DOI: 10.1113/EP090176

Lower transfer factor of the lung for carbon monoxide in women with a patent foramen ovale

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Running Title: Lower TL_{CO} in women with PFO

Competing Interests: The authors have no conflicts of interest.

Word Count: 3,927 (manuscript body only)

Keywords: intracardiac shunt, diffusion, diffusing capacity, alveolar volume

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NEW FINDINGS (94/100 words)

- **What is the central question of this study?**

Whether or not individuals with a patent foramen ovale (PFO+) have a lesser lung transfer factor for carbon monoxide than those without (PFO-).

- **What is the main finding and its importance?**

We found a lesser rate constant for carbon monoxide uptake in PFO+ women compared to PFO- women, which was physiologically relevant (≥0.5 Z-score difference), but not for PFO+ vs. PFO- men. This suggests factors independent of the patent foramen ovale are responsible for our findings, possibly differences inherent structural differences in the lung.

ABSTRACT (250/250 words):

Transfer factor of the lung for carbon monoxide measure assumes all cardiac output flows through the pulmonary circuit. However, right-to-left blood flow through a shunt can result in a lower transfer factor than predicted. A patent foramen ovale (PFO) is a potential source of right-to-left shunt present in ~35% of the population, but the effect of PFO on transfer factor of the lung for carbon monoxide is unknown. We sought to determine the effect of PFO on the transfer factor for carbon monoxide. We conducted a retrospective analysis of transfer factor for the lung for carbon monoxide data from 239 (101 women) participants. Anthropometrics, lung function including spirometry, plethysmography, and transfer factor of the lung for carbon monoxide were compiled from our previously published work. Women, but not men, with a PFO had a significantly lesser transfer factor of the lung for carbon monoxide and rate constant for carbon monoxide uptake (% predicted and Z-score) than women without a PFO. Women and men with a PFO had normal alveolar volumes that did not differ from those without a PFO. Correcting the data for hemoglobin in a subset of subjects did not change the results *(n* = 58; 25 women). The lesser rate constant for carbon monoxide uptake in women with compared to without a PFO was physiologically relevant (≥0.5 Z-score difference). There was no

effect of PFO in men. This suggests factors independent of the PFO are responsible for our findings, possibly inherent structural differences in the lung.

INTRODUCTION

According to Fick's law for diffusion, the rate and volume of movement of a gas, i.e., O_2, across the alveolar-capillary membrane is dependent upon the surface area for diffusion, diffusivity of the gas, the pressure gradient across the alveolar-capillary membrane, and the thickness of the alveolar-capillary membrane (Borland & Hughes, 2019). Quantifying the diffusing capacity or transfer factor of the lungs for carbon monoxide (DL_{CO} and TL_{CO}, respectively), when corrected for differences in hemoglobin concentration, is an important clinical measure as the components of this measure [V_A (alveolar volume) and K_{CO} (rate constant for carbon monoxide uptake)] provide important information on any underlying lung pathophysiology present (Hughes & Pride, 2012).

Measurement of DL_{CO}/TL_{CO} uses a small amount of alveolar CO, which then diffuses into the pulmonary capillary blood and binds to hemoglobin (Hb). Thus, one assumption of this test is that all of the cardiac output flows through the pulmonary microcirculation (Borland & Hughes, 2019). However, if there is a right-to-left shunt, then some portion of the blood will not be exposed to alveolar CO and the magnitude of CO diffused across the alveolar-capillary membrane would be less. In support of this idea, previous work has demonstrated a lower TL_{CO} in patients with pulmonary arteriovenous malformations (PAVMs), which increased towards normal values after the PAVMs were embolized (Dutton *et al.*, 1995; Whyte *et al.*, 1998; Rodríguez-Roisin & Krowka, 2008). PAVMs are most commonly associated with rare diseases such as hereditary hemorrhagic telangiectasia and hepatopulmonary syndrome, but other, more common, right-to-left shunts could also have an impact on the measured DL_{CO}/TL_{CO}.

A patent foramen ovale (PFO) is an intracardiac shunt present in ~30-40% of the general population (Hagen *et al.*, 1984; Marriott *et al.*, 2013; Elliott *et al.*, 2013). Previous work has demonstrated that individuals with a PFO compared to those without a PFO have a variety of physiologic differences including thermoregulation (Lovering *et al.*, 2011; Davis *et al.*, 2015, 2017) and ventilatory responsiveness (Davis *et al.*, 2019). Most relevant to the current investigation,

individuals with a PFO have impaired pulmonary gas exchange efficiency compared with their counterparts without a PFO (Lovering *et al.*, 2011; Fenster *et al.*, 2013; Elliott *et al.*, 2015; Duke *et al.*, 2020). Despite these findings, the effect of a PFO on TL_{CO} has not been previously investigated. Accordingly, the purpose of this retrospective study was to compare TL_{CO}, K_{CO}, and V_A between otherwise healthy young men and women with and without a PFO. Based upon our previous research, we hypothesized that individuals with a PFO will have a lower TL_{CO} and K_{CO} than those without a PFO.

METHODS

Ethical Approval and Participants

We performed a retrospective analysis of anthropometric, lung function (spirometry and plethysmography), and diffusing capacity of the lung for CO data from previously published studies conducted in our lab. Much of these data have been previously published (Laurie *et al.*, 2012, 2018; Lovering *et al.*, 2013, 2014; Norris *et al.*, 2014, 2016; Duke *et al.*, 2014, 2016, 2017*b*, 2017*a*, 2018, 2019, 2020; Elliott *et al.*, 2014, 2015; Davis *et al.*, 2015, 2017, 2019), but the comparison between individuals with and without a PFO on DL_{CO} and TL_{CO} have not been previously examined or published. All studies from which these data were derived received approval from the University of Oregon's Office for Protection of Human Subjects (approval numbers: 04282011.081, 02032011.021, 12152011.013, 09292015.023, 05162011.087, 12182010.051, 09072011.076, 09292014.024, 05012015.001, 01132011.023). All subjects were recruited as healthy individuals without any history of cardiovascular or respiratory disease and reported not being cigarette smokers at the time of testing. All participants provided oral and written informed consent and all experimental procedures were conducted in accordance with the Declaration of Helsinki except for registration in a database. In total, data from 239 participants were analyzed and included in this study. A total of 103 subjects (46 female) had a PFO and the remaining individuals, n = 136 (55 female), did not have a PFO. The overall prevalence of PFO in our data set was 43% which is slightly greater than the 25-35% of the population because not everyone who participates in our studies performs all measures, i.e., some subjects without a PFO may have been screened out before performing the TL_{CO} measure.

Echocardiographic Screening

In all studies, an initial echocardiographic (Philips Sonos 7500, The Netherlands or Philips iE33, The Netherlands) screening and bubble study was performed on all participants at the time of initial study as previously described (Lovering & Goodman, 2012). Briefly, participants were connected to a 3-lead ECG and an intravenous catheter was placed in a peripheral vein for injection of saline contrast with and without a Valsalva maneuver to determine the absence or presence and grade of a PFO. The use of a Valsalva is done to facilitate the conditions for blood to flow across the PFO, i.e., right atrial pressure is greater than left atrial pressure, and does not "open" a PFO, per se. These conditions do occur naturally, i.e., at the end of inspiration during atrial diastole, but it is not possible to time an injection to occur at precisely this time. We considered a PFO to be present if bubbles appeared in the left ventricle ≤3 cardiac cycles following right heart opacification with saline contrast. We considered a PFO to be absent if no bubbles appeared in the left ventricle. Using our previously published 0-5 scoring system (Lovering & Goodman, 2012), we classified those with a PFO as having either a large shunt (scores 3-5) or a small shunt (scores 1-2), as we have done before (Davis *et al.*, 2015). Of the n = 103 individuals with a PFO, we were unable to identify PFO size in only one individual. There were n = 21 women with a small shunt (PFO+) and n = 24 women with a large shunt. There were n = 30 men with a small shunt and n = 27 with a large shunt. We compared the median bubble score between sexes in the PFO+ [2 (women) vs. 2 (men)] and PFO++ [(4 (women) vs. 3 (men)] groups and found no differences (p = 0.565 and p = 0.122, respectively).

Pulmonary Function and Lung Diffusion Capacity

Prior to the pulmonary function testing, mass and standing height were measured (Ohaus Corporation, ES200L, Pinebrook, NJ). Baseline pulmonary function testing was done at the time of initial study according to American Thoracic Society/European Respiratory Society (ATS/ERS) standards (Miller *et al.*, 2005; Wanger *et al.*, 2005; MacIntyre *et al.*, 2005) and included measures of forced vital capacity (FVC), slow vital capacity (SVC), forced expiratory volume in 1 s (FEV_1), and mid-expiratory flows. Lung volumes and capacities, i.e., residual volume, functional residual capacity, and total lung capacity, were determined using whole-body plethysmography (MedGraphics Elite DL, MedGraphics, St. Paul, MN). Predicted values for lung function values were calculated as described by the Global Lung Initiative (Quanjer *et al.*, 2012).

Lung diffusion capacity for carbon monoxide (DL_{CO}) was determined by the single-breath, breath-hold method (Knudson *et al.*, 1987) using the Meade (Jones & Meade, 1960) method for timing and alveolar sample collection. Measurements were made with a computerized spirometry system (MedGraphics Elite DL, MedGraphics, St. Paul, MN). Computations of the rate constant for CO uptake (K_{CO}), alveolar volume (V_A) and DL_{CO} were calculated, as before (Hughes & Pride, 2012): $K_{CO} = \log_e (CO_0/CO_t)/BHT$, where CO_0 and CO_t are the alveolar concentrations of CO at the beginning and end of the breath hold and BHT is the breath-holding time; $V_A = (V_I - V_D) \times (He_0/He_t)$ where V_I is inspired volume, V_D is anatomical dead space and He_0 and He_t are the alveolar concentrations of helium at the beginning and head of the breath hold; and $DL_{CO} = (V_A \times K_{CO})/(Pb - P_{H2O})$ where Pb is barometric pressure and P_{H2O} is water vapor pressure of alveolar gas at 37 degrees C. DL_{CO} values were converted to transfer factor of the lung for CO (TL_{CO}), and corrected for dead space, as before (Stanojevic *et al.*, 2017). Barometric pressure in Eugene, OR is ~755 mmHg, and all measures were made at this altitude. Previous work has suggested that correction of TL_{CO} for hemoglobin concentration is not necessary so this was not done for all subjects in the present study (Stanojevic *et al.*, 2017). Nevertheless, in a subset of participants (n = 58; 25 women), we have hemoglobin data and corrected TL_{CO} for hemoglobin concentration. We found no difference, within these participants, on TL_{CO} whether or not hemoglobin was corrected for, respectively (12.3 ± 5.3 *vs.* 12.5 ± 5.6 mmol/min/kPa; p = 0.10). Accordingly, we report only the uncorrected TL_{CO} data hereafter. Predicted values for TL_{CO}, K_{CO}, and V_A, as well as the lower-limit of normal values for each, were calculated as described by the Global Lung Initiative (Stanojevic *et al.*, 2017).

Statistical Analyses

Data were analyzed using GraphPad Prism v9.1.1. To compare variables of interest, i.e., anthropometrics, spirometry, TL_{CO}, etc., two-way (sex x PFO group) ANOVAs were computed. When appropriate, pairwise comparisons were computed using the Holm-Bonferroni posthoc test. This method is uniformly more powerful than the classic Bonferroni correction and adjusts the rejection criteria for each individual comparison. Specifically, t-tests are computed for all pairwise comparisons of interest and then the p-values are ranked by smallest to largest. Adjusted p-values of each comparison is computed via the formula: p = p-value x (# of comparisons – rank +1). Alpha was

set, *a priori*, to 0.05.

RESULTS

Anthropometrics and Spirometry

Anthropometric and spirometry data are displayed in **Table 1**. Overall, those with a PFO were younger ($p = 0.045$), weighed less ($p = 0.021$), and had a lesser body mass index ($p < 0.024$). There were no difference on height ($p = 0.376$), FVC [$p = 0.513$; $p = 0.955$ (percent of predicted)], FEV_1 [$p = 0.194$; $p = 0.755$ (percent of predicted)], FEV_1/FVC ratio [$p = 0.203$; $p = 0.730$ (percent of predicted)] ,or $FEF_{25-75\%}$ [$p = 0.175$; $p = 0.978$ (percent of predicted)] between those with and without a PFO. Women with a PFO weighed less ($p < 0.014$) and had a smaller body mass index ($p = 0.009$) than women without a PFO. Women without a PFO were shorter ($p < 0.0001$) and weighed less ($p < 0.0001$) than men without a PFO. Likewise, women without a PFO had a lesser FVC ($p < 0.0001$), FEV_1 ($p < 0.0001$), and $FEF_{25-75\%}$ ($p < 0.0001$) than men without a PFO. Women with a PFO were shorter ($p < 0.0001$), weighed less ($p < 0.0001$), and had a smaller body mass index ($p = 0.0003$) than men with a PFO. Likewise, women with a PFO had a lesser FVC ($p < 0.0001$), FEV_1 ($p < 0.0001$), and $FEF_{25-75\%}$ ($p = 0.0005$) than men with a PFO. When appropriately expressed as a percent of predicted, there were no sex differences on any spirometry parameter regardless of PFO absence (FVC: $p = 0.321$; FEV_1: $p = 0.435$; and $FEF_{25-75\%}$: $p = 0.997$) or presence (FVC: $p = 0.589$; FEV_1: $p = 0.731$; and $FEF_{25-75\%}$: $p = 0.833$).

TL_{CO}, K_{CO}, and V_A

Figure 1 displays TL_{CO} data between sexes and groups. In Panel A, women had smaller TL_{CO} values compared to men regardless of PFO group [$p < 0.0001$ (PFO-); $p < 0.0001$ (PFO+)], as expected. Panel B displays group and sex data as a function of PFO size, i.e., bubble score during screening. When compared in this manner, women with a large PFO had a significantly smaller TL_{CO} value than those without a PFO ($p = 0.048$). There was no difference on TL_{CO} between women with small and large PFO ($p = 0.992$). **Table 2** displays TL_{CO} data [absolute units, percent predicted, lower limit of normal (LLN), and Z-score] for individuals with and without a PFO by sex. Only Z-score for TL_{CO} differed significantly between PFO- and PFO+ ($p = 0.005$) when men and women were grouped

together within PFO statuses. Women had a lesser TL_{CO} [$p < 0.0001$ (percent predicted); $p < 0.0001$ (LLN); $p < 0.0001$ (Z-score)] compared with men regardless of PFO group (i.e., a main effect for sex), as expected. Women without a PFO achieved a significantly smaller percent of predicted ($p < 0.0001$), LLN ($p < 0.0001$), and Z-score ($p < 0.0001$) TL_{CO} compared to men without a PFO. Women with a PFO women achieved a significantly smaller percent of predicted ($p < 0.0001$), LLN ($p < 0.0001$), and Z-score ($p < 0.0001$) for TL_{CO} compared to men with a PFO. Women with a PFO achieved a significantly lesser percent predicted ($p = 0.0163$) and Z-score ($p = 0.004$) TL_{CO} compared to women without a PFO.

Figure 2 displays K_{CO} data between sexes and groups. In Panel A, women had smaller K_{CO} values compared to men regardless of PFO group [$p < 0.0001$ (PFO-); $p < 0.0001$ (PFO+)], as expected. Panel B displays group data as a function of PFO size, i.e., bubble score during screening. When compared in this manner, women with a large PFO had a significantly smaller K_{CO} value than those without a PFO ($p = 0.030$). There was no difference on K_{CO} between women with small and large PFO ($p = 0.985$). **Table 2** displays K_{CO} data [absolute units, percent predicted, lower limit of normal (LLN), and Z-score] for individuals with and without a PFO by sex. Only percent of predicted for K_{CO} differed significantly between PFO- and PFO+ ($p = 0.0235$) when men and women were group together within PFO statuses. Women had a lesser K_{CO} [$p < 0.0001$ (percent predicted); $p < 0.0001$ (LLN); $p < 0.0001$ (Z-score)] compared with men regardless of PFO group (i.e., a main effect for sex). Women without a PFO achieved a significantly small percent of predicted ($p = 0.003$), LLN ($p < 0.0001$), and Z-score ($p = 0.002$) K_{CO} compared to men without a PFO. Women with a PFO achieved a significantly smaller percent of predicted ($p < 0.0001$), LLN ($p < 0.0001$), and Z-score ($p < 0.0001$) K_{CO} compared to men with a PFO. Women with a PFO achieved a significantly lesser percent predicted ($p = 0.006$) and Z-score ($p = 0.003$) K_{CO} compared to women without a PFO.

Table 2 displays V_A data [absolute units, percent predicted, lower limit of normal (LLN), and Z-score] for individuals with and without a PFO by sex. V_A did not differ between PFO groups on absolute units ($p = 0.083$)), percent of predicted ($p = 0.193$), LLN ($p = 0.566$), and Z-score ($p = 0.174$). Women had a lesser V_A [$p < 0.0001$ (absolute units); $p = 0.009$ (percent predicted); $p < 0.0001$ (LLN); $p = 0.002$ (Z-score)] compared with men regardless of PFO group (i.e., a main effect for sex).

Women without a PFO had a significantly smaller V_A expressed as absolute units (p <0.0001), percent of predicted (p = 0.006), LLN (p < 0.0001), and Z-score (p = 0.002) compared to men without a PFO. Women with a PFO had a significantly smaller V_A expressed as absolute units (p < 0.0001) and LLN (p < 0.0001), but not percent of predicted (p = 0.935) or Z-score (p = 0.830), compared to men with a PFO. There were no differences on V_A between women with a PFO and women without a PFO [p = 0.995 (absolute units); p = 0.992 (percent of predicted); p = 0.789 (LLN); p = 0.991 (Z-score)].

DISCUSSION

The purpose of this retrospective analysis was to compare TL_{CO}, K_{CO}, and V_A between healthy men and women with and without a PFO. We found that TL_{CO} (Z-score) and K_{CO} (percent predicted) were significantly less in those with a PFO compared to those without a PFO when men and women were grouped together. Furthermore, when we consider sex as an independent variable, women had significantly lesser TL_{CO} (percent predicted and Z-score) and K_{CO} (absolute units, percent predicted, and Z-score). Likewise, when we sort participants into no, small, and large PFO we see a pattern emerge where women with a large PFO have smaller TL_{CO} (absolute units and Z-score) and K_{CO} (absolute units and Z-score) values than women without a PFO. Men, regardless of PFO presence or size, did not differ on TL_{CO}, K_{CO}, or V_A. Accordingly, there is a clear effect of sex and PFO presence on normalized data for TL_{CO} and K_{CO}.

The TL_{CO} is determined by both the V_A and K_{CO} and thus the TL_{CO} value is best interpreted when both the V_A and K_{CO} are known. For example, it is possible to have similar TL_{CO} values (% predicted) when the V_A is normal and the K_{CO} is low (e.g. pulmonary hypertension) and vice versa when the V_A is low and K_{CO} is normal (e.g., pneumonectomy) (Hughes & Pride, 2012). In the current study we found that women with a PFO had a lower K_{CO} in the presence of a normal V_A (>100% predicted). Thus, the lower TL_{CO} in women with a PFO must be attributed to a lower K_{CO} (Hughes & Pride, 2012). This is similar to what is expected in someone with right-to-left intrapulmonary shunt (e.g., PAVMs) and opposite that of someone with a left-to-right shunt (Hughes & Pride, 2012).

Larger PFOs appear to worsen pulmonary gas exchange efficiency (Fenster *et al.*, 2013). We found a significant effect of shunt size on TL_{CO} in women with a PFO, whereby a large shunt resulted in a significantly lower TL_{CO}, compared to those without a PFO or a small PFO. In this study we used

a shunt score from transthoracic saline contrast echocardiography results following the release of a Valsalva maneuver to demonstrate a maximum potential for intracardiac shunt. We and others have previously used this approach to demonstrate the importance of considering the potential amount of blood flow through a PFO on the measured outcome (Fenster *et al.*, 2013; Davis *et al.*, 2015). However, despite having a similar number of men (n = 27) and women (n = 24) with large shunts, we only found a significant effect of PFO on TL_{CO} in women. The reason for this is currently unknown, but it could be related to the differences in respiratory mechanics between men and women. Although speculative, it is possible that the greater work of breathing resulting from greater swings in intrathoracic (i.e., pleural) pressure in women compared to men may facilitate a greater volume of blood flow across the PFO in women compared to men. Likewise, it is possible that for a given bubble scores, e.g., 4, women have a greater volume of blood flow than men, but this is also speculative. Future research focusing on the interrelationship between respiratory mechanics and PFO blood flow, as would attempts to quantify the volume of blood flow across the PFO, would be of interest and importance.

Our findings in women with a PFO appear to be physiologically significant. Previous work determining normative values for TL_{CO} identified a 0.5 Z-score difference as a threshold for physiological relevance, or 10% relative change in TL_{CO} (Stanojevic *et al.*, 2017). Thus, the 0.7 Z-score difference in TL_{CO} between individuals with and without a PFO and 1.3 Z-score difference in TL_{CO} between women with and without a PFO identifies a physiologically significant difference. Although these differences between women with and without a PFO are physiologically significant, their clinical relevance remains unclear. We speculate that a PFO may worsen pulmonary gas exchange efficiency to a clinically relevant degree when combined with other pathologies such as intrapulmonary shunts due to lung disease. The importance of a PFO in such "two-hit" settings may be an avenue of future investigation.

We interpret these findings to mean that blood shunting through a PFO does not bind to CO and therefore results in a lower TL_{CO}. This occurs as a result of lower K_{CO} in the setting of constant V_A. Indeed, with right-to-left intrapulmonary shunt (e.g. PAVMs), a lower TL_{CO} is physiologically and clinically meaningful. This is because the shunted *pulmonary* blood flow is not participating in

pulmonary gas exchange because of a defect within the pulmonary circuit yet this pulmonary blood flow would have a chance to interact with CO in the absence of such defects. Conversely, with an intracardiac shunt, the blood flow never has a chance to interact with alveolar CO and therefore, although the K_{CO} is lower, it is artificially lower. That is, the shunted blood never would have interacted with the alveolar CO.

It is curious that we observed a lesser TL_{CO} in women with a PFO, but not men with a PFO. This may be due to cardiopulmonary differences between men and women, i.e., smaller lungs and airways (Sheel *et al.*, 2009; Dominelli *et al.*, 2018; Peters *et al.*, 2021) and heart sizes and pulmonary pressures in women (Islam *et al.*, 2021), leading to differences in cardiac output, pulmonary pressures, and pressure gradients across the right and left atrium during the TL_{CO} breath-hold maneuver. Ultimately, the combined effect(s) could be a greater blood flow across the PFO and a difference in TL_{CO} in women with compared to women without a PFO. Although we do not have those data for this retrospective investigation, we have previously reported no differences in resting cardiac output when TL_{CO} was lower in those with a PFO (Elliott *et al.*, 2015), which suggests it is unlikely that differences in cardiac output played a role in the differences reported here. In that same study, we also reported lower pulmonary pressures in participants with a PFO which would help to reduce right heart pressures limiting the blood flow through PFO so this is also not likely an explanation for the lower TL_{CO} reported in the current study. Alternatively, structural or physiological differences in the lung between women with and without a PFO may account for the lower K_{CO} despite normal V_A. This possibility would include microvascular changes such as microvascular underdevelopment, destruction, remodeling, or dilation. The precise cause remains unknown but would contribute to the growing body of work demonstrating physiologically significant differences between those with and without a PFO, including a higher core body temperature and blunted thermal hyperpnea (Davis *et al.*, 2015, 2017), blunted ventilatory acclimatization to high altitude (Elliott *et al.*, 2015), blunted hypercapnic ventilatory responses (Davis *et al.*, 2019), and worse pulmonary gas exchange efficiency (Lovering *et al.*, 2011; Elliott *et al.*, 2015; Duke *et al.*, 2020).

Limitations

As with all studies there are limitations to the current study that should be considered. First,

we only measured TL_{CO} and, thus, cannot comment on how the presence of a PFO influences the various components of diffusion capacity, e.g. membrane diffusion capacity or red blood cell resistance (Hughes & Pride, 2012; Borland & Hughes, 2019), which may be of value in future investigations. Likewise, the retrospective nature of the study is such that we do not have the same data, e.g. nuclear medicine images, exercise data, etc., on all participants so we are unable to explore all potential causes of the observe difference on TL_{CO} and K_{CO} between women with and without a PFO. Future, prospective work is needed to identify the precise mechanism(s) of our observed differences.

Summary and Conclusions

In summary, we report that when normalized as a percent predicted or Z-score, TL_{CO} is lower in women with PFO, specifically those with larger right-to-left shunts that allow for the greatest potential for blood flow across the shunt. These findings highlight the possibility for the presence of a PFO to potentially have an impact on interpretation of the TL_{CO} in the presence of normal V_A, but in the absence of other significant lung disease. Perhaps future iterations of the equations used to predict/estimate TL_{CO} and K_{CO} may include PFO status.

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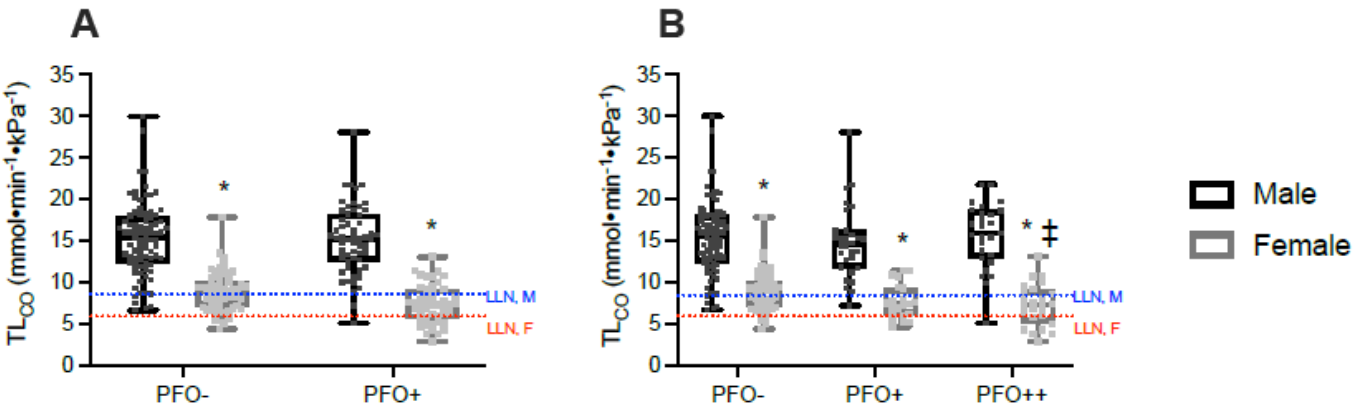

Figure 1. Box and whisker plot of TL_{CO} data. The line within the box represents the median, the box is the 25th and 75th percentiles, and the whiskers are the minimum and maximum. The dotted lines represent the average lower limit of normal (LLN) line for men (blue) and women (red). A two-way ANOVA (sex x PFO group) was computed to identify differences across sexes and PFO groups. A Holm-Bonferroni posthoc test was performed when a significant interaction or main effect was identified. A) TL_{CO} data in PFO- (n = 81) and PFO+ (n = 57) men (black) and women (n = 55, PFO-; n = 46, PFO+) (gray). Within PFO group, women had a significantly lower TL_{CO} ($p < 0.0001$) compared with men, denoted by an *. B) TL_{CO} data in PFO-, those with a small PFO (n = 30 men; n = 21; bubble score of 1-2; PFO+), and those with a large PFO (n = 27 men; n = 24 women; bubble score of 3-5; PFO++). Men are represented in black and women in gray. Within each PFO group, women had a significantly lower TL_{CO} [$p < 0.0001$ (PFO-); $p < 0.0001$ (PFO+); $p < 0.0001$ (PFO++)] compared with men, denoted by an *. PFO++ women had a significantly lower TL_{CO} ($p = 0.048$) compared with PFO- women, denoted by a ‡.

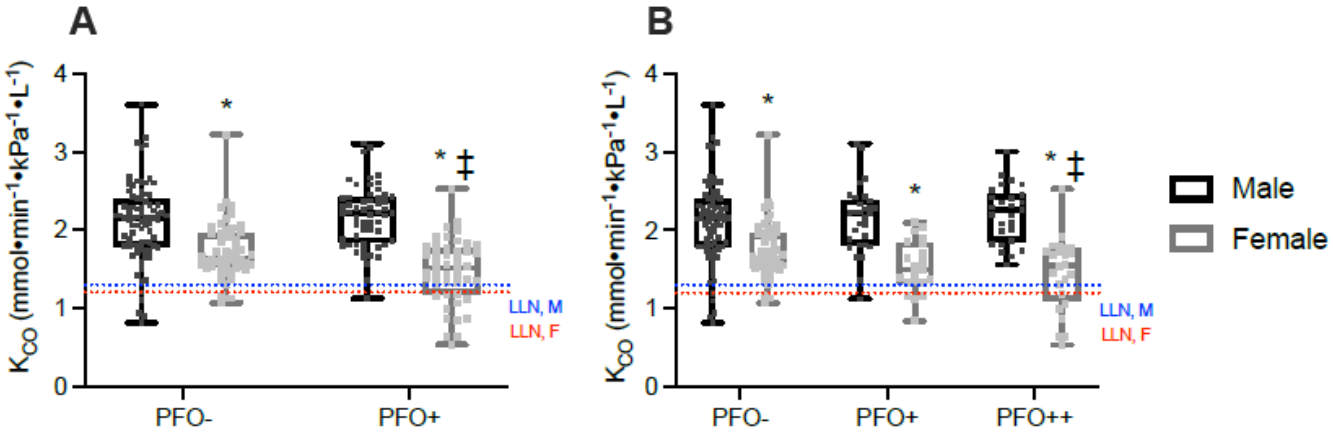

Figure 2. Box and whisker plot of K_{CO} data. The line within the box represents the median, the box is the 25th and 75th percentiles, and the whiskers are the 5th and 95th percentiles. The dotted lines represent the average lower limit of normal (LLN) line for men (blue) and women (red). A two-way ANOVA (sex x PFO group) was computed to identify differences across sexes and PFO groups. A Holm-Bonferroni posthoc test was performed when a significant interaction or main effect was identified. A) K_{CO} data in PFO- (n = 81) and PFO+ (n = 57) men (black) and women (n = 55, PFO-; n = 46, PFO+) (gray). Within PFO group, women had a significantly lower K_{CO} ($p < 0.0001$) compared with men, denoted by an *. PFO+ women had a significantly lower K_{CO} ($p < 0.0001$) compared to PFO- women, denoted by ‡. B) K_{CO} data in PFO-, those with a small PFO (bubble score of 1-2; PFO+), and those with a large PFO (bubble score of 3-5; PFO++). Men are represented in black and women in gray. Within each PFO group, women had a significantly lower K_{CO} [$p < 0.0001$ (PFO-); $p < 0.0001$ (PFO+); $p < 0.0001$ (PFO++)] compared with men, denoted by an *. PFO++ women had a significantly lower K_{CO} ($p = 0.030$) compared with PFO- women, denoted by a ‡.

Table 1. *Anthropometrics and spirometry data.*

	PFO-			PFO+		
	Male (n = 81)	Female (n = 55)	All (n = 136)	Male (n = 57)	Female (n = 46)	All (n = 103)
Age, yr	31 ± 18	30 ± 17	31 ± 17	27 ± 13	26 ± 14	26 ± 13 §
Height, cm	179 ± 7	166 ± 6 *	174 ± 9	179 ± 7	164 ± 7 *	172 ± 10
Mass, kg	77.0 ± 15.7	64.3 ± 9.4 *	71.9 ± 14.9	77.0 ± 10.9	56.4 ± 14.2 * ‡	67.8 ± 16.1 §
BMI, $kg \cdot m^{-2}$	24.0 ± 4.2	23.4 ± 2.9	23.8 ± 3.7	24.1 ± 3.2	20.9 ± 5.2 * ‡	22.7 ± 4.5 §
PFO grade, median	--	--	--	2	3	2.5
Spirometry						
FVC, L	5.4 ± 0.8 (102 ± 12)	3.8 ± 0.6 * (99 ± 9)	4.8 ± 1.1 (101 ± 11)	5.4 ± 0.8 (99 ± 10)	3.9 ± 0.6 * (103 ± 12)	4.7 ± 1.0 (101 ± 11)
FEV_1, L	4.4 ± 0.7 (100 ± 13)	3.2 ± 0.5 * (98 ± 8)	3.9 ± 0.9 (99 ± 11)	4.5 ± 0.7 (98 ± 11)	3.3 ± 0.5 * (101 ± 11)	4.0 ± 0.8 (99 ± 11)
FEV_1/FVC	81 ± 8 (97 ± 8)	84 ± 7 (98 ± 7)	82 ± 8 (98 ± 8)	83 ± 7 (98 ± 7)	85 ± 6 (98 ± 7)	84 ± 7 (98 ± 7)
FEF_{25-75}, $L \cdot s^{-1}$	4.3 ± 1.4 (96 ± 27)	3.3 ± 0.9 * (92 ± 21)	3.9 ± 1.3 (94 ± 24)	4.5 ± 1.1 (94 ± 21)	3.6 ± 0.9 * (94 ± 18)	4.0 ± 1.1 (94 ± 20)

Table 1. PFO-, no PFO present; PFO+, PFO present. All values are means ± SD. FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; FEV_1/FVC, ratio of FEV_1 to FVC; FEF_{25-75}, forced mid-expiratory flow. Values in parentheses are mean ± SD % predicted. § Denotes a significant difference between PFO- and PFO+. A two-way ANOVA (sex x PFO group) was computed to identify differences across sexes and PFO groups. A Holm-Bonferroni posthoc test was performed when a significant interaction or main effect was identified. * Denotes a significant difference between sexes within a PFO group. † Denotes a significant difference between males across PFO groups. ‡ Denotes a significant difference between females across PFO groups.

Table 2. *Lung diffusion and alveolar volume data.*

	PFO-			PFO+		
	Male (n = 81)	Female (n = 55)	All (n = 136)	Male (n = 57)	Female (n = 46)	All (n = 103)
TL_{CO}						
Absolute, mmol·min^{-1}·kPa^{-1}	15.2 ± 4.5	8.9 ± 2.4 *	12.6 ± 4.9	15.1 ± 4.1	7.4 ± 2.3 * ‡	11.7 ± 5.2
% predicted	141 ± 36	116 ± 25 *	131 ± 34	137 ± 30	98 ± 27 * ‡	119 ± 35
LLN	8.4 ± 1.1	5.9 ± 0.6 *	7.4 ± 1.6	8.6 ± 1.0	5.8 ± 0.6 *	7.4 ± 1.6
Z-score	2.4 ± 2.1	0.8 ± 1.2 *	1.7 ± 1.9	2.3 ± 1.9	-0.5 ± 2.1 * ‡	1.0 ± 2.4 §
K_{CO}						
Absolute, mmol·min^{-1}·kPa^{-1}·L^{-1}	2.1 ± 0.5	1.7 ± 0.4 *	1.9 ± 0.5	2.2 ± 0.4	1.5 ± 0.4 * ‡	1.9 ± 0.5
% predicted	127 ± 31	111 ± 22 *	121 ± 29	129 ± 23	93 ± 27 * ‡	113 ± 30 §
LLN	1.3 ± 0.1	1.2 ± 0.1 *	1.3 ± 0.1	1.3 ± 0.1	1.2 ± 0.1 *	1.3 ± 0.1
Z-score	1.8 ± 2.2	0.7 ± 1.4 *	1.4 ± 2.0	2.1 ± 1.7	-0.6 ± 2.0 * ‡	0.9 ± 2.3
V_A						
Absolute, L	7.2 ± 0.9	5.1 ± 0.8 *	6.4 ± 1.4	6.9 ± 1.1	5.0 ± 0.6 *	6.1 ± 1.3
% predicted	111 ± 13	104 ± 13 *	108 ± 13	106 ± 15	104 ± 11	105 ± 13
LLN	5.4 ± 0.5	4.0 ± 0.4 *	4.8 ± 0.8	5.4 ± 0.5	3.9 ± 0.4 *	4.7 ± 0.9
Z-score	0.9 ± 1.1	0.2 ± 0.9 *	0.7 ± 1.1	0.5 ± 1.4	0.3 ± 0.8	0.4 ± 1.2

Table 2. PFO-, no PFO present; PFO+, PFO present. All values are means ± SD. TL_{CO}, transfer factor of the lung for carbon monoxide; LLN, lower limit of normal; K_{CO}, transfer factor for carbon monoxide; V_A, alveolar volume. A two-way ANOVA (sex x PFO group) was computed to identify differences across sexes and PFO groups. A Holm-Bonferroni posthoc test was performed when a significant interaction or main effect was identified. § Denotes a significant difference between PFO- and PFO+. * Denotes a significant difference between sexes within a PFO group. † Denotes a

significant difference between males across PFO groups. ‡ Denotes a significant difference between females across PFO groups.

Additional Information Section:

Acknowledgements: The authors would like to extend their gratitude to the participants for their enthusiastic participation in the study.

Funding: American Heart Association Scientist Development Grant #2280238 (ATL); the American Physiological Society's Giles F. Filley Memorial Award for Excellence in Respiratory Physiology and Medicine (ATL); U.S. Department of Defense Grants W81XWH-10-2-0114 to ATL); American Lung Association in Oregon/American Thoracic Society (ATS; grant no. C-10 014); The Eugene and Clarissa Evonuk Memorial Graduate Fellowship (SSL, JEE, JTD); American Heart Association pre-doctoral fellowship (JEE).

Competing Interests: The authors have no conflicts of interest to report.

Data Availability: The data that support the findings of the study are available from the corresponding author upon reasonable request.

Author Contributions: All authors contributed to the design of the investigation, as well as the acquisition, analysis, and interpretation of data. All authors contributed to the intellectual content by drafting and revising the manuscript accordingly and have approved the final version. All authors agree to being held accountable for all aspects of the work