Regional hypoxic pulmonary vasoconstriction in prone pigs

I. R. Starr, W. J. E. Lamm, B. Neradilek, N. Polissar, R. W. Glenny, and M. P. Hlastala. Regional hypoxic pulmonary vasoconstriction in prone pigs. J Appl Physiol 99: 363–370, 2005. First published March 17, 2005; doi:10.1152/japplphysiol.00822.2004.—Hypoxic pulmonary vasoconstriction (HPV) is known to affect regional pulmonary blood flow distribution. It is unknown whether lungs with well-matched ventilation (V)/perfusion (Q) have regional differences in the HPV response. Five prone pigs were anesthetized and mechanically ventilated (positive end-expiratory pressure = 2 cmH2O). Two hypoxic preconditions [inspired oxygen fraction (FIO2) = 0.13] were completed to stabilize the animal’s hypoxic response. Regional pulmonary blood flow distribution was determined at various FIO2 (0.21, 0.15, 0.13, 0.11, 0.09) using the fluorescent microsphere technique. Q and V in the lungs were quantified within 2-cm3 lung pieces. Pieces were grouped, or clustered, based on the changes in blood flow when subjected to increasing hypoxia. Unique patterns of Q response to hypoxia were seen within and across animals. The three main patterns (clusters) showed little initial difference in V/Q matching at room air where the mean V/Q range was 0.92–1.06. The clusters were spatially located in cranial, central, and caudal portions of the lung. With decreasing FIO2, blood flow shifted from the cranial to caudal regions. We determined that pulmonary blood flow changes, caused by HPV, produced distinct response patterns that were seen in similar regions across our prone porcine model.

HIGHLIGHTED TOPIC | Pulmonary Circulation and Hypoxia

Early studies demonstrated an increase in pulmonary perfusion nonuniformity (becoming more heterogeneous) during global hypoxia. Kuwahira et al. (15), in an exercised murine model, found that initially high flow areas were overperfused due to nonuniform HPV. Marshall and Marshall (17) found that the larger the hypoxic region is the less effective HPV is in diverting blood flow away from the hypoxic region. The complete HPV response has been described in both an isolated porcine model (28) and in an intact canine model (4). Bribioule et al. (4) found HPV to have a protective effect on arterial oxygenation. These studies additionally showed that, at sufficiently low arterial PO2 (Pao2), pulmonary vascular resistance (PVR) was reduced such that further reduction of Pao2 resulted in a decrease in resistance.

Two prior studies of human subjects exposed to hypoxia (30, 31) found that HAPE most severely affected peripheral lung regions, findings consistent with permeability and/or perfusion edema. Additionally, this group hypothesized that, at the initiation of HPV, the presence of uneven vasoconstriction is seen, further supporting the overperfusion/permeability leak theory of lung edema formation.

A recent article by Kleinsasser et al. (14) demonstrated that the pig is an excellent model of human HAPE because of the pig’s brisk HPV response (29). Two studies from our laboratory, one in supine pigs (12) and one in prone dogs (16), demonstrated a marked change in regional pulmonary blood flow with increasing hypoxia. By reconstructing the spatial blood flow distribution with the use of fluorescent microspheres, we found the hypoxic response to be unique across lung regions. Nonuniform HPV is in part related to regional nonuniformity of V/Q, as seen in our supine pig study (12), where regions of the lung were compressed by abdominal content. The prone dog study (16), and this present study in pigs, were designed to investigate the nonuniform and intrinsic response to hypoxia without large regional V/Q variation. To study the effects of normobaric hypoxia on pulmonary blood flow, we studied pigs in the prone posture. The prone porcine model, like the prone dog study, was chosen due to the lungs having originally well-matched V/Q. Comparison of these studies allows us to test whether regional HPV patterns are seen across species.

Furthermore, by measuring V and Q to small (~2 cm3) lung volumes, regional O2 (Prad) can be determined. Our study is designed to provide insight into the nature of the HPV nonuniformity in the relatively uniform (prone) lung in an animal...
with a brisk HPV response. We hypothesize that HPV is heterogeneous in the pig even in the prone position, which normally minimizes V/Q heterogeneity.

METHODS

Experimental protocol. The experimental protocol was approved by the University Washington Animal Care Committee. Pigs (n = 6) of either sex weighing ~20 kg were studied in the prone position. Ketamine/xylazine (im) was used to initially anesthetize the animals, and then a continuous thiopental drip (10–15 mg·kg⁻¹·h⁻¹) was titrated to suppress hemodynamic and motor responses to noxious stimuli. A tracheotomy was performed, and arterial and three venous lines were placed. Animals were mechanically ventilated with a constant-volume Harvard piston pump (Harvard Apparatus, South Natick, MA) with the following parameters: tidal volume (VT) of ~13 ml/kg, respiratory rate (RR) of ~20 breaths/min, and positive end-expiratory pressure of ~2 cmH2O. RR was adjusted to maintain arterial PCO2 at ~40 Torr. The pigs were ventilated with air during surgery.

A 7-Fr Swan-Ganz thermodilution catheter (Baxter, Irvine, CA) was advanced into the pulmonary artery to measure pulmonary arterial pressure (Ppa), pulmonary capillary wedge pressure, and temperature for mixed venous blood sampling. Cardiac output (CO) measurements (via the thermodilution technique) and blood temperature were measured with a CO computer (Sat-2, Baxter). Femoral venous catheters were inserted for infusion of anesthetic drugs, maintenance fluids, and fluorescent microspheres. Airway pressure, VT, RR, and minute ventilation were measured continuously with a digital spirometer (KORR, Medical Technologies Research Spirometry System, Salt Lake City, UT). End-expiratory CO2, RR, and hemoglobin saturation were continuously monitored with a CO2SMO (Novametrix, Medical System, Wallingford, CT). Systemic arterial pressure, Ppa, airway pressure, and heart rate were recorded with a Graphtec Mark 12 Data Managed System, DMS 1000 (Dewetron, Charlestown, RI). Vascular and airway pressures, end-expiratory CO2, and VT were digitally recorded with PowerLab (A-D Instruments, Colorado Springs, CO) on a Power personal computer. Arterial and mixed venous blood gases and hemoglobin were analyzed by the ABL-5 and OSM-3 hemoximeter machines, respectively (Radiometer, Copenhagen). Inspired O2 concentration was measured with a mass spectrometer (MGA-1100, Inspire-Elmer Medical Instruments, Norwalk, CT). Pigs were rotated to the prone posture and maintained on 2 cmH2O positive end-expiratory pressure during the duration of the experiment. Pigs were paralyzed initially with 1.5 mg of pancuronium bromide followed by 0.5 mg every 30 min thereafter.

To assure a consistent lung volume history and prevent regional atelectasis, lungs were briefly inflated (3 stacked breaths) to near total lung capacity and held for 2–4 s. After another 1–2 min of normal ventilation, expired CO2 concentration and lung volume were recorded in PowerLab at 100 samples/s. CO2 concentration was plotted against expired volume, and dead space was estimated by Fowler’s method (1, 6). To obtain a more stable hypoxic response, two 10-min preconditioning hypoxic periods [inspired O2 fraction (FiO2) = 0.13 and 0.09] were completed for each animal.

To study HPV response curves, six 20-min periods with FiO2 levels equal to 0.21, 0.15, 0.13, 0.11, 0.09, and 0.21 repeat were completed. The sequence of conditions (or runs) was as follows: air was always run 1 and air repeat was always run 6. The intervening hypoxic conditions were randomized. Fluorescent microspheres were administered as aerosols to mark ventilation as aerosols (1 mm) and injected intravenously (15 mm) to measure blood flow with a different color marking Q or VT during each condition. The only exception was the repeat air condition, where only Q was measured because of limited numbers of microsphere colors. Fluorescent microspheres were administered during the final 5 min of each condition. Systemic arterial pressure, Ppa, pulmonary capillary wedge pressure, CO, heart rate, airway pressure, FiO2, VT, RR, minute ventilation, end-expiratory CO2, and core body temperature were recorded during each air and hypoxic condition.

Postexperiment processing. After the final run, the animals were given heparin (5,000 U) and papaverine (30 mg) intravenously before being exsanguinated under deep anesthesia. The lungs were excised and dried with warm air for 3 days at ~25 cmH2O. Once dry, the lungs were coated with Kwik Foam (DAP, Dayton OH). The foamed lungs were then suspended vertically in a plastic-lined square box and embedded in rapidly setting urethane foam (2 lb. of Polyol and Isocyanate, International Sales, Seattle, WA). A rigid form was created to which a three-dimensional coordinate system was applied. Lungs were diced into ~2-cm³ pieces (854 ± 184 pieces), with spatial coordinates assigned and weights recorded for each piece.

Recovery of fluorescence. The fluorescent signal for each color was determined by extracting the fluorescent dyes from each piece with an organic solvent (Cellosolve Æ, Sigma-Aldrich, St. Louis, MO) and measuring the concentration of fluorescence in each sample (7). Spillover from adjacent colors was corrected using a matrix inversion method (25).

To compare V and Q among pieces, some being incomplete tissue cubes, each piece was weight normalized (WN) by dividing piece fluorescence by piece weight. To minimize the effect of nonalveolar tissue on weight normalization, pieces consisting of >20% airway tissue (as determined by eye) were not included. Q and V per piece were expressed as WN relative to the mean Q, (WNRQ) and WN relative to V (WNRV). We defined piece resistance as the overall pressure divided by the piece flow: (Ppa − pulmonary capillary wedge pressure)/piece Q, as measured by intravenous fluorescent microspheres. Pieces were clustered on the response pattern of the residuals of their WNRQ (WNRQ at each condition minus their mean piece WNRQ across all conditions) to increasing hypoxia using K mean clustering (without regard to spatial information).

Cluster analyses. The data were grouped into clusters using the K means method in the statistical software JMP (SAS Institute, Cary, NC). Pieces in a given group showed similar patterns of change in flow over time. The clustering was carried out on the residuals of WNRQ around the mean across the five hypoxia measurements (see Fig. 1). Before clustering, it was evident that there were many pieces whose flow changed little over time, and a cluster of nearly constant flow was formed; all lung pieces with a square root of the sum of the five squared WNRQ residual values of <0.22 were grouped into a single cluster and considered flat responders. The value of 0.22 was chosen as a cutoff because a piece with WNRQ residuals of ±0.1 (10% variation for a typical piece with flow 1.0) would have this cutoff, a level that would include normal variation over time (9). The remaining lung pieces were clustered with respect to their pattern of change. Cluster analysis is a statistical method for grouping items, such as pieces of lung, into “clusters” that share similar characteristics (5). A description of the K means clustering algorithms can be found in Hartigan’s study (11). Pieces that differed substantially from the mean profile of the cluster were removed from that cluster and did not appear in any cluster. For these excluded pieces (3–6% of the total number of pieces in the lung), the Euclidean distance was >0.41 from the mean of the five flows across the cluster, indicating a very substantial difference of a piece from its cluster mean. Again, the purpose was to identify some common patterns of flow across the hypoxic states and not to exhaustively classify every piece within the lung.

The entire clustering process was completed without reference to the spatial location of any piece. Once a cluster of pieces was defined by their flow response, the spatial location of these pieces was investigated as the potential locus for the associated regional HPV response.

Metacluster analyses. To identify lung pieces with common flow patterns across animals, all data sets were merged and the WNRQ residuals for each pig were adjusted by a multiplicative factor such
that the mean within-piece variance was the same for all animals. This multiplicative factor was close to unity for all pigs (see Ref. 12 for details). Metacluster analysis was then performed on this combined data set similar to the method above for the individual lung, resulting in clusters that included pieces from multiple animals.

**Testing for spatial clustering.** We used the permutation test for the null hypothesis that there is no spatial clustering of the pieces in the flow clusters. We have previously used and described this test (9). Briefly, if there is no spatial clustering, then the cluster designation of pieces can be randomly interchanged among the identified clusters without appreciably changing an appropriate quantitative measure of spatial clustering. Our measure of spatial clustering is a test statistic D, defined as the mean Euclidean distance between all possible pairs of pieces from the clusters, but only using pairs of pieces drawn from the same cluster. The value of the statistic D becomes smaller as the clusters become spatially more compact. To test the null hypothesis, the cluster labels were randomly permuted among all pieces of the lung (999 random permutations), and the statistic D was recomputed for each permutation. The P value was then calculated as (n + 1)/1,000, where n was the number of random permutations with the statistic D smaller than the value of D observed in the original, unpermuted data. We first carried out the permutation test for each of the animals separately (using the clusters defined from the meta-lung). For the entire meta-lung permutation test, cluster labels were randomly permuted only within each animal, and the pairs of pieces used in calculating D, the mean interpiece distance, consisted of all possible pairs drawn from within each cluster and within each animal. The grand mean interpiece distance, D, was calculated as a mean across all the resulting pairs from all animals.

**Statistical analyses.** All data presented are means ± SD, except where noted. The coefficient of variation (CV), where CV = SD/mean, is used to characterize the heterogeneity of V and Q at each FIO2. Repeated-measures ANOVA and Fisher’s protected least significant difference post hoc test were used to test changes in hemodynamic values from baseline at a FIO2 of 0.21. The P values from the repeated-measures ANOVA were adjusted for deviation from compound symmetry using the Greenhouse-Geisser epsilon. Quantile-quantile plots of residuals from the ANOVA models showed no material departure from normality. The paired t-test was used to compare the initial hemodynamic values at baseline (FIO2 = 0.21) to a repeat FIO2 of 0.21. Repeated-measures ANOVA and Fisher’s protected least significant difference post hoc test were used to test for differences in physiological and flow heterogeneity variables across FIO2 states. For each of these analyses, the observations analyzed were the summary measurements per animal (such as pH or the CV of perfusion) and not measurements of individual pieces. The several FIO2 levels per animal serve as the repeating factor in the ANOVA analysis. Statistical significance was designated by P < 0.05.

**RESULTS**

**Physiological data.** One study animal was withdrawn from experimental analysis due to questionable baseline FMS values due to a technical problem during the experiment, probably due to failure to fully reexpand the caudal-dorsal area of the lungs compressed while supine during surgery. At baseline the ln SD (V/Q) for this animal (0.74) was eight SD units above the mean of the other five animals (0.33). Therefore, this animal was not included in any table or analysis.

The physiological data shown in Table 1 shows changes between hypoxia runs. Additional data shown is the repeat air run 6, demonstrating how well the animals came back to initial baseline values. Data is presented as means ± SD as an average of all five pigs. With progressive hypoxia, vasoconstriction results in an increase in total PVR. Most hemodynamic and blood gas measures returned to baseline values after four 30-min periods of hypoxia.

Hypoxia had no significant effect on systemic arterial pressure. CO and Ppa increased with increasing hypoxia. At the most severe hypoxia condition (FIO2 = 0.09), CO was roughly 32% greater than at baseline. Arterial pH and arterial PO2 were not statistically altered by hypoxia. PaO2 decreased with increasing hypoxia.

**Fluorescent microsphere data.** The average perfusion and ventilation heterogeneity for all five animals are presented in Table 2. Progressive hypoxia led to an increase in perfusion nonuniformity as measured by the CV of WNRV. There was little effect on ventilation heterogeneity. Likewise, the V-to-Q correlation and the SD ln V/Q indicate an increasing mismatch of V/Q with increasing hypoxia. This increasing mismatch was seen in our laboratory’s prior two studies (12, 16).
Cluster analyses. Use of an accepted methodology for defining the number of clusters showed a broad continuum of responses rather than natural clusters (26). A division into three clusters (decreasing, increasing, and nearly constant across FIO2 levels) was a meaningful representation of the continuum, as shown in Fig. 1 for one representative animal. The nearly constant cluster was defined as noted in METHODS, and the increasing and decreasing clusters were then defined using K means clustering. With increasing hypoxia, the relative flow of most pieces fit into one of three clusters representing relatively constant flow (cluster 1), decreased flow (cluster 2), and increased flow (cluster 3). Differences in changing flow patterns with hypoxia indicate variable HPV in the lung.

Figure 2 presents the WNRQ and WNRV response to increasing hypoxia in one animal, with pieces grouped into their respective clusters (same animal as in Fig. 1). With hypoxia, WNRQ increased 30% in pieces in clusters 3 and decreased 30% in cluster 2. WNRV fluctuated <5% with increasing hypoxia. Each of our five study animals showed similar patterns of change in their WNRQ for the three clusters.

If regional HPV response is heterogeneous, our ability to measure PrO2 will allow us to examine whether the variation in response is due to the magnitude of constriction or sensitivity to PrO2. The complete HPV response has been described both in pigs (28) in the isolated lung and in intact dogs (4). Using a blood-perfused isolated pig preparation, Sylvester et al. (28) demonstrated a PrO2-dependent HPV response. The preparation allowed blood PrO2 to equilibrate with ventilated air causing PrO2 to be homogeneous throughout the entire lung. These studies also showed that, at a sufficiently low PrO2, PVR was reduced such that further reduction of PrO2 resulted in a decrease in resistance. We characterize the total lung resistance curve to be the flow-weighted sum of the contribution of individual response curves, each with differing magnitude and/or PrO2 at peak resistance. In doing so, we implicitly assume that the input and output pressures for each region are Ppa and pulmonary venous pressure, respectively.

We chose to compare our data to those of the previous studies by analyzing the resistance for each region. This allows better comparison within and across animals with varying COs.

A similarity in resistance response to increasing hypoxia was seen across pigs. Changes in WN resistance (related to the inverse of flow) with increasing hypoxia for each cluster within the same representative animal are shown in Fig. 3.

Lung vascular resistance increased with increasing hypoxia, with resistance reaching a maximum at an FIO2 of ~0.11–0.13. The HPV response to increasing hypoxia is similar in each cluster (Fig. 3). However, the clusters vary in the magnitude of their HPV response. Generally, cluster 3 showed the least increase in resistance, whereas cluster 2 showed the greatest increase in resistance. The range of mean PrO2 values averaged only 4 ± 2 Torr at the initial baseline FIO2 of 0.21 for the three clusters (Fig. 3). This indicates that these lungs had relatively uniform V/Q matching throughout the lung at the initial baseline condition.

The spatial distribution of pieces within each cluster is shown in the accompanying Fig. 4 for this one animal. Clusters were found to be in anatomically defined locations, which were similarly located across all five study animals. Pieces that increased their relative flow with progressive hypoxia were located in the dorsal/caudal lung region. Pieces that decreased their relative flow with progressive hypoxia were positioned in

Table 1. Physiological variables with progressive hypoxia

<table>
<thead>
<tr>
<th>FIO2</th>
<th>Psa, mmHg</th>
<th>Ppa, cmH2O</th>
<th>CO, l/min</th>
<th>PVR, cmH2O/min/</th>
<th>pH</th>
<th>PaCO2, Torr</th>
<th>PaO2, Torr</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.21</td>
<td>99±2</td>
<td>22±3</td>
<td>2.8±0.8</td>
<td>5.8±1.8</td>
<td>7.47±0.05</td>
<td>38±2</td>
<td>109±4</td>
</tr>
<tr>
<td>0.15</td>
<td>92±14</td>
<td>35±5</td>
<td>3.3±1.2</td>
<td>9.6±3.1</td>
<td>7.42±0.07</td>
<td>42±4</td>
<td>63±5</td>
</tr>
<tr>
<td>0.13</td>
<td>87±23</td>
<td>46±5</td>
<td>3.0±1.2</td>
<td>14.1±4.4</td>
<td>7.43±0.07</td>
<td>41±3</td>
<td>47±6</td>
</tr>
<tr>
<td>0.11</td>
<td>107±23</td>
<td>49±7</td>
<td>3.5±1.3</td>
<td>12.5±3.4</td>
<td>7.43±0.06</td>
<td>42±3</td>
<td>32±5</td>
</tr>
<tr>
<td>0.09</td>
<td>101±33</td>
<td>46±5</td>
<td>3.7±1.1</td>
<td>10.9±3.3</td>
<td>7.42±0.07</td>
<td>42±3</td>
<td>26±2</td>
</tr>
<tr>
<td>P Value (overall)</td>
<td>0.31</td>
<td>&lt;0.001</td>
<td>0.057</td>
<td>&lt;0.001</td>
<td>0.28</td>
<td>0.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>0.21R</td>
<td>91±12</td>
<td>27±3</td>
<td>2.8±0.9</td>
<td>8.1±2.8</td>
<td>7.48±0.05</td>
<td>40±4</td>
<td>106±8</td>
</tr>
</tbody>
</table>

Values are means ± SD as averaged across all 5 animals. FIO2, inspired O2 fraction; Psa, systemic arterial pressure; Ppa, pulmonary arterial pressure; CO, cardiac output; PVR, pulmonary vascular resistance; PaCO2, arterial PaCO2; PaO2, arterial PaO2; 0.21R, repeat of room air at the end of the hypoxia periods. Statistical significance based on repeated-measure ANOVA using Greenhouse-Geisser epsilon (overall P value) and Fisher’s paired least significant difference post hoc test for FIO2 of 0.21–0.09. *P < 0.05 vs. all other FIO2 levels. †P < 0.05 vs. 0.15 FIO2. ‡P < 0.05 vs. 0.13 FIO2. Statistical significance based on paired t-test for 0.21 vs. 0.21R. §P < 0.05 vs. 0.21 FIO2.

Table 2. Heterogeneity of weight-normalized relative blood flow and ventilation vs. FIO2

<table>
<thead>
<tr>
<th>FIO2</th>
<th>0.21</th>
<th>0.15</th>
<th>0.13</th>
<th>0.11</th>
<th>0.09</th>
<th>P Value (overall)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WNRQ CV per piece</td>
<td>0.26±0.05</td>
<td>0.26±0.04</td>
<td>0.28±0.02</td>
<td>0.33±0.04*‡‡</td>
<td>0.35±0.03*‡‡</td>
<td>0.003</td>
</tr>
<tr>
<td>WNRV CV per piece</td>
<td>0.29±0.04</td>
<td>0.30±0.05</td>
<td>0.29±0.04</td>
<td>0.29±0.04</td>
<td>0.30±0.04</td>
<td>0.41</td>
</tr>
<tr>
<td>V-to-Q correlation</td>
<td>0.87±0.04</td>
<td>0.85±0.07</td>
<td>0.83±0.09</td>
<td>0.80±0.09</td>
<td>0.76±0.12</td>
<td>0.15</td>
</tr>
<tr>
<td>SD ln VΔ/Q</td>
<td>0.33±0.05</td>
<td>0.28±0.07</td>
<td>0.30±0.08</td>
<td>0.37±0.11†</td>
<td>0.40±0.10‡</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Values are means ± SD for the coefficient of variation (CV) in piece ventilation (V) or perfusion (Q) and the correlation of V to Q at each FIO2 across all 5 animals. WNRQ, weight-normalized relative blood flow; WNRV, weight-normalized relative ventilation. Statistical significance based on repeated-measures ANOVA using Greenhouse-Geisser epsilon (overall P value) and Fisher’s paired least significant difference post hoc test. *P < 0.05 vs. 0.21 FIO2. †P < 0.05 vs. 0.15 FIO2. ‡P < 0.05 vs. 0.13 FIO2.
the ventral/cranial lung region. Pieces whose relative flow stayed nearly constant with progressive hypoxia were located in the central lung region.

Metacluster analyses. To determine how consistently these HPV response patterns were represented across animals, we performed metaclustering by combining the data from each animal into one data set (see METHODS). As it appeared for the individual analysis, there seemed to be three meaningful HPV response patterns that were well represented in each of the five animals (Table 3). Roughly 2–3% of the total lung pieces grouped into clusters 2 and 3 were excluded (see METHODS), whereas no pieces were excluded from cluster 1.

The mean WN resistance plotted against the mean PR$_O_2$ for each metacluster is shown in Fig. 5. Figure 6 presents the corresponding three-dimensional plot(s) for each individual lung color coded to the metacluster analysis. Pieces that were excluded are shown in gray.

All of the permutation tests for spatial clustering were highly significant. Whether it was each animal considered individually or the meta-lung from all animals combined, the null hypothesis of a random uniform distribution of cluster pieces within the lung or meta-lung was rejected with $P < 0.001$. In every case, the observed mean spatial distance between pairs of cluster pieces (D) was smaller than the 999 corresponding means from the random permutations of cluster labels. Furthermore, using the SD of the permutation distribution of D, the observed D was smaller than the mean permutation D by 16–63 SDs.

**DISCUSSION**

The key findings of this study are that the relative flow response to hypoxia is nonuniform, that HPV varies in the prone porcine lung in anatomically defined regions, and that regional HPV differences are not due to differences in the sensitivity of PR$_O_2$ but rather to the magnitude of regional constriction.

Methods evaluation. The methods used in this study have been evaluated before (8, 18–20, 24) and in our laboratory’s prior papers on this topic (12, 16). Fifteen-micrometer diameters are completely entrapped in the small pulmonary arterioles (19) and adequately reflect the regional pulmonary perfusion (3, 18). Aerosolized fluorescent microspheres were used to measure regional V˙ (19, 24).

At present, it is not possible to directly measure regional PO$_2$ within individual 2-cm$^3$ pieces. We determine PR$_O_2$ indirectly based on the Q˙ and V˙ to a region based on the fluorescent microspheres measurements. This approach was initially developed by Altemeier et al. (1) based on the mathematical relationships of Olszowka and Farhi (22). The method implicitly assumes that there is a negligible degree of heterogeneity within each of the individual 2-ml pieces. The ability of this method to predict measured PAO$_2$ under hypoxic conditions is good (12, 16).

In this study, we used pigs in the prone posture to minimize V/Q heterogeneity. The SD ln V/Q with room air was 0.33 ± 0.05, which is small for the normal lung. In contrast to our laboratory’s earlier study in supine pigs (12), in which the control SD ln V/Q on room air was 0.58, the current animals have quite uniform V/Q in the prone posture. In the supine pigs, we were able to show a dependence of HPV response on regional V/Q. In the present study, in animals with relatively homogeneous V/Q, we were able to show that the HPV response is also associated with spatial location. This association is striking because the cluster designations were developed completely independent of spatial location.
Fig. 4. Two views of the same lungs as in the previous figures showing lung pieces color coded by clusters. Gray pieces are excluded but in general fit originally into the cluster of the surrounding pieces.

**Sensitivity of magnitude and response to hypoxia.** As can be seen in the resistance graphs (Figs. 3 and 5), pieces in each cluster constrict at similar regional \( P_{O_2} \), although the shape of their resistance graph varies between clusters. We believe this to mean that pieces in a cluster differ from pieces in another cluster by the magnitude of constriction.

Two studies have examined the PVR vs. \( P_{O_2} \) profile in the pig (27) and the dog (4). Both studies showed a biphasic response with an increase to a maximum in PVR as \( P_{O_2} \) was reduced and a reduced PVR as \( P_{O_2} \) was further reduced below \( \sim 50 \) Torr. The biphasic pattern was seen in the response of the whole lung. In our experiments, this same biphasic pattern was seen in individual pieces (see Figs. 3 and 5). The reduced vascular resistance seen at extremely low \( P_{O_2} \) may be due to passive vasodilation due to regional anoxia and an elevated \( P_{Pa} \), or to vasodilation in response to regional nitric oxide (NO) release.

**Spatial variation in HPV response.** The HPV response shown as resistance vs. \( P_{O_2} \) in Fig. 5 shows an approximate relative resistance variation of \( \pm 23\% \) at the maximum resistance at a \( P_{O_2} \) of \( \sim 40 \) Torr. This study is the first to evaluate HPV within small regions in prone pigs and to show that variation in HPV is distributed in an anatomically defined pattern. The reasons for the spatial heterogeneity are not clear. Pelletier et al. (23) found regional variation in endothelium-mediated relaxation caused by a spatial difference in the magnitude of endothelial release of NO in horses. Regional release of NO may modulate the regional HPV response.

A few earlier studies have indicated spatial variation in the response to hypoxia. They observed differential blood flow increases in response to hypoxia, which was less in the upper regions and greater in the lower lung regions of sheep (21), human subjects (2), rats (15), and HAPE-susceptible subjects in the erect posture (10).

We speculate that regional differences in HPV may be due to uneven distribution of vascular smooth muscle arrangement and density, variation in production and/or receptor density of dilating mechanisms such as NO, and/or constricting mechanisms, such as endothelin. Our data suggests that there is a spatial variation in one or more of these mechanisms. In this context, the studies of Vock et al. (30, 31) in humans are particularly interesting in that they do not show a similar pattern of edema distribution with recurrent episodes of HAPE. This suggests that structural components may not be involved in the heterogeneity of HPV. It may be that the occurrence of HAPE alters the regional response of mediators or causes some restructuring to occur, altering subsequent responses.

The general response of regional resistance vs. regional \( P_{O_2} \) is similar, but not identical, among animals. Although the pig is thought to have a greater HPV response than the dog, the resistance response (shown in Fig. 5) is similar. The range of peak response of individual regions of between 0.33 and 0.52 cmH\(_2\)O·min·ml\(^{-1}\)·g\(^{-1}\) for the prone pig compares to a range of between 0.25 and 0.61 cmH\(_2\)O·min·ml\(^{-1}\)·g\(^{-1}\) in the prone dog. Although the mean response of pieces is similar between the two species, the heterogeneity of response magnitude is greater in the dog.

Our laboratory’s previous studies in supine pigs (12) and prone dogs (16) both showed regional differences in the HPV response, but there was a large variation between the two studies in the initial regional resistances and magnitude and even direction of the regional HPV response. Besides species difference, the supine position in the pig without positive end-expiratory pressure created lungs that were compressed under the abdominal content leading to areas with initially very high vascular resistance. Whereas hypoxia was shown to shift blood flow into these compressed areas, comparison of re-

**Table 3. Metacluster analysis: number of pieces in each metacluster within each animal**

<table>
<thead>
<tr>
<th>Animal (n)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>(%)*</td>
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<td>n</td>
</tr>
<tr>
<td>A (624)</td>
<td>254</td>
<td>(42%)</td>
<td>200</td>
<td>(33%)</td>
<td>150</td>
<td>(25%)</td>
<td>5</td>
</tr>
<tr>
<td>B (743)</td>
<td>341</td>
<td>(49%)</td>
<td>204</td>
<td>(29%)</td>
<td>150</td>
<td>(22%)</td>
<td>11</td>
</tr>
<tr>
<td>C (760)</td>
<td>320</td>
<td>(45%)</td>
<td>200</td>
<td>(28%)</td>
<td>191</td>
<td>(27%)</td>
<td>29</td>
</tr>
<tr>
<td>D (1008)</td>
<td>428</td>
<td>(45%)</td>
<td>281</td>
<td>(30%)</td>
<td>239</td>
<td>(25%)</td>
<td>19</td>
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<tr>
<td>E (945)</td>
<td>371</td>
<td>(41%)</td>
<td>290</td>
<td>(32%)</td>
<td>251</td>
<td>(27%)</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>1,714</td>
<td>(44%)</td>
<td>1,175</td>
<td>(30%)</td>
<td>981</td>
<td>(26%)</td>
<td>73</td>
</tr>
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</table>

*Expressed as % of total number of pieces for that individual animal or total number of pieces for all animals, without excluded pieces.
Regional HPV response to the more normal lungs of the prone dog was difficult to interpret. In this study of the prone pig, regional differences in the magnitude of the HPV response were again shown (Fig. 5). As in the prone dog, lung in the caudal dorsal area showed the lowest rise in magnitude of resistance due to hypoxia, whereas lung in the cranial ventral area showed the highest HPV response. This suggests that regional differences in HPV may be common across species but that other factors, like abdominal pressure, can alter the intrinsic spatial pattern of the HPV response.

It is entirely possible that part of the regional difference in HPV response is related, in some way, to variation in the time dependence of the HPV response in various regions. Although we cannot exclude this possibility at this time, we have found Ppa at 20 min after introducing a new hypoxia level, when the microspheres are infused, to be relatively stable.

A limitation of the permutation test for spatial clustering of the flow-based clusters is that it is sensitive not only to spatial clustering of a large number of pieces in a limited spatial region but, also, to spatial clustering in the form of a few clumps of neighboring pieces in dispersed spatial locations or to the pervasive local spatial clustering that may naturally occur due to the correlated flow changes of neighboring pieces. The null hypothesis of a spatially random distribution of pieces is violated by all three kinds of spatial clustering: “true” clustering of a number of pieces in a limited spatial region; several isolated, small clusters of neighboring pieces; and the local clustering due to the naturally occurring correlation of flow changes in neighboring pieces. Nonetheless, three-dimensional spatial displays reveal clustering on a large scale. The highly significant P values from the permutation test appear to reflect nonrandom spatial clustering in the scope of the entire lung rather than just a limited region.

Fig. 5. Means ± SE weight normalized resistance vs. P_{O_2} for all 3 metacollectors calculated from the means of the 5 animals.

Fig. 6. Lungs in Fig. 5 are again shown (A) along with views in the cranial to caudal axis of the other 4 lungs (B–E). Lung pieces are color coded by metaccluster shown in previous figures. Gray pieces are excluded but in general fit originally into the cluster of the surrounding pieces.
than as merely isolated local clustering or pervasive clustering due to correlation of neighboring pieces.

Potential significance of heterogeneity of HPV. Lung regions have been identified as showing regionally distinct HPV response patterns over increasing hypoxic conditions. Our porcine prone model (present study) showed a region with increasing flow, a region with decreasing flow, and a region where flow stayed relatively constant in response to progressive hypoxia. In the study by Hlastala et al. (12), we studied hypoxic changes in a supine porcine model. The model for this previous study had poor V/Q matching at baseline, likely due to abdominal contents impinging on the caudal portion of the lung. This study also found regional differences in HPV, variations likely augmented by the nonuniform V˙/Q˙ starting point. Another study from our laboratory (16) examined HPV in a prone canine model. Findings included five main patterns of relative pulmonary blood flow change with progressive hypoxia. In comparing these two prone studies, similar HPV responses are seen in the same spatial location across animal subjects (i.e., in both studies, the dorsal/caudal lung portion increased flow with increasing hypoxia).

REFERENCES