Pulmonary capillary recruitment and distention in mammalian lungs: species similarities

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Shareable abstract (@ERSpublications)
Lungs accept exercise blood flow by filling a larger fraction of their capillaries, then distending already filled capillaries as flow rises more. Our review shows the transition range is around 2.5 to 3.5 times resting flow in mammals, including humans. https://bit.ly/3JFWuG7

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Abstract

Pulmonary arterial pressure rises minimally during exercise. The pulmonary microcirculation accommodates increasing blood flow *via* recruitment of pulmonary capillaries and, at higher flows, by distention of already perfused capillaries. The flow transition range between recruitment and distention has not been studied or compared across mammalian species, including humans. We hypothesised that the range would be similar. Functional pulmonary capillary surface area (FCSA) can be estimated using validated metabolic techniques. We reviewed data from previous studies in three mammalian species (perfused rabbit lungs and dog lung lobes, and exercising humans) and generated blood flow–FCSA curves over a range of flows. We noted where the curves diverged from the theoretical line of pure recruitment (Recruitment) and determined the flow where the curve slope equalled 50% that of Recruitment, or equalled that of a theoretical curve representing full capillary distention (Distention). The three mammalian species have similar flow ranges for the transition from predominantly recruitment to predominantly distention, with dogs having the highest transition point. Within the physiological range of most daily activity, the species are similar and accommodate increasing blood flow mainly *via* recruitment, with progressive distention at higher flows. This is highly relevant to pulmonary physiology during exercise.

Introduction

The human pulmonary circulation accepts increased cardiac output, such as during exercise, with minimal increases in pulmonary arterial pressure [1–5]. Compared with the systemic circulation, the lung circulation is a low resistance high-capacitance bed, with approximately 280 billion capillary segments in a normal adult, and it can thus accommodate increased blood flow while maintaining a low driving pressure [6]. It has previously been unclear how this accommodation occurs in humans: are all capillaries filled at rest and distend with more flow, or is there a continuously changing perfused fraction of the capillary bed that is increased to accommodate higher flow? Previous experimental evidence has shown that the latter can occur [7–12].

Prior studies of functional pulmonary capillary surface area (FCSA) in animal models, and our recent studies in exercising humans, demonstrate that both phenomena occur, but at different phases of increasing flow [13–15]. At low to moderate flows, capillary recruitment is predominant and, at higher flows close to complete recruitment, capillary distention dominates. We hypothesised that we could identify blood flow levels that represent the transition range from predominantly recruitment to predominantly distention and that the transition range would be similar among various mammalian species. We therefore explored the

data from previous studies measuring FCSA in intact animals, including humans, or in isolated perfused lungs or lobes, at varying blood flow rates.

Methods

In previous studies included in the present analysis, the assay methods for estimating functional capillary surface area (FCSA) utilise metabolism of trace doses of an injected radiolabelled tri-peptide (^{3}H-benzoyl-Phe-Ala-Pro (BPAP)) that is haemodynamically inactive [16]. In this type of experiment, BPAP interacts on a first-order kinetic and first-pass basis with the endothelial ectoenzyme angiotensin-converting enzyme-1 (ACE-1) as it passes through the lung capillary bed.

Depending on the experimental model, the ^{3}H-BPAP solution is injected into the right atrium or into the pulmonary artery. Simultaneously, systemic arterial blood or pulmonary venous effluent is collected into a fraction collector that has vials containing 0.9% saline with 5 mmol·L^{-1} EDTA and 6.8 mmol·L^{-1} 8-hydroxyquinoline 5-sulfonic acid, to inhibit further activity of blood ACE, and heparin 1000 IU·L^{-1}. After centrifugation, the supernatant is transferred into a scintillation vial containing 5 mL Ecolite, and total ^{3}H radioactivity is measured (^{3}H Total), or, to determine radioactivity associated with metabolites, another 0.5 mL of the supernatant is transferred into a scintillation vial containing 2.5 mL HCl (0.12 N). After addition of 3 mL of 0.4% Omnifluor in toluene and mixing by inversion, the radioactivity (^{3}H Toluene) is measured after 48 hours of undisturbed equilibration in the dark. Slight technical differences which do not alter the measurements have been present between the three studies described in this review. Using this technique, approximately 60% of the ^{3}H-BPAP metabolite ^{3}H-benzoyl-Phe (^{3}H-BPhe) (f_p), and approximately 13% of the parent ^{3}H-BPAP (f_s), are extracted in the organic phase of the mixture (*i.e.* toluene).

Transpulmonary hydrolysis (v) is calculated as:

$$v = ln([^3H - BPhe]/[^3H]) = [E] \times t_c \times k_{cat}/K_m$$

with [E], t_c, k_{cat} and K_m being the enzyme concentration available for reaction, capillary transit time (*i.e.* enzyme–substrate reaction time), catalytic rate constant and Michaelis–Menten constant, respectively. FCSA is calculated as:

$$FCSA = A_{max}/K_m = E \times k_{cat}/K_m = PPF \times v$$

with E and PPF being total enzyme mass available for reaction and pulmonary plasma flow (=CO× (1−Hct)), respectively.

The technique has been previously extensively validated. It has been employed in basic physiological studies, but also for understanding human disease pathophysiology [13–15, 17–22]. In all studies, ethics approval was obtained for handling of the animals, or for human experimentation, with written, informed consent as appropriate.

The studies were chosen because they provided interpretable individual or group data for FCSA at varying pulmonary blood flows [13–15]. In both rabbit and dog studies, two groups of experiments had been performed, at low or higher blood flow. When the data were examined, low-flow and high-flow data represented a smooth continuum and could be combined into a single curve for each species studied. FCSA was plotted (ordinate) *versus* blood flow (abscissa). To provide a basis for analysing the data in studies of isolated perfused lungs or lobes, previously reported values of normal resting blood flows for each species were normalised to the portion of lung being studied. For rabbits, resting cardiac output has been estimated as 400 mL·min^{-1} and, if 47% of flow enters the left lung, then basal flow would be approximately 188 mL·min^{-1} [23]. In dogs, basal cardiac output measurements have ranged from 1.7 L·min^{-1} in anaesthetised dogs [24] to 3.4 L·min^{-1} in awake dogs [25]. For the present analysis, we used an average of 2.19 L·min^{-1} [26] The left lower lobe receives approximately 25% of pulmonary blood flow, yielding an estimated basal lobar blood flow of 0.54 L·min^{-1}. For the human studies, a basal cardiac output of 5.73 L·min^{-1} was used [17].

We calculated the theoretical expected change in FCSA with change in blood flow [13, 14] for the scenario where increased flow is accommodated solely by capillary recruitment (Recruitment). In the Recruitment model, fold increase in FCSA matches fold increase in flow [13]. Also, in theory, any observed component of distention can be either where increased flow is accommodated solely by capillary distention without exposure of new surface ACE molecules (Distention A), or where increased flow causes

distention of already filled capillaries with exposure of some new surface ACE molecules (Distention B). In the Distention A model, FCSA does not change with flow. In the Distention B model, the ratio of new to baseline FCSA is equal to the square root of new to baseline flow. All the above curves are validated models and, by definition, free of error, where all points fall directly on the lines or curves [13, 14]. With progressive distention, the slope of the experimental data regression curve decreases as compared with the slope of Recruitment and is seen as a divergence of the regression curve from the line of Recruitment.

In all the studies, the experimental data were fitted to a first-order polynomial curve, and 95% confidence intervals were calculated. The Distention B curve was also fitted to a first-order polynomial. For the above curves, the regression equation was in the form of $y=a+bx+cx^2$, with the derivative slope at any point therefore being equal to b+2cx. To describe the Recruitment line, a linear equation y=mx+b, with m being the constant slope, was used.

In each study, we identified three significant points: 1) where the 95% confidence intervals of the experimental data curve diverted from the Recruitment line; 2) where the slope of the experimental curve fell to <50% of the Recruitment line slope; and 3) where the slope of the experimental data curve equalled the slope of the Distention B curve. Those points were then expressed as proportion of basal flow for each experiment.

Specifics of each study

Perfused rabbit lungs

Rabbit lungs were perfused *in situ* at varying flow rates, either into both lungs (n=4), or after right pneumonectomy to achieve even higher flows into the left lung (n=9) [13]. The FCSA data and flow data for double lung perfusion were extrapolated to single lung levels and combined with the data for isolated left lung perfusion to establish a single data series.

Perfused dog lungs

Isolated blood-perfused left lower dog lung lobes were studied over a range of blood flow rates (n=6–8 per flow assessment point), in Zone III conditions [14]. Lobar flow was expressed in $L \cdot min^{-1}$.

Exercising humans

Ten humans performed symptom-limited supine exercise [15]. Their baseline and exercise data covered a wide range of cardiac output, and were combined into a single group FCSA/body surface area (BSA) *versus* flow curve. While capillary surface area is related to BSA, lung blood volumes correlate better with cardiac output than with cardiac index [17]. Thus, we compared FCSA/BSA with absolute cardiac output.

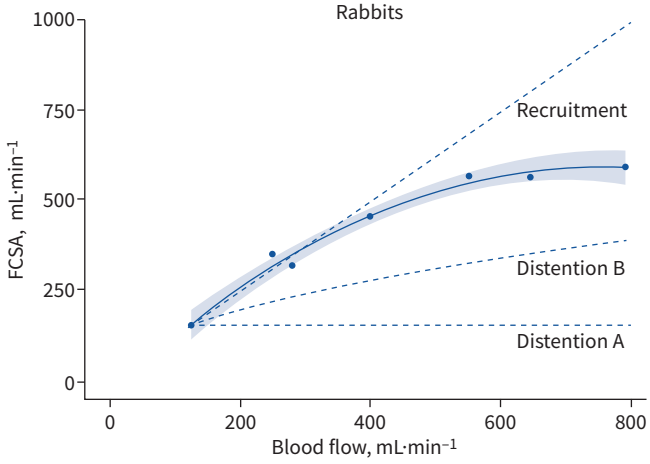

FIGURE 1 Pulmonary FCSA in perfused rabbit lungs over a range of blood flows. Blue circles (•) represent experimental data with fitted first-order polynomial curve and 95% CI (blue area). The theoretical lines or curves for pure capillary recruitment during increasing flow (Recruitment), capillary distention with some further exposure of FCSA during increasing flow (Distention B), and capillary distention without any ongoing further of FCSA during increasing flow (Distention A) are shown. FCSA: functional capillary surface area.

In a previous study, the average resting cardiac output was 5.73 L·min^{-1} and the FCSA/BSA was 2460 mL·min^{-1}·m^{-2} [17]. These values were used to generate the theoretical lines and curve.

Results

Perfused rabbit lungs

The rabbit lungs were perfused over a single lung flow range of approximately 125–791 mL·min^{-1} [13]. The FCSA/flow curve equation for the experimental data was y=−35.6+1676.8x−1119x^2, r>0.99, with the equation for Distention B being y=95.5+531.9x−209.5x^2, r>0.99, and for Recruitment y=1232.1x−0.17, r=1. The 95% confidence intervals of the experimental curve diverged from the Recruitment line at approximately 370 mL·min^{-1}. With normal basal single lung flow estimated at 170 mL·min^{-1} per single lung [27], the divergence occurred at 2.2× basal flow. The slope of the experimental curve was <50% that of the Recruitment line at a flow of 0.48 L·min^{-1} (2.55× basal flow) and the slope of the experimental curve equalled that of Distention B at a flow of 0.63 L·min^{-1} (3.35× basal flow) (figure 1 and table 1).

Perfused dog lung lobes

The isolated dog lobes were perfused over a flow range of 0.45–3.05 L·min^{-1} [14]. The FCSA/flow curve equation for the experimental data was y=93.8+1653.2x −219.7x^2, r=0.98, with the equation for Distention B being y=440.3+648.6x−61.8x^2, r>0.99, and for Recruitment y=1567.2x−0.36, r=1. The 95% confidence intervals of the FCSA/flow curve diverged from the Recruitment line at an approximate lobar flow of 1.67 L·min^{-1}. This represents 3.1× basal flow. The slope of the experimental curve was <50% that of the Recruitment line at a flow of 2.0 L·min^{-1} (3.6× basal flow) and the slope of the experimental curve equalled that of Distention B at a flow of 3.2 L·min^{-1} (5.8× basal flow) (figure 2 and table 1).

Exercising humans

The combined rest and exercise cardiac outputs ranged from 4.67–18.3 L·min^{-1}. The FCSA/flow curve equation for the experimental data was y=498.8+426.1x−6.4x^2, r=0.91, with the equation for Distention B being y=1339.9+214.1x−2.51x^2, r>0.99, and for Recruitment y=429.9x−0.05, r=1. The 95% confidence intervals of the experimental curve diverged from the Recruitment line at approximately 11.8 L·min^{-1}, which represent 2.1× resting flow. The slope of the experimental curve was <50% that of the Recruitment line at a flow of 16.7 L·min^{-1} (2.9× basal flow) and the slope of the experimental curve never paralleled that or equalled that of Distention B within the range of flows studied (figure 3 and table 1).

Discussion

The mammalian lung circulation, with a delicate microcirculation providing a large perfused surface area for efficient gas diffusion and for uptake or metabolism of circulating molecules, has evolved to accommodate exercise and stress-induced increases in pulmonary blood flow without major rises in pulmonary artery pressure [1–5]. In all species studied with our technique, it does so by recruitment of nonconcomitantly perfused capillaries as flow increases, reaching maximum recruitment, and any further flow increases then distend the already perfused capillaries [13–15]. Although several species have been studied, there has been no previous exploration of similarities and differences between them. We hypothesised that there would be an identifiable common range of blood flow at which the method of flow accommodation transitions from principally recruitment to principally distention.

The previous studies that provided data employed an extensively validated technique for estimating perfused FCSA which can distinguish between capillary recruitment and distention [13, 14, 28]. The first-pass interaction between ACE-1 and ^{3}H-BPAP provides a precise and repeatable method for studying FCSA and blood flow. Previous studies have demonstrated that ^{3}H-BPAP is exclusively metabolised by ACE-1, since its hydrolysis is completely blocked by ACE-1 inhibitors [29]. The technique was used identically in the three studies, providing a basis for comparison in the three species. The perfused lung

TABLE 1 Blood flows at points of transition from predominantly capillary recruitment to capillary distention.

	Estimated average basal flow, L·min^{-1}	Flow at slope=50% of Recruitment slope, L·min^{-1} (% of basal flow)	Flow at slope=that of Distention B slope, L·min^{-1} (% of basal flow)
Rabbit left lung	0.188	0.48 (255)	0.63 (335)
Dog left lower lobe	0.54	2.0 (370)	3.2 (590)
Exercising humans	5.73	16.7 (291)	Beyond studied range

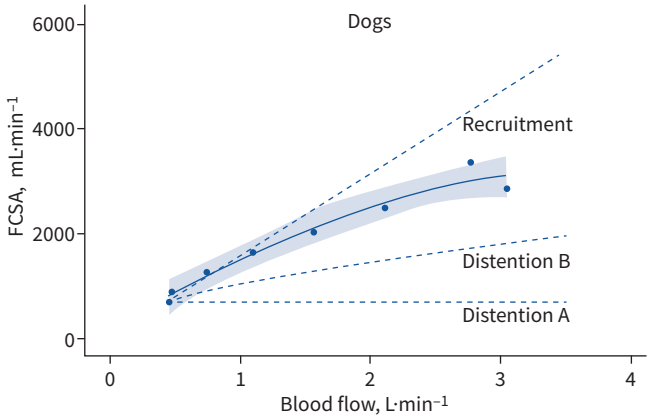

FIGURE 2 Pulmonary FCSA in perfused dog lung lobes over a range of blood flows. Blue circles (•) represent experimental data with fitted first-order polynomial curve and 95% CI (blue area). The theoretical lines or curves for pure capillary recruitment during increasing flow (Recruitment), capillary distention with some further exposure of FCSA during increasing flow (Distention B), and capillary distention without any further exposure of FCSA during increasing flow (Distention A) are shown. FCSA: functional capillary surface area.

experiments each studied several groups of animals: some at flows in the recruitment range and others at flows in the distention range. However, it was evident that those data and curves could be blended into a single curve for each species, which we did for the present review. The individual data for exercising humans were similarly blended to generate a single flow–FCSA curve. Thus, we could study the continuum of FCSA levels over the physiological range of flows. There is no discrete point of change between unique recruitment and unique distention. In theory, there is a range of individual capillary transition points, governed by gravitational gradients, flow distribution, ventilation, pulmonary venous pressure and other factors. Thus, for the lung as a whole, one can only speak of an overall transition range from predominant recruitment to predominant distention. Other groups have calculated a distensibility factor, alpha, to describe pulmonary vascular pressure–flow curves [30]. However, alpha is most closely associated with resistance vessels, is based on a fully distended vascular bed and includes the entire lung

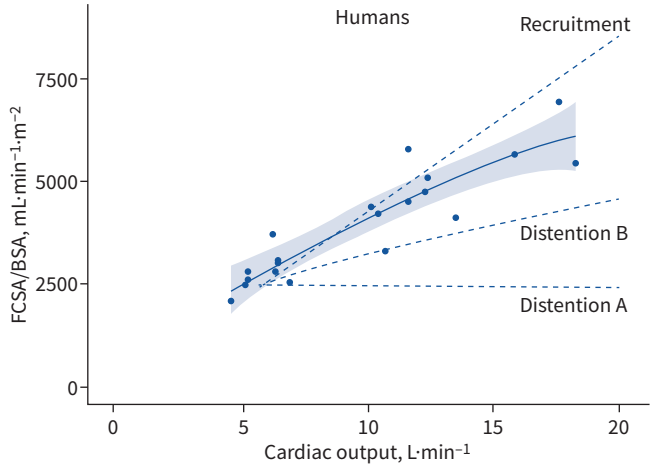

FIGURE 3 Pulmonary FCSA/BSA in humans performing supine exercise, over a range of blood flows. Blue circles (•) represent experimental data with fitted first-order polynomial curve and 95% CI (blue area). The theoretical lines or curves for pure capillary recruitment during increasing flow (Recruitment), capillary distention with some further exposure of FCSA during increasing flow (Distention B), and capillary distention without any further exposure of FCSA during increasing flow (Distention A) are shown. BSA: body surface area; FCSA: functional capillary surface area.

vasculature. Our results in healthy humans suggest that pulmonary capillary recruitment must contribute to alpha observations, and should be considered in the future. Also, using our technique, we cannot comment on the presence or absence of recruitment or distention of upstream arterioles or downstream veins.

The initial study in perfused rabbit lungs provided a guide as to the flow point where Distention would be greatly predominant and would represent the main mechanism for accommodating further increases in blood flow. In that study, the plateau for FCSA occurred at a single lung flow of 636 $mL \cdot min^{-1}$. This matched very nicely to the point on the curve we generated where the slope of the curve matched that of Distention B. We thus used this as a critical datapoint when examining the results from the other species. We also explored the flow at the point where the curve slope was 50% of the Recruitment slope, *i.e.* halfway between Recruitment and the flat line of Distention A, which indicates that Distention has become the major mechanism of flow accommodation. For the rabbit and dog studies, we used previously published measurements of cardiac output to establish basal flow. In the case of the rabbit lungs, that was 188 $mL \cdot min^{-1}$ to the left lung, and for the dogs lungs it was 540 $mL \cdot min^{-1}$ to both lungs. For the human data, we had actual measured resting cardiac output values to use as baseline (average 5.73 $L \cdot min^{-1}$).

In all three species, the data suggest that distention becomes the predominant mechanism of flow accommodation at between 2.5- and 3.7-times resting flow, depending on the species. In humans, the transition occurs at approximately 2.9 times basal flow. Thus, all three species maintain predominantly recruitment within what likely represents their normal range of physical activity, including mild to moderate exertion. It is also clear, from the divergence of the 95% confidence intervals of the curves away from the Recruitment line, that distention begins well before the 50% slope diversion from the Recruitment line, and that some recruitment continues in response to increased flow afterwards. In the rabbit and dog lungs, distention becomes the nearly unique mechanism of flow accommodation at 3.4–5.9 times basal flow. In humans, that phenomenon occurs at a flow point that was beyond the range of our observed data. That degree of flow would likely be achieved only by performance athletes during extreme exertion.

Our review has several potential areas for error, particularly in the assumptions we have made: first, the rabbit and dog measurements were made in perfused lungs, not awake exercising animals. Nonetheless, those analyses allowed for tightly controlled physiological measurements. Dupuis and colleagues [31], using a different metabolite for ACE-1, have studied FCSA in exercising dogs and show that recruitment is less predominant after blood flow exceeds three times basal flow. That compares well with our data. For logistical reasons, the human measurements were made during supine exercise, while most human exercise is upright. It is unknown whether, because of potentially less resting recruited lung in the upright position (mainly lower lobe perfusion) *versus* supine (mainly dorsal perfusion including upper lobes), the transition point from recruitment to distention might occur at a higher blood flow. Despite that, humans do perform horizontal exercise that requires large cardiac output increases, such as when swimming. What we do describe is a transition range for three species when horizontal, and it is quite similar. Second, using the data provided in each study, we recalculated the results in the rabbit and dog studies on the basis of single lung or lobe blood flow and average lung weights. We applied previous descriptions of resting blood flow as the basal flows. Furthermore, in the rabbit and dog studies the results of two different experimental groups (low flow and high flow) were combined to generate a single continuous data curve. What was reassuring is that, in each species, the results for the highest flow in the low-flow cohort matched those of the lowest flow in the high-flow cohort, suggesting the curves could be blended. Using those data together, the curves had excellent degrees of fit. In the humans, we took single-patient measurements at two flow points and combined all the points (rest and exercise) into a single blended curve over a range of flows. Again, the fit of that curve was excellent. Thus, our analysis makes many assumptions, but the data are consistent and informative. Finally, the techniques used are technically arduous and, while they provide a powerful research tool for understanding pulmonary circulatory physiology, they are unlikely to ever translate into an easily usable tool in the clinic.

In conclusion, in these three species including humans, we demonstrate that the transition point from mainly recruitment to mainly distention occurs at approximately 2.5–3.5 times resting blood pulmonary flow, with dogs having the highest transition point. Thus, within the physiological range of most daily activity, the species are similar and accommodate increasing blood flow mainly *via* recruitment, with progressive distention at higher flows. These mechanisms certainly contribute to the ability of the lung circulation to function at low driving pressures over a wide range of blood flows. With loss of perfused pulmonary microvasculature, as is found in pulmonary arterial hypertension, recruitment might be diminished or absent, and effective therapy might be able to restore it. However, those hypotheses remain to be tested.

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