted maximum, the exercise will be stopped.
APPENDIX V: CONSENT FORM
INFORMED CONSENT FORM

TITLE OF PROJECT: LUNG DIFFUSION AS A LIMITATION TO EXERCISE IN SARCOIDOSIS AND IDIOPATHIC PULMONARY FIBROSIS (IPF)

PRINCIPAL INVESTIGATOR: DR. R.T. ABBoud, MD, FRCP(C)
RESPIRATORY SPECIALIST, DEPARTMENT OF MEDICINE, UBC AT VGH, AND MEDICAL DIRECTOR, LUNG FUNCTION LABORATORY, VGH

OFFICE PHONE: 875-4122  LUNG FUNCTION PHONE: 875-4830

General purpose
The purpose of the study is to evaluate how gases diffuse from the lung into the blood during exercise in diffuse lung disease (sarcoidosis and idiopathic pulmonary fibrosis), because gas diffusion may play a significant role in causing a decrease in oxygen levels in the blood during exercise. Generally, the capacity to exercise is limited in diffuse lung disease patients because not enough oxygen can cross the lung membrane leading to a decrease in blood oxygen levels. The standard pulmonary function test used to measure the capacity of gases to diffuse in the lung involves the patient breathing in a gas mixture containing a tiny amount of carbon monoxide (0.3%), holding the breath for 10 seconds, and then giving a sample of exhaled gas. This allows calculation of the ease with which carbon monoxide diffuses from the air into the blood in the lung. Carbon monoxide is used because it is easy to measure, and because its diffusion is very similar to oxygen. The concentration of carbon monoxide is small and will not cause any side effect or problem.

Detailed procedure
It is planned to measure the diffusing capacity at rest, and at two levels of exercise, equivalent to 35% and 70% of my maximum exercise capacity on an electronic exercise bicycle. Each level of exercise will be maintained for about 2½ min to allow heart rate and lung function to be stable prior to measuring the diffusing capacity. The diffusing capacity will be determined with a modified technique, which does not require breath holding, and in which the carbon monoxide concentration is being continuously analyzed at the mouth along with an inert tracer gas. During this measurement, I will be asked to take a deep breath in, hold my breath for 1 or 2 seconds and then breathe out following the pattern shown on a video monitor.
On a separate day, prior to the actual measurement of diffusing capacity during exercise, my maximum exercise capacity will be determined on the exercise bicycle. I will start exercising at a low work load and the work load will be increased by a small increment every minute, until I reach my maximum capacity. My electrocardiogram, heart rate, oxygen saturation, ventilation, and gas exchange will be monitored continuously during all exercise testing. The exercise will be stopped if I feel unduly short of breath, or if my oxygen saturation drops significantly (below 85%), or if my heart rate increases to 90% of the predicted maximum exercise heart rate for my age.

**Exclusion from the study**

I am aware that if I have a history of heart disease, or an abnormal electrocardiogram, I will be excluded from the study. I will also excluded from the study if my breathing capacity is severely reduced, or if my oxygen saturation at rest is below 90%, or if my tests show that I have significant narrowing in my bronchial tubes (airflow obstruction).

**Reason for the study**

I have been requested to participate, in order to get a better understanding of the mechanisms limiting exercise in patients with sarcoidosis and idiopathic pulmonary fibrosis, and whether limitation of diffusion is a major factor contributing to a decrease in oxygen saturation during exercise in these diseases. If I am a control subject, I will be invited to participate in the study to determine how diffusing capacity increases during exercise in subjects of similar age and sex as the patients with lung disease.

**Confidentiality**

I have been assured that my identity will be kept confidential in the analysis and presentation of the results. My name will not be shown in the analysis or presentation of the data, but I will be assigned a confidential code number to indicate my data. The research records will be kept as part of the confidential records of the Lung Function Lab at VGH.

**Possible side effects**

I am aware that I may feel short of breath during the exercise test and that my oxygen saturation may decrease if I have diffuse lung disease. However the feeling of shortness of breath should be quickly relieved after stopping the exercise, and if my oxygen saturation decreases significantly (below 85%), I will be given oxygen for a few minutes to correct the decreased oxygen saturation and relieve the shortness of breath more quickly. My heart rate will go up with the exercise as would be expected, but this should recover promptly with cessation of exercise. Subjects with heart disease and abnormal electrocardiograms will be excluded from the study, so it is very unlikely that I would have cardiac side effects from the study. I am aware that the study will require me to spend a total of about 2-3 hours of additional time; about 1 hour will be required for the measurement of maximal exercise capacity which will be done on a separate day, and about 1-2 hours for the measurement of
diffusing capacity at rest and during the two levels of exercise. I am aware that my participation is purely voluntary, and there is no monetary compensation provided.

**Blood sampling for patients with diffuse interstitial lung disease**
If I am a patient with diffuse interstitial lung disease (sarcoidosis or idiopathic pulmonary fibrosis), I am aware that I will have an arterial sample of blood taken by arterial puncture twice, prior to exercise and during the higher level of exercise, to determine the actual oxygen level in the blood. The arterial puncture will be done from the artery at the wrist (radial artery) using a fine needle (gauge 23) and a small syringe. The amount of blood drawn each time will be about 1.5mL (about ⅓ of a teaspoon). Arterial puncture is commonly used to evaluate blood oxygenation in patients with lung disease. It may be slightly painful and may be followed by slight bruising or slight local swelling; these will resolve in a few days. To minimize pain with the needle puncture, a local anaesthetic (1% xylocaine) will be used.

**Rights as a research subject**
I am aware that the investigator is willing to answer any inquiries concerning the procedures to ensure that I fully understand them, or to answer any questions or concerns that I may have. I am also aware that if I have any concerns about my treatment or rights as a research subject, I may telephone Dr. R.D. Spratley, Director, Office of Research Services at UBC, at 822-8598. I am aware that I may refuse to enter into the study, and that I may withdraw from the research at any time, without any consequences to my continuing medical care.

**Consent**
I acknowledge receiving a copy of the consent form. By signing this form, I consent to participate, in this study and the study procedures as outlined above.

____________________________  ________________________________
Signature of Subject            Signature of Witness

____________________________  ________________________________
Signature of principal investigator  Date
APPENDIX VI: CLINICAL QUESTIONNAIRE

Clinical Questionnaire for DLCO Exercise Study

Date: __________

Name: ____________________________

Date of Birth (mm/dd/yy): _________ Weight (kg): ___ Height (cm): ___

Date last seen by physician: _______ Date of last CT: _______ CT#_____

Date of last PFT: ___________ PFT# _______

Clinical Symptoms:

A) Respiratory

1) Cough? N Y ____________________________

2) Phlegm? N Y ____________________________

3) Wheezing? N Y ____________________________

4) SOB? N Y ____________________________

5) Asthma? N Y ____________________________

6) Exercise Capacity ____________________________

B) History of heart disease or high blood pressure? N Y

If yes, please give details ____________________________________________

__________________________________________________________

__________________________________________________________

__________________________________________________________

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**Smoking History:**

Are you a current smoker?  N  Y  Age started ________

Have you ever smoked regularly?  N  Y  Age stopped ________

Number of years you smoked? ________  Cigarettes per day ________

**Medications and Allergies:**

_________________________________________________________________

_________________________________________________________________

_________________________________________________________________

_________________________________________________________________

_________________________________________________________________

**On Examination:**

BP: ______  Heart Rate: ______  Respiratory Rate: ______

A) Respiratory System:

B) Cardiovascular System:

C) Clubbing  N  Y

D) Edema  N  Y

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APPENDIX VII: EXERCISE EQUIPMENT CALIBRATION

Calibration procedures were performed daily and reverified before every exercise test. The barometric pressure, temperature, and percent relative humidity were entered into the customized acquisition software (PK Morgan, Chatham, Kent, UK). The temperature of the furnace of the \(O_2\) analyzer was checked to ensure stability. The \(O_2\) and \(CO_2\) gas analyzers were calibrated using a two point procedure. While aspirating dry atmospheric air at 3 L/min, the oxygen analyzer was zeroed to read 20.93±0.03% and the \(CO_2\) analyzer was adjusted to 0±0.03%. Both analyzers were calibrated against a sample of tank gas (PRAXAIR Canada Inc., Mississauga, ON) containing known concentrations of \(O_2\) (14.96%) and \(CO_2\) (5.03%). The absolute accuracy was ±0.02% for each gas component concentration. The response time of the analyzers including lag time from the point of sampling from the mixing chamber was measured by sampling room air and then switching to the calibration gas. A response time of less than 10 s was considered adequate for the gas analyzers.

The turbine ventilometer was attached on the inspiratory side of a two way Hans Rudolph valve and the transducer was internally calibrated by pumping a 1 L syringe four times. Appropriate adjustments to the calibration factor were made manually. The analog output from the ventilometer monitor was then verified by pumping the 1 L syringe ten times. The LED display on the cycle ergometer was zeroed and a 200 W LED reading was verified to produce a 2.00±0.05 volt full scale deflection. Similarly, an EKG calibration heart rate of 60 beat/min was confirmed to generate a 1.16±0.02 volt analog output. A finger or an ear probe was used to calibrate the oximeter in a range of 90-99%. All calibrations points were verified and stored in the exercise testing program.
APPENDIX VIII: DETERMINATION OF ANALYZER LAG AND RESPONSE TIMES

The lag time of a gas analyzer is the transport time of aspirated gas through the tubing to the sample chamber, whereas the response time is the time the analyzer takes to register 90% of the maximal response signal. The lag and response times were estimated by rapidly switching the gas being sampled from zero to full scale CO and CH$_4$ while the change in flow was measured simultaneously. The response times for the CO and CH$_4$ analyzers were confirmed to be under 250 ms. The +2 cmH$_2$O pressure flow transducer was replaced with a +225 cmH$_2$O differential pressure transducer (Model “MP45-14-871”; Validyne, Northridge, CA) to indicate sudden deflections in pressure. The zero and span settings on the carrier demodulator were adjusted to keep the signal inputs to the computer to the same range used for the low pressure transducer. A short sample tubing with a pressure release valve was used to connect the D$_{L}$CO$_{3EQ}$ gas mixture flowing at 12-15 Litres/min to the positive end of the pressure transducer. A three-way stopcock, placed just before the pressure transducer, directed the flow of test gas either towards the positive end of the transducer, or to a cut-off syringe where the gas analyzers sampled continuously. The test gas flow was rapidly switched from the pressure transducer to the cut-off syringe allowing the analyzers to sample from zero to full scale CO and CH$_4$ concentrations, while suddenly releasing the pressure on the transducer. The response curves for the CO and CH$_4$ analyzers, and the pressure signal were displayed on a computer screen with moveable cursors which indicated the start and end points of the respective pressure, CO, and CH$_4$ signals. The lag time of each analyzer was determined from the time interval between the onset of the pressure signal to the onset of the analyzer signal using the customized D$_{L}$CO$_{3EQ}$ software program (designed by Dr. 88
Brian Graham, University of Saskatchewan, Saskatoon). The 0-90% response time for each analyzer was determined by the software from the onset of the signal to 90% of the maximal deflection. The software simply added the lag and response time shift for each analyzer to adjust its timing with the flow and volume signals in the $D_L CO_{3eq}$ calculations.