Spatial pulmonary flow distribution in rabbit isolated lungs is a poor representation of the situation in vivo: ex vivo spatial blood flow distribution

M. Hübler, Axel R. Heller, J.U. Bleyl, T. Rössel, S.N. Stehr, Thea Koch

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SPATIAL PULMONARY FLOW DISTRIBUTION IN RABBIT ISOLATED LUNGS IS A POOR REPRESENTATION OF THE SITUATION *IN VIVO**

M Hübler, AR Heller, JU Bleyl, T Rössel, SN Stehr and T Koch

Department of Anaesthesiology and Intensive Care Medicine, Technical University Dresden, Dresden, Germany

SUMMARY

1. Isolated lung preparations are established to investigate effects on pulmonary vascular tone and spatial pulmonary flow ($\dot{Q}_{rel}$) distribution. In the present study, we hypothesized that $\dot{Q}_{rel}$ distribution in isolated lungs is only poorly correlated with the *in vivo* situation.

2. Fourteen rabbits were anaesthetized and mechanically ventilated with room air. Animals were held in an upright position for 15 min and $\dot{Q}_{rel}$ was assessed using fluorescent microspheres ($\dot{Q}_{rel\text{-}in\ vivo}$). A second injection of microspheres was made after isolation of the lungs ($\dot{Q}_{rel\text{-}ex\ vivo}$). Lungs were dried, cut into 1 cm^3 cubes and spatial $\dot{Q}_{rel}$ distributions were analysed.

3. The mean correlation of $\dot{Q}_{rel\text{-}in\ vivo}$ and $\dot{Q}_{rel\text{-}ex\ vivo}$ was 0.592 ± 0.188 (95% confidence interval 0.493–0.690). The $\dot{Q}_{rel}$ was redistributed to more ventral (the mean slope of $\dot{Q}_{rel}$ *vs* the dorsal–ventral axis changed from -0.289 ± 0.227 to -0.147 ± 0.114; $P = 0.03$), cranial (mean slope of $\dot{Q}_{rel}$ *vs* the caudal–cranial axis changed from -0.386 ± 0.193 to -0.176 ± 0.142; $P < 0.001$) and central (mean slope of $\dot{Q}_{rel}$ *vs* the hilus–peripheral axis changed from 0.436 ± 0.133 to -0.236 ± 0.159; $P = 0.003$) lung areas.

4. The results obtained from studies investigating $\dot{Q}_{rel}$ distributions in isolated lung models must be interpreted cautiously because the isolated lung set-up significantly affects the spatial distribution of pulmonary flow.

Key words: isolated lung, microspheres, perfusion distribution.

INTRODUCTION

The isolated lung preparation is an established model to investigate physiological and pathological changes in the pulmonary vascular bed because it allows the control of multiple variables.[1] The model is an especially useful tool to study mechanisms that influence pulmonary vascular tone.[2,3]

In addition, the model is frequently used to asses spatial relative flow distributions ($\dot{Q}_{rel}$).[4–8] So far, little is known about the degree to which the spatial distribution of $\dot{Q}_{rel}$ is affected by the experimental set-up and organ preparation.

Correspondence: Matthias Hübler, Department of Anaesthesiology and Intensive Care Medicine, Carl Gustav Carus University Hospital, Fetscherstr. 74, 01307 Dresden, Germany. Email: matthias.huebler@uniklinikum-dresden.de

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The *in vivo* distribution of pulmonary blood flow is influenced by various active and passive mechanisms. Active mechanisms induce contraction or relaxation of vascular smooth muscle, which lead to changes in pulmonary vascular tone and resistance. Examples of active factors are autonomic pulmonary innervations, humoral factors and partial pressures of gases. In contrast, passive mechanisms affect pulmonary blood flow independently of changes in pulmonary vascular tone. Passive factors include gravitational forces, interstitial and airway pressures, changes in lung volume, changes in cardiac output or pulmonary artery pressure, vascular obstruction or recruitment and left atrial pressure. Many of these active and passive factors are altered after removal of the lung from the thoracic cavity. Therefore, the validity of the data obtained in the isolated preparation remains unknown. No study has been performed previously comparing *ex vivo* and *in vivo* $\dot{Q}_{rel}$ distributions. In the present study, we hypothesized that the distribution of $\dot{Q}_{rel}$ in isolated lungs is only poorly correlated with the *in vivo* situation.

METHODS

The study was approved by the University Animal Care Committee and the responsible governmental institution (Regierungspräsidium Dresden, AZ 74-9168.11-1-2001-18). National Institutes of Health guidelines for animal use and care were followed throughout.

Animal preparation and experimental protocol

Experiments were performed on 14 chinchilla rabbits (*Orticolagus caniculus*) of both sexes, ranging in weight from 2.0 to 2.9 kg. Rabbits were anaesthetized with ketamine (50 mg/kg) and xylazine (4 mg/kg) and anticoagulated with heparin–sodium (1000 U/kg), injected in an ear vein. Animals were put in the supine position and a tracheotomy tube was placed under local anaesthesia (lignocaine 1%). Animals were mechanically ventilated with room air using a small animal ventilator (Hugo Sachs Elektronik, March, Germany). The ventilator settings were as follows: respiratory frequency 30/min; tidal volume 12 mL/kg; positive end-expiratory pressure (PEEP) 2 cmH_2O. Animals were then put in an upright position. The position was maintained without exerting pressure on the abdomen or thorax. This was achieved using a specially designed cast form, which supported the animals' hind limbs and pelvis. While in the upright position, the animals' upper limbs were held to avoid sliding in the cast form. After 15 min in the upright position, animals received the first injection of microspheres in an ear vein to assess $\dot{Q}_{rel}$ ($\dot{Q}_{rel\text{-}in\ vivo}$). The injection was performed over 1 min and was followed by a saline flush.

Thereafter, the lungs were isolated. The techniques of preparing and perfusing rabbit isolated lungs have been described in detail previously.[9,10] Briefly, a median sternotomy was performed and a catheter was inserted into the pulmonary artery. The left atrium was cannulated via the left ventricle to collect the pulmonary outflow. The lung organ preparation was removed and suspended from a weight transducer in a temperature-controlled (37°C) and humidified chamber. After the cannulation procedure, the lungs were perfused with a cell- and plasma-free perfusion medium. A 40 μm filter and a

bubble trap prevented pulmonary embolism by circulating particles or air. The perfusate consisted of a Krebs'–Henseleit hydroxyl-ethyl-starch buffer (KHB) solution with a colloid oncotic pressure between 23 and 25 mmHg, yielding final concentrations as follows (in mmol/L): Na^+ 138; K^+ 4.5; Mg^{2+} 1.33; Cl^- 135; Ca^{2+} 2.38; glucose 12; HCO_3^- 12. The osmolality of the solution was approximately 330 mOsmol/kg (Mikro-Osmometer; Roebling Meßtechnik, Berlin, Germany). The pH of the buffer solution was adjusted to 7.4 with 1 mol/L $NaHCO_3$.

First, low flow rates were used in an open circulatory system to remove remaining blood from the vascular bed. The perfusion fluid was exchanged twice for fresh buffer via two separate perfusion circuits, 2 and 15 min after the beginning of extracorporeal circulation. Then, the flow was increased to 150 mL/min. In previous experiments using the same model, the perfusion with KHB has been documented to maintain the integrity of the microcirculation for more than 5 h.[11] As a result of the constant perfusion flow, alterations of perfusion pressure directly reflect alterations of pulmonary vascular resistance. Pulmonary afterload was set to 3 mmHg using a height-adjustable fluid bridge open to ambient pressure. The lungs were ventilated with 4% CO_2 in air with unchanged ventilator settings. Because it has been shown that oxygenation of the lung by ventilation alone is satisfactory,[11] no additional oxygenator unit in the perfusion circuit is required. Mean pulmonary artery pressure (mPAP) and left atrial pressure (LAP) were recorded continuously via Statham strain gauge transducers (zero referenced at hilus level; Becton Dickinson, Sandy, UT, USA). The lungs showed a homogeneous white appearance, with no signs of haemorrhage or oedema formation, and no increase in weight during the steady state period was observed. Following a 30 min steady state period, the lungs had a constant mean (± SD) mPAP of 12 ± 3 mmHg. A second injection of microspheres was performed to assess $\dot{Q}_{rel}$ ($\dot{Q}_{rel\text{-}ex\ vivo}$). The microspheres were injected over 1 min into the pulmonary artery catheter. The time interval between the *in vivo* and *ex vivo* assessments of $\dot{Q}_{rel}$ distributions was approximately 50 min.

Determination of pulmonary flow

Fluorescent polystyrene microspheres (red, orange, yellow–green and crimson), 15 μm in diameter (Molecular Probes, Eugene, OR, USA), were used to measure regional flow to the lungs. Details of the method are described elsewhere.[12] Immediately before injection, the microspheres were vortexed and then sonicated for 90 s. The number of microspheres per injection was 600 000. The order that the different microspheres were injected was randomized in every experiment.

After completion of the experiments, catheters were removed, lungs were inflated and dried by a constant tracheal flow with air for 2 days (pressure limit 20 cmH_2O). When dry, the lungs were first coated with a one-component polyurethane foam (BTI Befestigungstechnik, Ingelfingen, Germany), then suspended vertically in a square box and embedded in rapidly setting urethane foam (Polyol and Isocyanate; a kind gift from Elastogran, Lemförde, Germany). The foam block was cut into uniformly sized 1 cm^3 cubes. Foam adhering to lung pieces was removed. Each cube was weighed and assigned a three-dimensional coordinate. Samples with airways occupying > 25% of the cube's volume were discarded. Samples were then individually soaked for 2 days in 2 mL 2-ethoxyethyl acetate (Aldrich Chemical, Milwaukee, WI, USA) to retrieve the fluorescent dye. The fluorescence was read in a luminescence spectrophotometer (LS-50B; Perkin-Elmer, Beaconsfield, Buckinghamshire, UK) fitted with a flow cell and a standard photomultiplier tube.

The weight-normalized relative flows at the two time points were calculated for each lung piece as follows:

$$\dot{Q}_{rel,i} = x_i/(\Sigma x_i/n)$$

where $\dot{Q}_{rel,i}$ is the weight-normalized relative flow of the piece i, x_i is the fluorescence divided by the weight of the piece and n is the number of pieces of lung. The dimensionless, mean normalized relative flow was therefore 1.0. A value below 1.0 indicates that the specific tissue sample received less flow than the average; values greater than 1.0 indicate that the tissue sample received a higher flow than average.

Table 1 Slopes of $\dot{Q}_{rel}$ versus spatial axes

	$\dot{Q}_{rel\text{-}in\ vivo}$	$\dot{Q}_{rel\text{-}ex\ vivo}$	*P* value
x-Axis (left–right)	−0.010 ± 0.101	0.041 ± 0.199	0.2
y-Axis (dorsal–ventral)	−0.289 ± 0.227	−0.147 ± 0.114	0.03
z-Axis (caudal–cranial)	−0.386 ± 0.193	−0.176 ± 0.142	0.001
h-Axis (hilus–peripheral)	0.436 ± 0.133	0.236 ± 0.159	0.003

Values are the mean ± SD.
$\dot{Q}_{rel}$, relative pulmonary blood flow.

Statistical analysis

A paired, two-sided *t*-test was used to compare changes in $\dot{Q}_{rel}$ distribution (slopes of regression lines) along spatial axes: left–right (*x*-axis); dorsal–ventral (*y*-axis); caudal–cranial (*z*-axis); and hilus–peripheral (*h*-axis). Pearson's correlation was used to further analyse changes in $\dot{Q}_{rel}$. A one-sample, two-tailed *t*-test was used to compare changes in flow distribution to a hypothesized mean of zero.

All data are presented as the mean ± SD. Throughout the present study, $P < 0.05$ was considered to represent statistical significance. All analyses were performed using SPSS Version 10.0.7. (SPSS, Chicago, IL, USA).

RESULTS

The mean number of tissue samples obtained per lung was 67 ± 10. The average weight of the samples was 0.019 ± 0.012 g. The mean correlation of $\dot{Q}_{rel\text{-}in\ vivo}$ and $\dot{Q}_{rel\text{-}ex\ vivo}$ was 0.592 ± 0.188 (95% confidence interval 0.493–0.690). Isolation of the lungs significantly changed the spatial distribution of $\dot{Q}_{rel}$: $\dot{Q}_{rel}$ was redistributed to more ventral, cranial and central lung areas (Table 1). Figure 1 shows the changes in $\dot{Q}_{rel}$ distribution along the *y*-, *z*- and *h*-axes in one representative lung. Further analysis of these changes showed that $\dot{Q}_{rel}$ decreased in lung specimens with an initially high flow and increased in lung specimens with an initially low flow: The mean slope of $\dot{Q}_{rel\text{-}in\ vivo}$ versus $\dot{Q}_{rel\text{-}ex\ vivo}$ minus $\dot{Q}_{rel\text{-}in\ vivo}$ was −0.473 ± 0.305. These changes were significantly different from zero ($P < 0.001$, one-sample, two-tailed *t*-test). Figure 2 shows these changes in $\dot{Q}_{rel}$ distribution. The redistribution of $\dot{Q}_{rel}$ also led to a slight decrease in the mean coefficient of variation between the two conditions from 0.786 ± 0.221 to 0.724 ± 0.170 ($P = 0.3$).

DISCUSSION

The results of the present study confirm the hypothesis that spatial $\dot{Q}_{rel}$ distribution in isolated lungs is only poorly correlated with the *in vivo* situation: the slopes of the regression lines versus all spatial axes become less steep. Accordingly, $\dot{Q}_{rel}$ is redistributed to more ventral, cranial and central lung areas. In addition, $\dot{Q}_{rel}$ decreases in lung specimens with an initially high flow and increases in lung specimens with an initially low flow. Hence, the overall $\dot{Q}_{rel}$ distribution becomes more homogeneous after removal of the lungs from the thoracic cavity, but spatial gradients are still measurable. A multitude of factors could be influencing the results and, thus, explain these effects.

Neural control of pulmonary vascular tone is completely abolished. Intrapulmonary arteries of rabbits have dense adrenergic[13] and cholinergic[14] innervation. Adrenergic stimulation causes an increase in pulmonary vascular resistance and a decrease in pulmonary

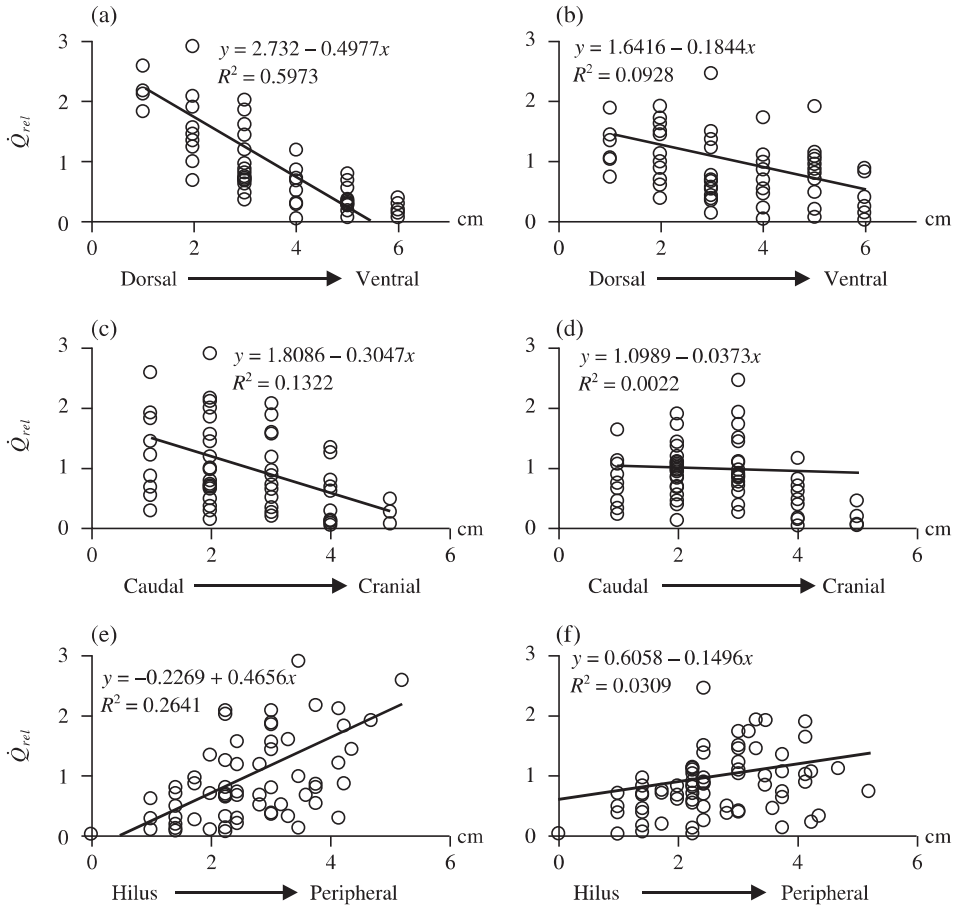

Fig. 1 Changes in relative pulmonary blood flow ($\dot{Q}_{rel}$) distribution along the (a,b) *y*- (dorsal–ventral), (c,d) *z*- (caudal–cranial) and (e,f) *h*-axes (hilus–peripheral) in one representative lung. The $\dot{Q}_{rel}$ (diminsionless) was redistributed to more ventral, cranial and central lung areas (see Table 1). A value less than 1.0 indicates that the specific tissue sample received less flow than the average; values greater than 1.0 indicate that the tissue sample received greater than average flow; $n = 75$ for all.

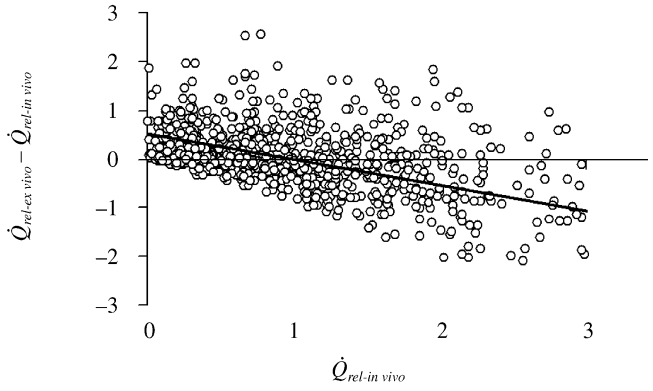

Fig. 2 Slope of $\dot{Q}_{rel\text{-}in\ vivo}$ versus $\dot{Q}_{rel\text{-}ex\ vivo} - \dot{Q}_{rel\text{-}in\ vivo}$ in all lungs ($y = 0.531 - 0.531x$; $R^2 = 0.3144$; $n = 942$). $\dot{Q}_{rel}$, relative pulmonary blood flow (dimensionless). These changes were significantly different from zero ($P < 0.001$; one-sample, two-tailed *t*-test).

vascular compliance and is considered to play a role in maintaining basal pulmonary vascular tone. The role of cholinergic stimulation is tone-dependent, inducing little change or a small contraction when tone is low, but vasodilatation when tone is elevated.[15] Almost all anaesthetics, including xylazine and ketamine, may affect pulmonary vascular tone, either by depressing the cardiovascular system, by modifying neural control[16] or by direct effects on vascular smooth muscles.[17–19] Accordingly, the anaesthesia performed may have affected *in vivo* $\dot{Q}_{rel}$ distribution.

The effects of partial pressures of respiratory gases on the regulation of pulmonary vascular tone are well known. They are important for local matching of ventilation and perfusion to maximize arterial oxygenation. Low inspiratory and mixed venous oxygen and high mixed venous carbon dioxide partial pressures induce contraction of vascular smooth muscle cells, whereas hyperventilation decreases pulmonary vascular tone. The isolated lung set-up is an established model to study the effects of changes in partial pressures of inspiratory gases on pulmonary vascular tone.[6,20] In our isolated lung preparation, alveolar partial pressures of these gases were maintained within the physiological range and, therefore, a major effect on spatial perfusion distribution can be excluded as factor. However, we did not measure blood gases prior to the first injection of microspheres and can therefore not exclude hypo- or hyperventilation during the *in vivo* assessment of $\dot{Q}_{rel}$.

Gravity influences the spatial distribution of pulmonary blood flow.[7,8] It was shown that probably 17–25% of the flow variability can be attributed to its influence[21] and that its influence can be even higher in specific conditions.[22] In our experimental set-up, we tried to minimize the effects of gravity on spatial blood flow distribution. We performed the *in vivo* measurement while holding the animals in an upright position, thus mimicking the lung position of the *ex vivo* preparation. Lung compression by mediastinal or abdominal structures reduces flow in the respective regions. Holding the animals in an upright position very likely decreased lung compression by abdominal structures. Caudal lung areas were probably distended and flow increased in these regions. Accordingly, the caudal–cranial gradient of $\dot{Q}_{rel\text{-}in\ vivo}$ was pronounced. Therefore, the difference seen

in the *ex vivo* preparation can probably be attributed to a decrease in lung distension. The distension caused solely by the lung weight in the excised lungs was probably less and therefore the caudal–cranial gradient of $\dot{Q}_{rel\text{-}ex\ vivo}$ decreased.

The geometry of the vascular bed is not static and blood flow within the pulmonary parenchyma is dependent on interstitial tissue pressures. These are determined by alveolar pressure, gravity and the interaction between the chest wall and the recoil pressure of the lung. In excised lungs, pleural pressure is uniform and alveolar distension is likely to be relatively uniform too. It is known that spatial gradients are abolished if lung volume is increased.[23] In our experimental set-up, we controlled the tidal volume by maintaining unchanged respiratory parameters before and after excision of the lung. Nevertheless, we observed a decrease in all spatial gradients. It is therefore likely that total compliance increases and total resistance decreases once the lungs are removed from the thoracic cavity. Therefore, a change in the size of the functional residual capacity appears possible.

It is well accepted that as perfusion pressure and, thus, flow increases, flow becomes more homogeneous. The mPAP after isolation of the lungs was 12 ± 3 mmHg. This is within the physiological range for rabbits[24] and we therefore believe that the perfusion pressures had only a minor affect on *ex vivo* $\dot{Q}_{rel}$ distributions. However, because we did not measure *in vivo* mPAP, we cannot exclude the possibility that some of the differences found in $\dot{Q}_{rel}$ distribution are not due to differences in perfusion pressures. We shortened the instrumentation time as much as possible and therefore did not cannulate central venous or arterial vessels. Holding the animals in an upright position may have caused a decrease in perfusion pressures, with different zonal conditions compared with the pump-perfused condition. Although we did not observe any indication that the animals did not tolerate the head-up posture, an effect on *in vivo* $\dot{Q}_{rel}$ distributions cannot be excluded.

The number of humoral factors known to affect pulmonary vascular tone and blood flow distribution increases continuously. A detailed discussion of all possible factors was not the aim of the present study. However, one critique of our experimental set-up could be the fact that we used a blood-free perfusate and non-pulsatile perfusion. Nitric oxide (NO) plays an important role in maintaining the physiologically low basal pulmonary vascular tone.[25] Its release is triggered by shear stress,[2] which is different in pulsatile compared with non-pulsatile perfusion. The role of erythrocytes appears to be at least twofold: mechanical deformation of erythrocytes induces ATP release, which stimulates NO production.[26] The consecutive decrease in pulmonary vascular tone is altered by an NO scavenger effect of haemoglobin.[1,27] Although it is likely that the pulmonary vascular tone was influenced by our experimental set-up, the effects should have been independent of the spatial coordinates of the individual lung piece.

Recently, Lamm and Albert investigated the effect of zonal conditions and posture on pulmonary flow distribution.[4] Analogous to the present experiments, they compared perfusion distribution before and after excision of the lungs. They found that weight-normalized flow to interior regions exceeded that to subpleural regions, whereas we found lower relative flows in central pulmonary areas. Their observed pattern of distribution was independent of posture and zonal condition. The principal, but not only, difference with the present study is that they used papaverine to ensure maximally dilated pulmonary vessels prior to the *in vivo* injection of microspheres. Lamm and Albert[4] perfused the lungs using a cell-free medium, as we did, but the perfusion was pressure controlled and not set to a fixed flow. In addition, no pulmonary afterload was applied and the animals/lungs were ventilated using a much higher tidal volume of 30 mL/kg. All this makes the comparison of these two studies difficult. Lamm and Albert[4] investigated the role of hydrostatic pressure and posture on flow distribution. Our aim was to show that the experimental set-up itself influences $\dot{Q}_{rel}$ distribution.

Spatial $\dot{Q}_{rel}$ distribution is not stable and may vary over time.[28,29] Unfortunately, we did not perform two time-separated injections and are therefore unable to quantify the changes over time. It is known that temporal heterogeneity of $\dot{Q}_{rel}$ is spatially clustered[28] and we therefore believe that this source of error is of minor importance in the present study.

The microsphere technique is well established for the investigation of pulmonary flow distribution. However, the technique itself incorporates several possible sources of error, which can be divided into stochastic and methodological errors.[30] The stochastic nature of microsphere entrapment follows a Poisson distribution and is usually minimized by increasing the number of microspheres injected,[31] as done in the present study. Regarding the methodological errors, two issues need to be addressed when interpreting the presented data: (i) non-uniform mixing; and (ii) viscosity. In the *in vivo* preparation, the microspheres were injected into a peripheral vein, whereas in the *ex vivo* preparation the microspheres were injected into the pulmonary artery catheter. Therefore, blood mixing in the *in vivo* condition was likely more homogeneous compared with the *ex vivo* condition using KHB. During the injections, the syringes were constantly gently moved but streaming effects cannot be ruled out completely. In addition to this, the possibility of slight bias because of rheological factors exists[32] and the use of cell- and plasma-free solution may have influenced the redistribution of perfusion. In our experiments, no changes in the coefficients of variation of the two injections were observed (see Results). This does not prove that the dispersion was not affected by the injection site, but an obvious bias could not be found. These additional methodological sources of errors further underline the limited use of isolated lung models to investigate spatial pulmonary flow distribution.

In conclusion, removal of the lung out of the thoracic cavity may significantly affect the spatial distribution of pulmonary flow. Therefore, results obtained from studies investigating $\dot{Q}_{rel}$ distribution in isolated lung models must be interpreted cautiously. An uncritical extrapolation of the results to the *in vivo* situation cannot be encouraged. The mechanisms by which the experimental set-up changes the $\dot{Q}_{rel}$ distribution are numerous and include physiological differences, such as loss of neural control, gravitational effects and changes in airway and interstitial pressures, as well as technical issues and methodological errors.

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REFERENCES

1. Deem S, Gladwin MT, Berg JT, Kerr ME, Swenson ER. Effects of *S*-nitrosation of hemoglobin on hypoxic pulmonary vasoconstriction and nitric oxide flux. *Am. J. Respir. Crit. Care Med.* 2001; **163**: 1164–70.

2. Ogasa T, Nakano H, Ide H *et al.* Flow-mediated release of nitric oxide in isolated, perfused rabbit lungs. *J. Appl. Physiol.* 2001; **91**: 363–70.
3. Weissmann N, Ebert N, Ahrens M *et al.* Effects of mitochondrial inhibitors and uncouplers on hypoxic vasoconstriction in rabbit lungs. *Am. J. Respir. Cell Mol. Biol.* 2003; **29**: 721–32.
4. Lamm WJE, Albert RK. Effect of zonal conditions and posture on pulmonary blood flow distribution to subpleural and interior lung. *J. Appl. Physiol.* 2000; **88**: 120–5.
5. Loer SA, Schlack W, Ebel D, Tarnow J. Effects of partial liquid ventilation on regional pulmonary blood flow distribution of isolated rabbit lungs. *Crit. Care Med.* 2000; **28**: 1522–5.
6. Oyamada Y, Mori M, Kuwahira I *et al.* Effects of active vasoconstriction and total flow on perfusion distribution in the rabbit lung. *Am. J. Physiol.* 1997; **273**: R1465–73.
7. West JB, Dollery CT. Distribution of blood flow and ventilation–perfusion ratio in the lung, measured with radioactive carbon dioxide. *J. Appl. Physiol.* 1960; **15**: 405–10.
8. West JB, Dollery CT, Naimaek A. Distribution of blood flow in isolated lung; relation to vascular and alveolar pressures. *J. Appl. Physiol.* 1964; **19**: 713–24.
9. Heller A, Fiedler F, Schmeck J, Luck V, Iovanna JL, Koch T. Pancreatitis-associated protein protects the lung from leukocyte-induced injury. *Anesthesiology* 1999; **91**: 1408–14.
10. Seeger W, Walmrath D, Grimminger F *et al.* Adult respiratory distress syndrome: Model systems using isolated perfused rabbit lungs. *Methods Enzymol.* 1994; **233**: 549–84.
11. Koch T, Duncker HP, Rosenkranz S, Neuhof H, van Ackern K. Alterations of filtration coefficients in pulmonary edema of different pathogenesis. *J. Appl. Physiol.* 1992; **73**: 2396–402.
12. Glenny RW, Bernard S, Brinkley M. Validation of fluorescent-labeled microspheres for measurement of regional organ perfusion. *J. Appl. Physiol.* 1993; **74**: 2585–97.
13. Cech S, Dolezel S. Monoaminergic innervation of the pulmonary vessels in various laboratory animals (rat, rabbit, cat). *Experientia* 1967; **23**: 114–15.
14. Cech S. Cholinesterase-containing nerve fibres on blood vessels in lungs of some laboratory mammals. *Z. Mikrosk. Anat. Forsch.* 1973; **140**: 91–100.
15. Hyman AL, Kadowitz PJ. Tone-dependent responses to acetylcholine in the feline pulmonary vascular bed. *J. Appl. Physiol.* 1988; **64**: 2002–9.
16. Ding X, Damron DS, Murray PA. Ketamine attenuates acetylcholine-induced contraction by decreasing myofilament Ca^{2+} sensitivity in pulmonary veins. *Anesthesiology* 2005; **102**: 588–96.
17. Hayashi Y, Minamino N, Isumi Y, Kangawa K, Kuro M, Matsuo H. Effects of thiopental, ketamine, etomidate, propofol and midozolam on the production of adrenomedullin and endothelin-1 in vascular smooth muscle cells. *Res. Commun. Mol. Pathol. Pharmacol.* 1999; **103**: 325–31.
18. Shimizu S, Ding X, Murray PA. Intravenous anesthetics inhibit capacitative calcium entry in pulmonary venous smooth muscle cells. *Anesthesiology* 2006; **104**: 791–7.
19. Teng B, Muir WW. Effects of xylazine on canine coronary artery vascular rings. *Am. J. Vet. Res.* 2004; **65**: 431–5.
20. Yamamoto Y, Nakano H, Ide H *et al.* Role of airway nitric oxide on the regulation of pulmonary circulation by carbon dioxide. *J. Appl. Physiol.* 2001; **91**: 1121–30.
21. Glenny RW, Lamm WJ, Albert RK, Robertson HT. Gravity is a minor determinant of pulmonary blood flow distribution. *J. Appl. Physiol.* 1991; **71**: 620–9.
22. Treppo S, Mijailovich SM, Venegas JG. Contributions of pulmonary perfusion and ventilation to heterogeneity in VA/Q measured by PET. *J. Appl. Physiol.* 1997; **82**: 1163–76.
23. Engel LA, Prefaut C. Cranio-caudal distribution of inspired gas and perfusion in supine man. *Respir. Physiol.* 1981; **45**: 43–53.
24. Hübler M, Souders JE, Shade ED, Polissar NL, Bleyl JU, Hlastala MP. Effects of perfluorohexane vapor on relative blood flow distribution in an animal model of surfactant-depleted lung injury. *Crit. Care Med.* 2002; **30**: 422–7.
25. Persson MG, Gustafsson LE, Wiklund NP, Moncada S, Hedqvist P. Endogenous nitric oxide as a probable modulator of pulmonary circulation and hypoxic pressor response *in vivo*. *Acta Physiol. Scand.* 1990; **140**: 449–57.
26. Sprague RS, Olearczyk JJ, Spence DM, Stephenson AH, Sprung RW, Lonigro AJ. Extracellular ATP signaling in the rabbit lung: Erythrocytes as determinants of vascular resistance. *Am. J. Physiol. Heart Circ. Physiol.* 2003; **285**: H693–700.
27. Heller A, Ragaller M, Schmeck J *et al.* Role of NO and endothelin in hemoglobin-induced pulmonary vasoconstriction. *Shock* 1998; **10**: 401–6.
28. Glenny RW, Polissar NL, McKinney S, Robertson HT. Temporal heterogeneity of regional pulmonary perfusion is spatially clustered. *J. Appl. Physiol.* 1995; **79**: 986–1001.
29. Glenny RW, McKinney S, Robertson HT. Spatial pattern of pulmonary blood flow distribution is stable over days. *J. Appl. Physiol.* 1997; **82**: 902–7.
30. Austin JR, Hauck WW, Aldea GS, Flynn AE, Coggings DL, Hoffmann JIE. Quantitating error in blood flow measurements with radioactive microspheres. *Am. J. Physiol.* 1989; **257**: H280–8.
31. Buckberg GD, Luck JC, Payne BD, Hoffmann JIE, Archie P, Fixler DE. Some sources of error in measuring regional blood flow with radiaactive microspheres. *J. Appl. Physiol.* 1971; **31**: 598–604.
32. Bassingthwaighte JB, Malone MA, Moffett TC *et al.* Validity of microsphere depositions for regional myocardial flows. *Am. J. Physiol.* 1987; **253**: H184–93.