

## Exposure to Hypoxia Results in Uneven Pulmonary Blood Flow Distribution Prior to Pulmonary Edema

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The effects of hypoxia on pulmonary arterial pressure (PAP) and on development of pulmonary edema, ascertained by changes in lung water and pulmonary vascular permeability were studied in rats using bronchoalveolar lavage (BAL). Rats were exposed to hypobaric hypoxia ( $P_B=290$  Torr) for 24 h followed by 4 h of normobaric hypoxia ( $F_{I_{O_2}} 0.07$ ) (Hx). Controls were rats maintained in a normoxia (Nx). Mean PAP was  $28.3 \pm 0.8$  mmHg in Hx, and  $18.8 \pm 1.7$  mmHg in Nx (mean  $\pm$  SD). The wet-to-dry lung weight ratio was significantly higher in Hx. The ratio of fluorescence activity between BAL fluid and plasma 4 h after i.v. injection of FITC-albumin was higher in Hx, suggesting an increased pulmonary microvascular permeability in Hx. In a separate study, pulmonary blood flow distribution, measured after 10 min of hypoxia ( $F_{I_{O_2}} 0.07$ ) using non-radioactive microspheres, was significantly more heterogeneous than Nx, suggesting a non-homogeneous hypoxic pulmonary vasoconstriction. The combined data of both studies suggest that hypoxia induces heterogeneous pulmonary blood flow distribution which is followed by increased vascular permeability and the development of pulmonary edema.

**Key words:** high-altitude pulmonary edema (HAPE), hypoxic pulmonary vasoconstriction (HPV), non-radioactive microsphere, rat, fluorescein isothiocyanate (FITC)-albumin

### INTRODUCTION

Rapid ascent to altitudes above 2450 m can lead to high-altitude pulmonary edema (HAPE) in nonacclimatized individuals [7, 25]. HAPE occurs in previously healthy young individuals and occasionally may be severe and even fatal [1, 7, 21, 25]. Clinical investigations suggest that HAPE is a form of non-cardiogenic pulmonary edema with increased pulmonary vascular permeability and pulmonary hypertension but normal wedge pressure [9, 14]. In spite of intensive research, the mechanisms responsible for HAPE remain uncertain. On the one hand, some researchers attribute the pathogenesis of HAPE to hydrostatic mechanisms leading to capillary leak and pulmonary edema [2, 7, 8, 13, 31]. The normal wedge pressure in HAPE

suggests that the pulmonary hypertension is due to precapillary vasoconstriction, which is not consistent with elevated pulmonary capillary pressure and hydrostatic edema. The assumption underlying the hydrostatic mechanism of HAPE, however, is that hypoxic pulmonary vasoconstriction (HPV) is uneven [7, 31], resulting in overperfusion with dilatation and capillary injury and edema downstream from non-constricted areas [7, 31]. While this hypothesis is supported by the patchy X-ray appearance of HAPE [7], the role of a heterogeneous HPV on the production of HAPE has not been directly proven yet. Furthermore, support for this mechanism requires demonstration that heterogeneous HPV precedes the development of pulmonary edema. On the other hand, several researchers have proposed that an

inflammatory process plays an important role in the pathogenesis of HAPE [12, 15, 23]. The role of inflammatory mediators and activated neutrophils on other forms of pulmonary edema such as in the acute respiratory distress syndrome (ARDS) and re-expansion pulmonary edema is well known [10, 20, 28, 29]. Especially activated neutrophils play the most important role in the pathogenesis of lung injury, in which pulmonary vascular permeability increases [17, 18]. Myeloperoxidase (MPO) is contained only in azurophilic granules of neutrophils. MPO is released from activated neutrophils. For these reasons, measurement of MPO activity is used as an index of neutrophil activation in inflammatory disease such as ARDS and sepsis [6, 24].

The purposes of the present study were: 1) to determine the effects of severe hypobaric hypoxia on pulmonary arterial pressure, pulmonary vascular permeability, and lung fluid balance, 2) to ascertain whether there is an activation of neutrophils in the pathogenesis of HAPE, and 3) to evaluate the changes in regional pulmonary blood flow distribution induced by severe hypoxia are compatible with a hydrostatic mechanism of HAPE.

## METHODS

### Experimental Design

Two series of experiments were carried out: Study 1 was directed to study the effects of severe hypobaric hypoxia on the development of pulmonary edema and on tissue markers of inflammatory-dependent microvascular injury. Study 2 was directed to study the effect of severe hypoxia on the distribution of pulmonary blood flow using non-radioactive microspheres.

### Study 1.

#### Experimental Protocol (Fig. 1)

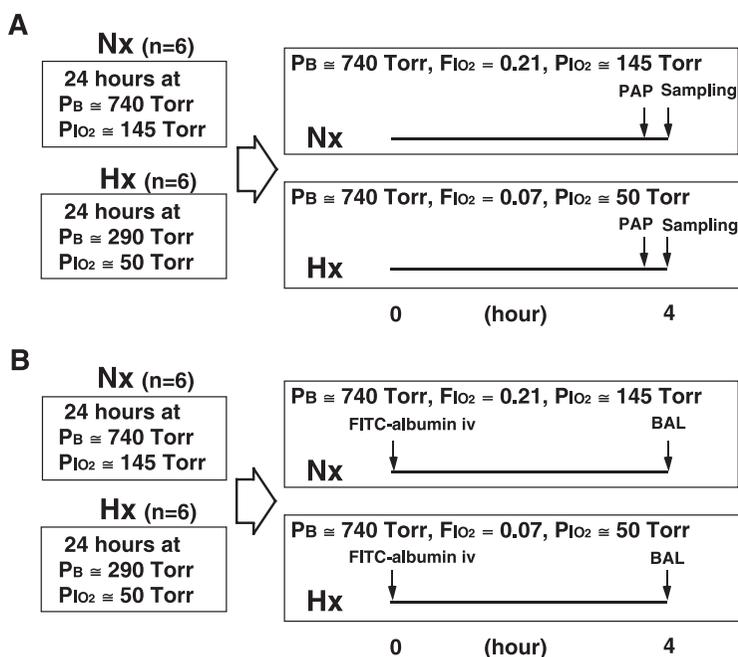
Twenty-four male Sprague-Dawley rats, 250-325 g (8-10 weeks of age) were assigned to a hypoxic (Hx) or a normoxic (Nx) group. The animals were anesthetized with sodium pentobarbital 40 mg/kg *i.p.*. Surgery was carried out using aseptic procedures. In one half of the animals (Fig. 1A), a polyethylene (PE50) catheter was implanted into the aortic arch via the left carotid artery, and a PE10 catheter was placed in the pulmonary artery (PA) via the right external jugular vein, with the use of a J-shaped plastic introducer.

Adequate positioning of the catheter in the PA was determined by the pressure wave form; placement of PA and other vascular catheters was confirmed at autopsy. In the remaining half of the animals (Fig. 1B), a PE50 catheter was placed into the superior vena cava (SVC) instead of in the pulmonary artery. In all cases, the catheters were filled with heparin, exteriorized at the back of the neck, cut at ~ 4 cm after emergence from the skin, and occluded with a short length of wire. The animals were allowed 24 h of recovery after surgery, after which the rats belonging to the Hx group were placed for an additional 24 h in a hypobaric chamber where air was circulated at a barometric pressure of 290 Torr, which resulted in an inspired  $P_{O_2}$  ( $P_{iO_2}$ ) of moist inspired air of ~ 50 Torr. The animals of the Nx group were kept in the same room at ambient barometric pressure (~ 740 Torr). All animals were allowed free access to water and standard rat chow. After 24 h in the chamber, the animals of the Hx group were maintained for 4 h in normobaric hypoxia ( $F_{iO_2}$  0.07) in a sampling chamber through which air circulated at the same  $P_{iO_2}$  as that of the hypobaric chamber (~ 50 Torr). The animals of the Nx group were also placed in the sampling chamber for 4 h at a  $P_{iO_2}$  of ~145 Torr. After 4 h in the sampling chamber, the following procedure was carried out in the Hx and Nx groups with a PA catheter (Fig. 1A): the mean pulmonary arterial pressure (PAP) value was recorded, and an arterial blood sample was obtained for determination of blood gases and total leukocyte count. The animals then received 1000 U/kg of heparin and were euthanized with an overdose of anesthetic (sodium pentobarbital *i.v.*, 80 mg/kg). The lungs were removed and blotted free of blood; the left lobe was used to measure tissue water content and the caudal lobe to measure tissue myeloperoxidase (MPO) activity.

In the Hx and Nx groups with an SVC catheter (Fig. 1B), fluorescein isothiocyanate (FITC)-labeled albumin (Sigma Chemical Co, St Louis MO), 50 mg/kg, was injected when the animals were placed in the sampling chamber. Four h later, a 5 ml blood sample was obtained for the determination of FITC concentration, and BAL was performed.

#### Bronchoalveolar Lavage (BAL)

The Nx and Hx groups fitted with SVC catheters were anesthetized with sodium



**Fig. 1** Experimental design and protocol of study 1.

- A.** Rats with a pulmonary arterial (PA) catheter and an arterial catheter. Nx: normoxic group ( $n=6$ ), Hx: hypoxic group ( $n=6$ ). Rats were exposed to normobaria or hypobaria for 24 h, then mean pulmonary arterial pressure (PAP) was measured at 4 h of normoxia or hypoxia, blood was sampled and lung tissue was harvested.
- B.** Rats with a superior vena caval (SVC) catheter and an arterial catheter. Nx: normoxic group ( $n=6$ ), Hx: hypoxic group ( $n=6$ ). At the time 0 h, FITC-albumin (50 mg/kg) was injected via a SVC catheter. At the time of 4 h, bronchoalveolar lavage (BAL) was performed after blood sampling.

pentobarbital 80 mg/kg i.v., 4 h after FITC-albumin injection. Immediately a tracheostomy was performed and a silicone tube (1 mm I.D., 2 mm O.D.) was placed in the trachea above the carina. BAL was performed using phosphate-buffered saline solution (PBS, pH 7.4). Ten ml of PBS were infused slowly into the lungs and withdrawn into the syringe. This procedure was repeated two more times. The amount of fluid recovered was ~80-90% of the infusion, and no difference in recovery was detected among groups. The BAL fluid was centrifuged at 3000 rpm for 5 min. FITC fluorescence and total protein concentration were measured in the supernatant. The pellet was suspended in 0.5 ml saline and the white blood cells (WBC) in the pellet were counted.

#### Assessment of Pulmonary Vascular Permeability

Pulmonary vascular permeability was

estimated from the fluorescence activity ratio of FITC-albumin in BAL fluid to  $100 \times$  diluted plasma (B/P ratio). Fluorescence was measured in plasma (diluted 100 fold with PBS, pH 7.4), and in BAL fluid, using a fluorometer (Hitachi F-3010, Hitachi Ltd. Tokyo, Japan) using excitation and emission wavelengths of 497 and 523 nm, respectively, with excitation and emission bandpass of 5 nm.

#### Measurement of Lung Water

After excision, the left lung was weighed before and after drying at  $90^\circ\text{C}$  for 48 h. Lung water was expressed as the wet-to-dry weight ratio (W/D ratio).

#### Measurement of Tissue Myeloperoxidase (MPO) Activity

The lung tissue was homogenized using an Omni 5000 tissue homogenizer. To release MPO from the primary granules of neutrophils, the tissue was homogenized in 0.5% HTAB buffer in 50 mM potassium phosphate buffer,

pH 6.0. The homogenate was freeze-thawed 3 times with 10 sec sonication before each freeze-thaw cycle, and then centrifuged at 16,000 G for 30 min at 4°C. The supernatant was assayed spectrophotometrically for MPO activity after mixing 50 ml of supernatant with 200 ml of substrate containing 0.167 mg/ml o-dianiside HCl and 0.0005% hydrogen peroxide in 50 nM potassium buffer at pH 6.0. Absorbance was determined at 450 nm every 3 min in an EL340 Automated Microplate Reader (Bio-Tek Instruments, Winooski, VT). Values at the 3rd minute were used for MPO since the relationship of MPO activity vs absorbance was linear at that time. One unit of MPO was defined as the quantity of MPO that would degrade 1 mmol of peroxidase per min at 25°C.

#### **Measurement of White Blood Cell (WBC) Count in Blood**

Blood WBC count was determined with a cell counter (Coulter Counter System ZM, Coulter Scientific Instruments, Houston, TX).

#### **Measurement of Total Protein Concentration**

We determined total protein concentration in BAL fluid and plasma by micro protein determination kit (Sigma Chemical Co. St. Louis MO).

## **Study 2**

### **Experimental Protocol**

Twenty male Sprague-Dawley rats were assigned to a normoxic (Nx) control group (n=10) or to a hypoxic (Hx) group, (n=10). Arterial and SVC catheters were placed using the method described in Study 1, using inhaled halothane as an anesthetic. Twenty-four h after recovery from surgery the animals were placed in a sampling chamber where  $P_{O_2}$  was maintained at 145 Torr. Arterial blood pressure and heart rate were recorded continuously. Once stable hemodynamic conditions were achieved, barium (Ba)- labeled microspheres suspended in 1 ml saline were injected in the SVC of the Hx group at  $P_{O_2}$  145 Torr. The  $P_{O_2}$  of the sample chamber was then reduced to ~50 Torr by mixing  $N_2$  and air at ambient  $P_B$ . Iodine (I)-labeled microspheres in equal amount and volume as in the first injection, were infused through the SVC 10 min after chamber  $P_{O_2}$  had reached 50 Torr. The same protocol was followed for the Nx group, except that  $P_{O_2}$  remained at 145 Torr during both microsphere injections. After the second microsphere injection, an arterial blood

sample was obtained for blood gas analysis. The animals were sacrificed with an overdose of anesthetic (sodium pentobarbital 80 mg/kg i.v.) and the lungs removed.

### **Determination of Pulmonary Blood Flow (PBF) Distribution**

PBF distribution was assessed from the distribution of 15  $\mu$ m diameter, inert plastic microspheres labeled with the stable elements Ba and I (Sekisui Plastic Co. Tokyo, Japan), as described in detail previously [16]. Each injection consisted of ~600,000 non-radioactive microspheres suspended in saline with Tween 80. The suspensions were sonicated for 5 min and mixed mechanically in a syringe immediately prior to and during the infusion to prevent microsphere aggregation. Microsphere infusion, followed by a flush of 0.5 ml saline, was carried out over a 2 min period. After the second infusion, the animals were heparinized, exsanguinated under deep anesthesia (sodium pentobarbital 40 mg/kg i.v.) and euthanized with a sodium pentobarbital overdose (another 40 mg/kg i.v.). A tracheostomy was performed and the lungs were inflated to a reproducible volume by the tracheal injection of 10 ml of 10% formalin solution. The chest was then opened, the lungs removed and immersed in 10% formalin solution for 3 days. After fixation, the lungs were cut into a total of 28 pieces following the procedure described by Kuwahira *et al.* [16]. Briefly, using a sharp surgical knife, each lung was cut into 5 transverse slices, 3.5 to 4.0 mm thick, along the caudal-ventral axis. These slices were cut into 3 pieces each except for the lowest slices of each lung, which were not subdivided, and the postcaval lobe of the right lung, which was cut into two slices. A total of 28 samples were obtained from both lungs. In order to decrease variability, the same person always performed the procedure. The samples were dried at 60°C for 48 h, weighed, and dissolved with 2 N KOH. After centrifugation, the supernatant was discarded and the microspheres were aspirated and trapped in filter papers. The X ray fluorescence activity of the microspheres trapped in the filter paper was measured by spectrometry using a wavelength dispersive spectrometer (PW 1480, Phillips). PBF distribution was estimated from the relative scatter (sr) of specific pulmonary blood flow in each sample (PBFsr), calculated as follows:  $PBFsr = (\text{sample activity} / \text{sample}$

**Table 1** Arterial blood gas in Study 1

	Nx (n =12)	Hx (n =12)
pH	7.47 ± 0.02	7.57 ± 0.03*
Paco <sub>2</sub> (Torr)	29.2 ± 2.1	14.5 ± 0.5*
Pao <sub>2</sub> (Torr)	79.5 ± 1.9	22.8 ± 2.1*
[HCO <sub>3</sub> <sup>-</sup> ] (mmol/l)	20.7 ± 1.6	12.7 ± 0.4*

Values are means ± SD

\*Significantly different ( $p < 0.05$ ) from the Nx group

dry weight) / (total lung activity / total lung dry weight)

Total activity was determined by adding up the activity of the 28 samples. A PBFsr value of 1 means that the sample blood flow is identical, per unit dry weight, to the blood flow of the lung as a whole; lower and higher PBFsr values mean that the sample receives less or more flow than the lung as a whole.

### Statistical Analysis

All data are presented as means ± SD. Student's t test for non-paired samples was used to compare the value of a given variable in the Hx group with that of its Nx counterpart. Pearson's correlation coefficient was used to assess correlation between variables. Fisher's r to z transformation was used to determine if a correlation coefficient was different from zero.

## RESULTS

### Study 1

Exposure to 24 h of hypobaria plus 4 h of hypoxic normobaria resulted in marked arterial blood hypoxemia (Pao<sub>2</sub> 22.8 ± 2.1 Torr) with the partially compensated respiratory alkalosis characteristic of prolonged hypocapnia (Table 1). Mean pulmonary arterial pressure (PAP), measured immediately before sampling 4 h after placement in the sampling chamber, was significantly higher in the Hx group (28.3 ± 0.8 mm Hg) than in the Nx group (18.8 ± 1.7 mmHg,  $p < 0.05$ , Fig. 2A). Severe hypoxia resulted in pulmonary edema, as demonstrated by the significantly higher W/D ratio of the Hx group (5.76 ± 0.11) compared to that of the Nx group (5.33 ± 0.11; Fig. 2B), and by the increased pulmonary vascular permeability as shown by the significantly higher fluorescence B/P ratio of

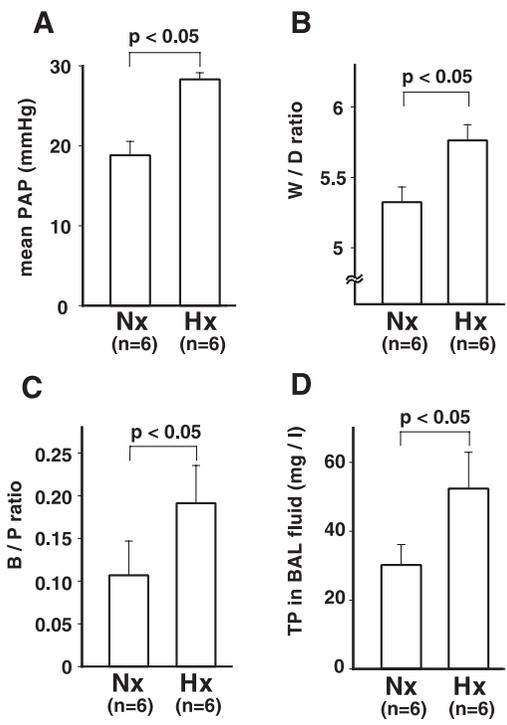
the Hx group vs the Nx group (Fig. 2C), and the significantly higher protein concentration of BAL fluid in the Hx group than in the Nx group (Fig. 2D). The protein concentration in BAL fluid was highly correlated with the B/P ratio (Fig. 3). Hypoxia resulted in a significantly higher WBC count in blood (Fig. 4A). Although the number of leukocytes in BAL fluid from the Hx group was higher than the Nx group, the difference was not significant (Fig. 4B). Lung tissue MPO activity, on the other hand, was significantly higher in the Hx group than in the Nx group (Fig. 4C).

### Study 2

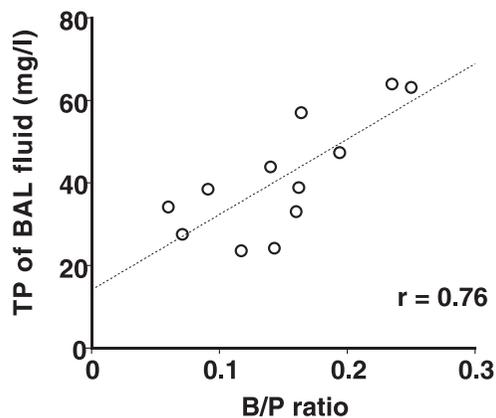
Severe hypoxia of 10 min duration resulted in marked arterial blood hypoxemia (Table 2) with marked hypocapnia and alkalosis. Due to the short length of exposure, no evidence of compensation of the alkalosis was observed. PBF distribution was markedly influenced by severe hypoxia. Figure 5 shows the values of PBFsr obtained with the second microsphere injection plotted as a function of PBFsr observed with the first microsphere injection. In the left panel (Nx group), both injections were made under normoxic conditions and show a high degree of reproducibility, as demonstrated by a correlation coefficient of 0.925 and a slope close to unity. The panel on the right shows the data in the Hx group, in which the second injection was made after 10 min of hypoxia. A correlation coefficient of the Hx group was 0.717, lower than that of the Nx group. These data suggest that severe hypoxia changed the pulmonary blood flow distribution.

## DISCUSSION

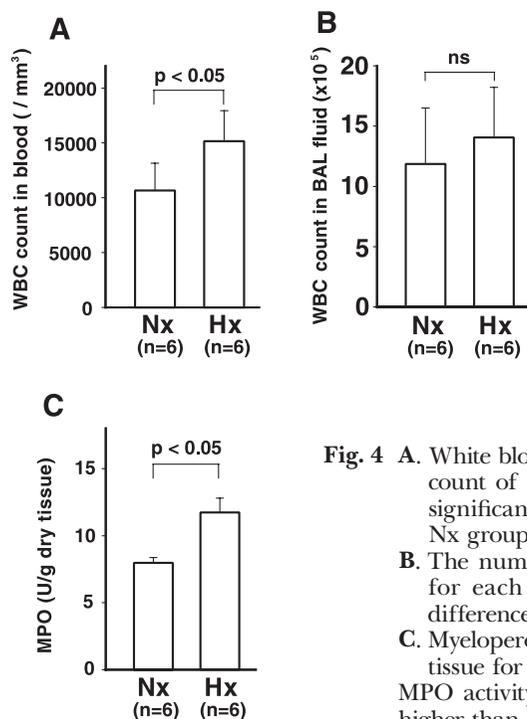
The present studies show that severe hypoxia of 28 h duration (Study 1) leads to



**Fig. 2** A. Effect of exposure to hypoxia on mean pulmonary arterial pressure (PAP). Hypoxia induced pulmonary hypertension due to HPV. B. Lung wet-to-dry weight (W/D) ratio. W/D ratio of the Hx group is significantly higher than that of the Nx group ( $p < 0.05$ ). The results suggest that pulmonary edema was induced in the Hx group. C. FITC-albumin leakage in alveolar space as represented by BAL fluid/plasma (B/P) ratio of fluorescent intensity in each group. B/P ratio of the Hx group is significantly higher than that of the Nx group ( $p < 0.05$ ). The results suggest that hypoxia increased pulmonary vascular permeability. D. Total protein concentration (TP) in BAL fluid. BAL fluid of the Hx group contained more protein than that of the Nx group ( $p < 0.05$ ).



**Fig. 3** Correlation and linear regression analyses between B/P ratio and TP in BAL fluid of all animals of both groups; Hx group ( $n = 6$ ), Nx group ( $n = 6$ ). There was a significant correlation between B/P ratio and TP in BAL fluid ( $r = 0.76$ ).



**Fig. 4** A. White blood cell (WBC) count in blood. WBC count of the Hx group ( $15,200 \pm 2,800$ ) was significantly higher ( $p < 0.05$ ) than that of the Nx group ( $10,700 \pm 2,500$ ).  
 B. The number of WBC in collected BAL fluid for each group. There was no significant difference in each group.  
 C. Myeloperoxidase (MPO) activity in dry lung tissue for each group. MPO activity of the Hx group was significantly higher than that of the Nx group ( $p < 0.05$ ).

**Table 2** Arterial blood gas in Study 2

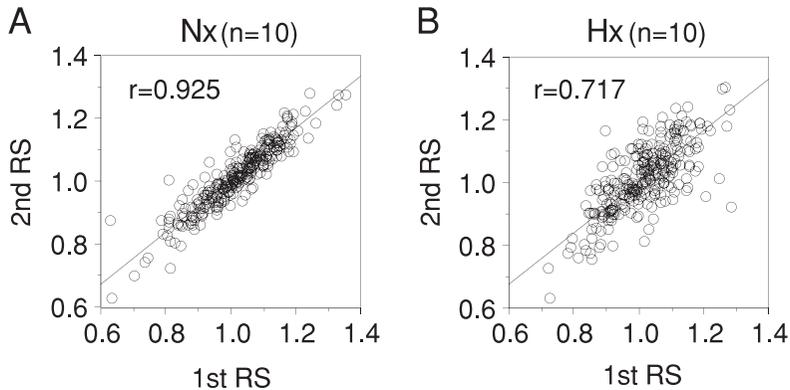
	Nx (n = 10)	Hx (n = 10)
pH	$7.44 \pm 0.04$	$7.61 \pm 0.04^*$
Paco <sub>2</sub> (Torr)	$37.2 \pm 4.6$	$22.5 \pm 1.9^*$
PaO <sub>2</sub> (Torr)	$94.5 \pm 7.8$	$23.7 \pm 0.8^*$
[HCO <sub>3</sub> <sup>-</sup> ] (mmol/l)	$24.7 \pm 2.5$	$22.5 \pm 0.6$

Values are means  $\pm$  SD

\* Significantly different ( $p < 0.05$ ) from the Nx group

marked pulmonary hypertension (Fig. 2A) which is accompanied by pulmonary edema. Production of pulmonary edema in this animal model is supported by the following observations: animals in the Hx group showed a significantly higher W/D ratio (Fig. 2B), indicating increased water content of lung tissue. The increase in lung tissue water necessary to produce the observed increase in W/D ratio associated with hypoxia is slightly higher than 10% of the total water content of the lung. This was accompanied by increased appearance of FITC-albumin in the BAL fluid, as evidenced by the higher fluorescence B/P ratio in the Hx group (Fig.

2C), and increased total protein concentration in the BAL fluid of the Hx group (Fig. 2D). Furthermore, BAL protein content was highly correlated with fluorescence B/P ratio (Fig. 3) suggests that the behavior of FITC-albumin is representative of the behavior of plasma protein, and supports the notion of increased pulmonary vascular permeability in the Hx group. These independent results can be interpreted as evidence of increased efflux of plasma protein across the capillary endothelium, the interstitium, and the alveolar epithelium to eventually be recovered by BAL. Taken together, these data indicate that severe hypoxia of this duration results in



**Fig. 5** Correlation and linear regression analyses between the 1st and 2nd relative scatter of specific pulmonary blood flow (PBFsr) in each sample. **A.** In the Nx group the 1st MS (Ba-MS) and the 2nd MS (I-MS) were injected at an interval of 10 min in normoxic state ( $n=10$ ). **B.** In the Hx group the 1st MS (Ba-MS) were injected in normoxic state and the 2nd MS (I-MS) were injected 10 min after the onset of hypoxia ( $n=10$ ). Correlation coefficient for the Hx group was lower than that for the Nx group. These data suggest that the pulmonary blood flow distribution was changed by exposure to severe hypoxia.

pulmonary edema, and that this represents a valid experimental animal model of HAPE.

In this study, mean PAP in the Hx group was  $28.3 \pm 0.8$  mmHg and that in the Nx group was  $18.8 \pm 1.7$  mmHg (Fig. 2A). Stelzner and coworkers [27] showed that hypobaric hypoxia ( $P_B=380$  mmHg) for 48 h increased mean pulmonary arterial pressure from  $21.4 \pm 0.7$  mmHg to  $27.8 \pm 0.9$  mmHg in Sprague-Dawley rats. West and coworkers [31] showed that hypobaric hypoxia ( $P_B=236$  mmHg) for 8 h increased pulmonary arterial or right ventricular systolic pressure from  $30.5 \pm 0.5$  mmHg to  $48.0 \pm 2.0$  mmHg.

The objective of the experiments of Study 2 was to determine if hypoxia results in changes in pulmonary blood flow consistent with heterogeneous HPV. HPV develops rapidly, and is well manifested within 1 min of hypoxia [3, 11, 19]. To support a role of heterogeneous HPV on HAPE, it is important to demonstrate that the change in blood flow distribution precedes the development of HAPE. Flow measurements made later in the exposure to hypoxia, once HAPE has started to develop, may reflect the effects of edema itself on the distribution of the blood flow, and lead to erroneous conclusions on the role of this factor as a mechanism of HAPE. In the present studies we chose to measure the distribution of pulmonary blood flow after 10 min of hypoxia, a time when HPV is already developed but in which it is unlikely that

edema may have developed to any significant extent. The results clearly show that hypoxia of this severity substantially alters the distribution of blood flow. Measurements made 10 min apart during normoxia are tightly correlated (Fig. 5A), indicating that pulmonary blood flow distribution is very stable in these conditions. In contrast, the lower correlation between normoxic and hypoxic blood flow distribution in the same animal indicates that the proportion of blood flow received by any given lung sample varies markedly between normoxia and hypoxia (Fig. 5B), such that areas which received a low proportion of blood flow in normoxia receive more in hypoxia, and viceversa. These results are consistent with a heterogeneous distribution of HPV, and the fact that the change in the distribution of pulmonary blood flow occurs early after the onset of hypoxia and is likely to precede the development of edema supports a role of a hydrostatic component in hypoxia-induced pulmonary edema. Capillary pressure is likely to be higher in the areas of higher blood flow, thus contributing to fluid extravasation. West and coworkers [32] raised pulmonary capillary transmural pressure in anesthetized rabbits to 40 mmHg and found disruption of the capillary endothelium and alveolar epithelium. Uneven HPV could result in overperfusion of some lung regions and dilatation of the capillaries and high flow

could result in capillary injury leading to pulmonary capillary leak and pulmonary edema [7, 31].

While the pattern of PBF distribution is consistent with a hydrostatic mechanism of HAPE, it is not clear if other mechanisms also participate. An inflammatory response to hypoxia is suggested by the elevated circulating WBC count (Fig. 4A), and the increased MPO activity in lung tissue (Fig. 4C). Inflammatory responses, with activation of leukocytes and increased adherence of leukocytes to vascular endothelial cells result in microvascular damage and increased vascular permeability as observed in other forms of lung disease such as ARDS and re-expansion pulmonary edema [17, 18, 20]. It is well known that activated neutrophils play an important role in the pathogenesis of increase in pulmonary vascular permeability [28, 29]. Tumor necrosis factor (TNF)- $\alpha$  is believed that one of the most important cytokines. The first step of ARDS is the activation of macrophages and the release of TNF- $\alpha$  from activated macrophages. Released TNF- $\alpha$  activates neutrophils, which play an important role in the pathogenesis of ARDS. Exposure to 10% O<sub>2</sub> in the inspired air results in increased leukocyte-endothelial interaction in venules of the mesenteric microcirculation of rats [33]. A comparable response to hypoxia in the pulmonary microcirculation would eventually result in the development of pulmonary edema, which could be exacerbated by the effects of increased capillary hydrostatic pressure. Clinical cases of HAPE are characterized by increased cell counts, neutrophils or alveolar macrophages, and elevated cytokines concentration in BAL fluid [15, 26]. Kaminsky and coworkers [12] reported that urinary leukotriene E<sub>4</sub> levels were elevated in patients with HAPE. Prevost and coworkers [23] showed that alveolar macrophages might be activated by hypobaric hypoxia in rabbits. Some researchers [4, 5] suggested that a preexisting upper respiratory infection predisposed the development of HAPE. Ono and coworkers [22] reported that neutropenia prevented the development of HAPE in rats. These studies indicate that inflammatory process plays important roles in pathogenesis of HAPE primarily or secondary. Accordingly, a role of increased endothelial-leukocyte interactions on the development of hypoxia-induced pulmonary edema should

be the subject of further research. Tsukimoto and coworkers [30] reported that exposing pulmonary capillaries to high transmural pressure resulted in a high-permeability form of edema and high level of leukotriene B<sub>4</sub> in BAL fluid. These results suggest an alternative possibility, namely that exposure to the reactive capillary endothelial basement membrane could induce release of chemical mediators. The present studies, nevertheless, show that in an animal model of HAPE, the initial vascular blood flow response to severe hypoxia is compatible with uneven hypoxic pulmonary vasoconstriction and a participation of a hydrostatic factor in the development of pulmonary edema.

In conclusion, the present studies show that severe hypoxia induces heterogeneous pulmonary blood flow distribution which is followed by increased vascular permeability and development of pulmonary edema. These features support a role of a hydrostatic component of HAPE secondary to overperfusion of some capillaries due to heterogeneous HPV. Additionally, the association of elevated blood WBC counts and increased lung tissue MPO activity suggest the presence of an inflammatory process which may contribute to the development of HAPE.

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