Splenic contraction augments diffusive O₂ transport in the lung of dogs

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Dane, D. Merrill, Connie C. W. Hsia, Eugene Y. Wu, Richard T. Hogg, Deborah C. Hogg, Aaron S. Estrera, and Robert L. Johnson, Jr. Splenectomy impairs diffusive oxygen transport in the lung of dogs. J Appl Physiol 101: 289–297, 2006. First published April 6, 2006; doi:10.1152/japplphysiol.01600.2005.—The spleen acts as an erythrocyte reservoir in highly aerobic species such as the dog and horse. Sympathetic-mediated splenic contraction during exercise reversibly enhances convective O₂ transport by increasing hematocrit, blood volume, and O₂-carrying capacity. Based on theoretical interactions between erythrocytes and capillary membrane (Hsia CCW, Johnson RL Jr, and Shah D. J Appl Physiol 86: 1460–1467, 1999) and experimental findings in horses of a postsplenectomy reduction in peripheral O₂-diffusing capacity (Wagner PD, Erickson BK, Kubo K, Hiraga A, Kai M, Yamaya Y, Richardson R, and Seaman J. Equine Vet J 18, Suppl: 82–89, 1995), we hypothesized that splenic contraction also augments diffusive O₂ transport in the lung. Therefore, we have measured lung diffusing capacity (DLCO) and its components during exercise by a rebreathing technique in six adult foxhounds before and after splenectomy. Splenectomy eliminated exercise-induced polycythemia, associated with a 30% reduction in maximal O₂ uptake. At any given pulmonary blood flow, DLCO was significantly lower after splenectomy owing to a lower membrane diffusing capacity, whereas pulmonary capillary blood volume changed variably; microvascular recruitment, indicated by the slope of the increase in DLCO with respect to pulmonary blood flow, was also reduced. We conclude that splenic contraction enhances both convective and diffusive O₂ transport and provides another compensatory mechanism for maintaining alveolar O₂ transport in the presence of restrictive lung disease or ambient hypoxia.

Blood volume; red cell volume; hematocrit; lung diffusing capacity; membrane diffusing capacity; pulmonary capillary blood volume; cardiac output; maximal oxygen uptake; exercise; spleen; canine

The spleen is a major erythropoietic organ in invertebrate animals but has become primarily a lymphoid organ in adult reptiles, birds, and mammals. In some marine diving animals or in highly aerobic land mammals (trained dogs, Thoroughbred horses, etc.) that depend on a high O₂ delivery for survival, the spleen also functions as a blood reservoir, as described by Barcroft and colleagues (2, 3). In response to sympathetic stimulation invoked by exercise, hypoxia, or hemorrhage, the spleen rapidly contracts to release extra erythrocytes (at a hematocrit of 80–90%) into the circulation (4) and then relaxes when the stimulus recedes (28, 39). Splenic contraction increases circulating hematocrit, blood volume, and O₂-carrying capacity during exercise 1.3- to 1.5-fold above resting levels; these indexes quickly return to baseline when exercise stops (40, 42). This form of reversible autologous blood doping is an efficient way of augmenting O₂ delivery during periods of metabolic stress. In athletic animals, systemic hematocrit can rise from ~40% at rest to nearly 60% at exercise (41, 42), and their maximal O₂ uptake (above 140 ml·min⁻¹·kg⁻¹) is almost twice that in the best endurance Olympic athletes, for whom blood doping is prohibited. The rise in blood volume and hematocrit constitutes one mechanism that allows these animals to achieve supra-high exercise capacities without the complications caused by hyperviscosity, because baseline hematocrit at rest remains normal. Although classically believed to enhance O₂ delivery, autologous erythrocyte infusion has also been reported to enhance peripheral tissue diffusing capacity in exercising Thoroughbred horses (40). The effect of erythrocyte infusion on O₂ transport in the lung has not been described.

Our laboratory has investigated the compensatory mechanisms in response to gas exchange impairment caused by 1) pneumonectomy and 2) high-altitude exposure. In both models, we observed superior adaptation in the dog compared with the human subject. We speculated that autoinfusion of blood by splenetic contraction contributes to a significant portion of the adaptive advantage in the dog; this mechanism could also reduce the disability imposed by existing pulmonary impairment such as the loss of a lung because of pneumonectomy. To understand better how dynamic autologous erythrocyte infusion by splenic contraction regulates O₂ transport in athletic species, we studied trained adult foxhounds before and after splenectomy to address the following questions: 1) How much augmentation of blood volume and hematocrit occurs by splenic contraction during exercise in the foxhound? 2) How does exercise-induced splenic contraction affect pulmonary gas exchange? Our results show that splenic contraction improves not only convective O₂ delivery but also lung carbon monoxide (CO)-diffusing capacity (DLCO) and its recruitment during exercise. Splenic contraction also mitigates the reduction in lung diffusing capacity imposed by pneumonectomy.

METHODS

Animals

The Institutional Animal Care and Use Committee approved all protocols and procedures. We studied six purpose-bred adult male foxhounds before and 7–10 mo after splenectomy. Two to 3 yr before the present study and as part of a separate project, two of these animals (19 and 26 kg) had undergone right pneumonectomy and four (22–24 kg) had undergone right thoracotomy without lung resection (Sham). In addition, all animals had bilateral carotid artery loops constructed under general anesthesia by an established method (32) to...
permit acute catheterization without the complications of chronic indwelling vascular catheters. The animals had been trained to run on a treadmill (see Exercise Training below); exercise studies were performed before and after pneumonectomy. To collect autologous blood, the jugular vein was catheterized under local anesthesia, and one unit (250 ml) of blood was removed, separated into erythrocyte and plasma fractions, and stored at −70°C for later reinfusion. After phlebotomy, blood volume was measured by a CO rebreathing method (see Blood Volume Measurement below). These procedures were repeated approximately once a month for ~6 mo. Iron supplementation was provided during this interval.

Surgery

After the completion of blood collections, splenectomy was performed. The animal was fasted overnight and sedated (acepromazine 0.2 mg/kg im). Anesthesia was induced with intravenous thiopental (up to 10 mg/kg) or propofol (4 to 8 mg/kg) and maintained with isoflurane inhalation. The animal was intubated and ventilated. The abdomen was shaved and prepared with betadine and alcohol. Through a midline abdominal incision, the spleen was isolated and the splenic vessels were ligated close to the hilum with silk ties. The vessels were severed between ligatures, and the spleen was removed. The abdomen was irrigated with sterile saline and closed with silk suture. Approximately 50–100 ml of normal saline were administrated intravenously during the operation. After surgery the animal was monitored in a recovery room until awake. Buprenorphine was administered daily for 7 days. The wound was dressed daily, and the sutures were removed in 10–14 days.

Exercise Training

The physical training program has been described previously (22). Before undergoing splenectomy, each dog had been trained to run voluntarily on a motor-driven treadmill for 30 min a day, 3–5 days a week at a workload equivalent to 60–80% of predicted or measured maximal O₂ uptake. Dogs were considered “trained” when reproducible values of O₂ uptake were obtained at each speed and incline, and when a maximum speed and incline was established beyond which exercise cannot be sustained for 5 min. It took ~3 mo to fully train a dog. Training continued throughout the course of the study and resumed 2 wk after splenectomy. The present exercise studies were carried out before and 4–9 mo after splenectomy.

Breathing Circuit

Each animal wore a customized, leak-free respiratory mask, which permits open-mouth breathing and protrusion of the tongue to facilitate cooling and salivary drainage (1). The mask was sealed around the muzzle with a modified latex glove and duct tape. A built-in cylindrical breathing orifice connects to a set of pneumographic respiratory valves and a rebreathing bag. Inspired and expired ventilation was measured by separate heated screen pneumotachographs (model 3813, Hans Rudolph, Kansas City, MO). Expired gas concentrations were sampled continuously by a mass spectrometer distal to a mixing chamber. Minute ventilation, O₂ uptake, CO₂ production, and respiratory rate were followed breath by breath and averaged over a predetermined number of breaths. Heart rate and rectal temperature were continuously recorded. All signals were digitized at 50 Hz.

Blood Volume Measurement

Before splenectomy, we measured blood volume in the conscious animal using a CO rebreathing method, which is inexpensive and suitable for monitoring blood volumes during repeated phlebotomy to collect autologous blood. However, this method is inconvenient for use during exercise; CO also alters the oxyhemoglobin dissociation curve and precludes simultaneous blood gas measurements. After splenectomy, we employed the Evans blue dye dilution method, which is less restrictive during exercise and has less effect on the oxyhemoglobin dissociation curve but is too expensive for repeated use. Direct comparisons were made to ensure that the two techniques yielded comparable results (Table 1).

CO rebreathing method. While wearing the respiratory mask, the animal rebreathed through a carbon dioxide absorber from a 5-liter anesthetic bag containing 100% O₂; sufficient O₂ was bled in to maintain a near-constant bag volume. Blood was drawn from a peripheral vein for the measurement of baseline fractional carboxyhemoglobin saturation (S₉⁵), hemoglobin concentration ([Hb], in g/dl), and hematocrit (in fraction). Then a known volume of CO ([QCO], ~25 ml ATPD) was added to the rebreathing bag and allowed to equilibrate for 10–15 min. A repeat venous blood sample was taken to measure the change in S₉⁵ (ΔS₉⁵), [Hb], and hematocrit. Blood volume (Q₉⁵, in ml) was calculated by mass balance:

\[
P₉⁵ \cdot \frac{273}{760} \cdot \frac{Q₉⁵}{[Hb]} \cdot 1.39 \cdot Q₉⁵ \cdot \Delta S₉⁵ = 1
\]

where P₉⁵ is barometric pressure (mmHg), t is room temperature (°C), and 1.39 is CO binding capacity of hemoglobin [ml (STPD)/g hemoglobin].

Total red cell volume (in ml) = Q₉⁵ \cdot hematocrit

Plasma volume (in ml) = Q₉⁵ \cdot (1 – hematocrit)

Evans blue dilution method. A venous catheter was inserted into each external jugular vein under local anesthesia. Evans blue dye (2 ml, 5 mg/ml) was injected from a calibrated syringe into one catheter; the syringe and catheters were flushed well with normal saline. Blood (3 ml) was sampled from the contralateral jugular catheter at 2, 5, 10, and 15 min after injection and centrifuged to separate plasma. The optical density of the dye in plasma was measured with a spectrophotometer (Beckman DU 640B, Beckman Coulter, Fullerton, CA) at 620 nm for first estimation of Evans blue concentration and then repeated at 740 nm to correct for any turbidity and free hemoglobin in the samples (15). The dye-disappearance curve was used to extrapolate the changes in concentration back to the time of dye injection for calculating plasma volume. Total blood volume was calculated from

Table 1. Hematocrit and blood volumes measured at rest and moderate exercise

<table>
<thead>
<tr>
<th>Method</th>
<th>CO 1 Pre-Rest</th>
<th>CO 2 7 mo Post-Rest</th>
<th>CO 3 7 mo Post-Rest</th>
<th>Evans Blue 1 8 mo Post-Rest</th>
<th>Evans Blue 2 8 mo Post-Rest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit, %</td>
<td>43.4±1.5</td>
<td>41.5±2.3</td>
<td>40.3±2.1*</td>
<td>40.2±2.4*</td>
<td>40.6±1.3†</td>
</tr>
<tr>
<td>Blood volume, ml/kg</td>
<td>107±13</td>
<td>106±15</td>
<td>103±20</td>
<td>109±18</td>
<td>102±20</td>
</tr>
<tr>
<td>Red cell volume, ml/kg</td>
<td>46±6</td>
<td>44±8</td>
<td>42±8</td>
<td>44±9</td>
<td>42±9</td>
</tr>
<tr>
<td>Plasma volume, ml/kg</td>
<td>61±7</td>
<td>62±8</td>
<td>62±13</td>
<td>65±10</td>
<td>61±12‡</td>
</tr>
</tbody>
</table>

Measurements were obtained in the conscious animal standing at rest or during exercise (50% of maximal workload). Values are means ± SD; combined data from all animals (n = 6) were used in paired t-test. CO, carbon monoxide. See text for explanation of groups. *P < 0.05 and †P < 0.01 vs. presplenectomy. ‡P < 0.05 Evans blue exercise vs. Evans blue rest postsplenectomy.
plasma volume and the systemic hematocrit. Erythrocyte volume was the difference between plasma volume and total blood volume. Measurements using this method were made post-splenectomy with the animal standing at rest and during moderate exercise (50% of maximal O₂ uptake).

Maximal O₂ Uptake

Under local anesthesia, a 5-Fr catheter was inserted into a jugular vein, flushed with heparinized saline, sutured to the skin, and connected to a manifold for blood sampling. After a 5-min warm-up period with the animal running at 6 mph, 0% grade, the treadmill speed was elevated to a predetermined constant level. The treadmill grade was incremented by 5% every 2 min until maximal workload was reached, signaled by volitional termination, heart rate exceeding 300 beats/min, or rectal temperature exceeding 41°C. Before exercise, during the last 30 s of each workload and every 2 min for 6 min after cessation of exercise, 3 ml of blood were drawn from the carotid artery for measuring lactate (YSI, Yellow Springs, OH), hemoglobin concentration (OSM-3, Radiometer, Copenhagen, Denmark), and hematocrit (by microcapillary centrifuge).

Rebreathing Measurement During Exercise

These methods have been described in detail elsewhere (23, 37). A rebreathing bag was prefilled with a volume of gas equal to average tidal volume at a given exercise intensity + 200 ml ATTD. The rebreathing gas mixture contained 0.6% acetylene, 0.3% C18O, 8–9% He, 30% O₂, in a balance of N₂ or in a balance of 100% O₂. At end expiration, a pneumatic valve was switched so the animal inspired from the bag and then rebreathed for 6–8 s while gas concentrations were monitored at the mouth. At a respiratory rate of 100–120 breaths/min during heavy exercise, we could reliably obtain 8–10 invocations were performed by paired t-test or repeated-measures ANOVA (StatView v.5.0, SAS Institute, Cary, NC). DLCO, DmCO, and Vc were plotted with respect to pulmonary blood flow; the slopes and intercepts of individual regression lines were compared as described by Zar (45). Because there was no significant difference between pneumonectomized and Sham-control animals in their responses to splenectomy, data from all animals were combined for the analysis of post-splenectomy changes relative to presplenectomy by using each animal as its own control. Where the absolute measurement in pneumonectomized animals differs significantly from that in Sham-operated animals, the results were summarized separately for each group to ensure an accurate report of their respective means and variance. A P value ≤0.05 was considered significant.

RESULTS

Hematology Measured at Rest and Moderate Exercise

These results did not differ between pneumonectomized and Sham-operated animals; therefore they were pooled for pre- to post-splenectomy comparisons (Table 1). The resting hematocrit was significantly reduced after splenectomy compared with before. Blood volumes measured at rest by the CO rebreathing method before (presplenectomy, CO 1) and on two occasions 7 mo postsplenectomy (CO 2 and CO 3) are shown; duplicate postsplenectomy results were highly reproducible (CO 2 vs. CO 3). Blood volumes measured at rest by the Evans blue dilution method at ~8 mo postsplenectomy at rest (Evans blue 1) and during moderate exercise (50% maximal O₂ uptake, Evans blue 2). Blood volumes measured at rest by the two independent techniques were similar (CO 2 and 3 vs. Evans blue 1). Splenectomy did not significantly alter circulating blood, plasma, or erythrocyte volumes at rest (CO 1 vs. CO 2, CO 3, or Evans blue 1). After splenectomy, circulating blood and erythrocyte volumes did not increase from rest to exercise whereas plasma volume declined by ~6% (Evans blue 1 vs. Evans blue 2).

Blood and Erythrocyte Volumes Estimated at Peak Exercise

Because we could not directly measure plasma volume at peak exercise and the measured change in plasma volume during moderate exercise was small (Table 1), for practical
purposes we assumed that plasma volume remained constant and used the measured changes in hematocrit to index the change in circulating erythrocyte volume at peak exercise. In all animals before splenectomy, systemic hematocrit increased as O2 uptake increased; after splenectomy hematocrit no longer increased and actually decreased from rest to peak exercise (Fig. 1). Before splenectomy, there was a direct relationship between changes in erythrocyte volume and O2 uptake in (Fig. 1). Before splenectomy, there was a direct relationship between changes in erythrocyte volume at peak exercise and O2 uptake during exercise before splenectomy. Assuming plasma volume was unchanged from rest to exercise, the increase in hematocrit directly reflects a similar proportional increase in erythrocyte volume in Sham and pneumonectomized (PNX) animals. Regression lines are through pooled data; Sham, solid line; right PNX, dashed line.

**Maximal O2 Uptake After Splenectomy**

Minute ventilation, O2 uptake, and CO2 output at peak exercise were significantly lower in pneumonectomized animals compared with Sham-operated animals (Table 2). Postsplenectomy maximal O2 uptake declined by a similar extent in Sham-operated and pneumonectomized animals (30 and 25%, respectively) compared with presplenectomy. There was a direct relationship between maximal O2 uptake and circulating erythrocyte volume estimated at exercise; the slope of this relationship was similar between Sham-operated and pneumonectomized animals as well as between foxhounds and published reports of Thoroughbred horses studied before and after splenectomy (40) (Fig. 3). In comparison, circulating erythrocyte volume changes relatively little from rest to exercise in human athletes, and maximal O2 uptake is only moderately enhanced when blood volume is elevated by autologous blood transfusion (8, 9) (Fig. 3).

**Pulmonary Diffusing Capacity After Splenectomy**

The absolute measurement of Dl,CO and its components at a given pulmonary blood flow was significantly lower in pneumonectomized animals than in Sham-operated animals (Table 3). At a given pulmonary blood flow postsplenectomy, Dl,CO, Dm,CO, Vc, as well as the Dl,CO estimated from Dm,CO and Vc declined by a similar extent in both groups of animals compared with their respective presplenectomy control values (Fig. 4). Pulmonary capillary blood volume measured at a given pulmonary blood flow changed variably, and the difference from pre- to postsplenectomy did not reach statistical significance. In addition, the slope of the relationships of Dl,CO and Dm,CO with respect to pulmonary blood flow changed variably, and the difference from pre- to postsplenectomy did not reach statistical significance. In addition, the slope of the relationships of Dl,CO and Dm,CO with respect to pulmonary blood flow changed variably, and the difference from pre- to postsplenectomy did not reach statistical significance.

**DISCUSSION**

**Summary of Results**

This study directly demonstrates the effect of splenectomy on exercise performance of foxhounds and provides the first experimental evidence of physiological interaction between blood volume and pulmonary O2 uptake by diffusion. After splenectomy, circulating hematocrit and blood volume were normal at rest, but exercise-induced polycythemia was eliminated. Hematocrit actually declined slightly postsplenectomy. These changes correspond to a 25–30% reduction in maximal O2 uptake in both Sham-operated and pneumonectomized animals before splenectomy, there was a direct relationship between maximal O2 uptake and circulating erythrocyte volume estimated at exercise; the slope of this relationship was similar between Sham-operated and pneumonectomized animals as well as between foxhounds and published reports of Thoroughbred horses studied before and after splenectomy (40) (Fig. 3). In comparison, circulating erythrocyte volume changes relatively little from rest to exercise in human athletes, and maximal O2 uptake is only moderately enhanced when blood volume is elevated by autologous blood transfusion (8, 9) (Fig. 3).

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membrane; \( V_c \), pulmonary capillary blood volume; \( D_{LO2} \), lung \( O_2 \)-diffusing capacity. Data from all animals were combined for paired
tomy (solid lines; Sham
erthrocyte (RBC) volume in individual foxhounds before and after splenec-
tomy, whereas pulmonary capillary blood volume changed
variably. The slope of recruitment in lung and membrane
tomy, whereas pulmonary capillary blood volume changed
animals. At any given cardiac output, lung and membrane
diffusing capacities were significantly reduced after splenectomy,
whereas pulmonary capillary blood volume changed
variably. The slope of recruitment in lung and membrane
diffusing capacity with respect to pulmonary blood flow was
also reduced after splenectomy, indicative of impaired alveolar
microvascular recruitment. The Sham and pneumonectomy
groups exhibited similar responses to splenectomy. Thus the
autoinfusion of erythrocytes by splenic contraction signifi-
cantly augmented convective as well as diffusive \( O_2 \) transport.

**Critique of Methods**

The present report is one component of a chronic series that
required 5 yr to complete. The two survival operations (thorac-
totomy with or without pneumonectomy and splenectomy) were separated by 2.5 to 3 yr. In pneumonectomized animals,
splenectomy did not impair maximal \( O_2 \) uptake more than in
Sham-operated animals and postpneumonectomy maximal \( O_2 \) up-
take remained relatively high (\( \sim 70 \text{ ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1} \)). Because
of the chronicity and the consistent response pattern to splenec-
tomy in 6 animals, we did not feel it necessary to study
more pneumonectomized animals. All animals continued reg-
ular exercise training throughout the study and remained
healthy, fit, and well socialized. With special permission from
our institution, two animals were adopted by staff members at the end of the study.

Our estimates of hematocrit (43.4%) and erythrocyte volume
(46.3 ml/kg) in unsedated resting animals presplenectomy were
significantly higher than that reported in sedated or anesthe-
tized adult dogs (average hematocrit 37.2–39.8% and erythro-
cyte volume 29.3–34.2 ml/kg) (13, 26), consistent with the basal sympathetic tone that keeps the spleen partially
contracted in the awake resting state. We estimated the erythrocyte
volume at peak exercise from the measured hematocrit, assum-
ning that resting plasma volume remained unchanged during
exercise. From rest to moderate exercise, we found only a
modest change (\( \sim 6\% \)) in plasma volume postsplenectomy in
these foxhounds, whereas Sarelius (33) had reported no change
or a modest increase in plasma volume (0 to +11.8%) in
splenectomized greyhounds. These modest changes in plasma
volume do not alter our conclusions in the present study.

Simulation studies have suggested that \( O_2 \)-dependent distri-
bution of CO flux across the erythrocyte membrane could alter
\( D_{mCO} \), lung \( O_2 \)-diffusing capacity; \( D_{mCO2} \), diffusing capacity of the alveolar-capillary
membrane; \( V_c \), pulmonary capillary blood volume; \( D_{LO2} \), lung \( O_2 \)-diffusing capacity. Data from all animals were combined for paired \( t \)-test (\( n = 6 \)); *\( P < 0.05 \),
\( \dagger P < 0.01 \), ‡\( P < 0.001 \) presplenectomy vs. postsplenectomy.

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**Table 3. Rebreathing data at heavy exercise**

<table>
<thead>
<tr>
<th></th>
<th>Pre- or Postsplenectomy</th>
<th>Sham</th>
<th>Post</th>
<th>Postpneumonectomy</th>
<th>Combined</th>
<th>Pre vs. Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>23.0±0.9</td>
<td>25.2±3.5</td>
<td>22.7±2.5</td>
<td>28.4±7.1</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>( O_2 ) uptake, mlin^{-1}kg^{-1}</td>
<td>108±43</td>
<td>74±18</td>
<td>81±15</td>
<td>56±18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( CO_2 ) output, mlin^{-1}kg^{-1}</td>
<td>98±42</td>
<td>60±14</td>
<td>71±15</td>
<td>41±7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventilation, lmin^{-1}kg^{-1}</td>
<td>6.9±3.3</td>
<td>5.4±1.2</td>
<td>5.6±1.3</td>
<td>4.0±0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>191±15</td>
<td>254±13</td>
<td>275±5</td>
<td>239±17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary blood flow, lmin^{-1}kg^{-1}</td>
<td>735±92</td>
<td>708±17</td>
<td>558±132</td>
<td>568±227</td>
<td></td>
<td></td>
</tr>
<tr>
<td>End-expiratory lung volume, ml/kg</td>
<td>117±23</td>
<td>101±17</td>
<td>92±17</td>
<td>71±26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>End-inspiratory lung volume, ml/kg</td>
<td>166±19</td>
<td>149±21</td>
<td>137±12</td>
<td>106±42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( DL_{CO} ), mlmin^{-1}mmHg^{-1}kg^{-1}</td>
<td>1.92±0.17</td>
<td>1.57±0.36</td>
<td>1.16±0.47</td>
<td>0.89±0.27</td>
<td>†</td>
<td></td>
</tr>
<tr>
<td>( DL_{CO2} ), mlmin^{-1}mmHg^{-1}kg^{-1}</td>
<td>2.84±0.35</td>
<td>2.11±0.43</td>
<td>1.57±0.58</td>
<td>1.14±0.28</td>
<td>‡</td>
<td></td>
</tr>
<tr>
<td>( V_c ), ml/kg</td>
<td>6.40±0.73</td>
<td>8.85±4.69</td>
<td>4.07±0.87</td>
<td>7.98±5.26</td>
<td>‡</td>
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<tr>
<td>( DL_{CO} ), mlmin^{-1}mmHg^{-1}kg^{-1}</td>
<td>3.12±0.34</td>
<td>2.37±0.52</td>
<td>1.75±0.63</td>
<td>1.34±0.36</td>
<td>‡</td>
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<tr>
<td>Septal volume, ml/kg</td>
<td>28.9±11.4</td>
<td>25.2±4.2</td>
<td>13.2±1.5</td>
<td>12.9±4.7</td>
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Values are means ± SD: Sham (\( n = 4 \)), pneumonectomized (\( n = 2 \)); \( DL_{CO} \), lung \( CO \)-diffusing capacity; \( DL_{CO2} \), diffusing capacity of the alveolar-capillary
membrane; \( V_c \), pulmonary capillary blood volume; \( DL_{CO} \), lung \( O_2 \)-diffusing capacity. Data from all animals were combined for paired \( t \)-test (\( n = 6 \)); *\( P < 0.05 \),
\( \dagger P < 0.01 \), ‡\( P < 0.001 \) presplenectomy vs. postsplenectomy.

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**Fig. 3.** Comparison of the relationship of maximal \( O_2 \) uptake to circulating
erthrocyte (RBC) volume in individual foxhounds before and after splenec-
tomy (solid lines; Sham
erthrocyte (RBC) volume in individual foxhounds before and after splenec-
tomy, whereas pulmonary capillary blood volume changed
variably. The slope of recruitment in lung and membrane
diffusing capacity with respect to pulmonary blood flow was
also reduced after splenectomy, indicative of impaired alveolar
microvascular recruitment. The Sham and pneumonectomy
groups exhibited similar responses to splenectomy. Thus the
autoinfusion of erythrocytes by splenic contraction signifi-
cantly augmented convective as well as diffusive \( O_2 \) transport.
Fig. 4. Relationships of lung CO-diffusing capacity (DLCO; A), membrane CO-diffusing capacity (DmCO; B), capillary volume (Vc; C), and lung O2-diffusing capacity (DLO2) derived from DmCO and Vc (D) to pulmonary blood flow pre- and postsplenectomy are shown for Sham-operated (left) and pneumonectomized (right) animals. Means ± SD. *P < 0.05 pre- vs. postsplenectomy.

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Table 4. Average slope of recruitment with respect to pulmonary blood flow

<table>
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<th>Postpneumonectomy</th>
<th>Combined</th>
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<td>Post</td>
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<td>DLco</td>
<td>2.18±0.60</td>
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<td>Dmco</td>
<td>3.45±0.71</td>
<td>2.37±0.38</td>
<td>1.72±0.42</td>
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<tr>
<td>Vc</td>
<td>4.88±3.42</td>
<td>9.08±4.89</td>
<td>6.69±0.60</td>
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<td>Dol2</td>
<td>3.71±0.85</td>
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<td>2.06±0.36</td>
<td>1.52±0.80 *</td>
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Values are means ± SD; Sham (n = 4), pneumonectomized (n = 2). The units are as shown in Figure 4. Data from all animals were combined (n = 6) for statistical comparison. *P < 0.05 presplenectomy vs. postsplenectomy.

ment also holds in the present animals (unpublished observations).

Splenic Contribution to Exercise Capacity

In aerobic animals. Joseph Barcroft noted in the 1920s that the spleen of dogs and cats contracts in response to physiological stress (2, 3) and thought the contraction might mobilize erythrocytes to enhance O2 transport. In seals, horses, and dogs (4, 14, 38), the spleen sequesters nearly 50% of total erythrocyte volume or 13% of blood volume at a hematocrit of 85–90%. α-Adrenergic-mediated splenic contraction during exercise, hypoxia, or blood loss releases the sequestered erythrocytes into the circulation. Vatner et al. (39) studied untethered instrumented dogs during exercise before and after splenectomy and reported that mobilization of erythrocytes raised systemic hematocrit from ~40% at rest to ~49% at peak exercise. After splenectomy, hematocrit no longer increased, but the capability for severe exercise in the dog appeared identical to that before splenectomy, leading to the conclusion that the spleen contributed to the exercise response by raising hematocrit and O2 transport sufficiently to obviate the necessity for reducing visceral blood flow but did not actually augment exercise capacity (39). Later, Longhurst et al. (31) measured exercise capacity and maximal O2 uptake in mongrel dogs before and after splenectomy and again before and after administering α-adrenergic receptor blockade to prevent splenic contraction. They found a 13% reduction in maximal O2 uptake after splenectomy, significantly less than our finding of 25–30% reduction in Sham and pneumonectomized foxhounds. The difference between our finding and that of Vatner et al. and Longhurst et al. most likely reflects differences in aerobic conditioning; these investigators studied mongrel dogs with a presplenectomy maximal O2 uptake of 95 ml·min⁻¹·kg⁻¹ of body weight. On the other hand, the Thoroughbred horses studied by Wagner et al. (40) and shown in Fig. 3 reach a maximal O2 uptake of 148 ml·min⁻¹·kg⁻¹, similar to that in our Sham-operated foxhounds (144 ml·min⁻¹·kg⁻¹). In these horses, the average reduction in maximal O2 uptake by splenectomy (31%) is also identical to that in Sham-operated foxhounds.

In humans. Compared with aerobic species, the small human spleen has a volume around 100 ml at rest (35) and serves mainly as an immunological organ and a site for erythrocyte breakdown and iron storage. Even so, maximal exercise in normal subjects induces a 58–66% reduction in spleen volume (29, 36); total body erythrocyte and blood volume do not change from pre- to postexercise, but plasma volume decreases by 18.9% associated with a 4–5% absolute increase in hematocrit (36). Maximal O2 uptake correlates with hemoglobin mass and blood volume, which are 35–40% higher in endurance athletes (average 15.3 g/kg and 107.1 ml/kg, respectively) than in untrained persons (average 11.0 g/kg and 78.3 ml/kg, respectively) (17). These average values in athletes are similar to our measurements in foxhounds. The change in maximal O2 uptake due to blood volume manipulation by venesection or autologous transfusion in human subjects is modest and variable, ~10–13% (8, 9).

Splenic Contribution to Lung Diffusing Capacity

Splenic contraction is generally thought to augment convective O2 transport by increasing cardiac output as well as the O2-carrying capacity of blood. Splenectomy in dogs abolishes the increase in cardiac output induced by acute hypoxemia (30). The increase in DL CO with blood volume or hematocrit is usually attributed to a higher Vc and pulmonary blood flow that recruits previously closed capillaries and enlarges the hemoglobin sink for CO uptake. However, a comparative study from our laboratory found a much larger increase of DL CO and Dm CO in exercising foxhounds than in average human subjects (42), leading us to suggest that polycythemia from splenic contraction could potentially explain the superior recruitment of diffusing capacity in dogs, a consequence of the physical properties of erythrocytes as discrete particles nonuniformly distributed within and among capillaries. Geiser and Betticher (11) found a 36% higher steady-state DL CO in excised rabbit lungs perfused with hemoglobin solutions than in lungs perfused with erythrocyte suspensions at the same concentration. These investigators (11) attributed the higher DL CO to the elimination of diffusion resistance offered by unindurbed layers around the flowing erythrocytes, although it is equally possible that packaging hemoglobin into discrete erythrocytes imposes additional membrane resistance to O2 uptake. About the same time, Federspiel (10) demonstrated in a two-dimensional capillary model a lower computed DL CO, when the spacing between erythrocytes is increased because of lower fluxes across the tissue-erythrocyte barrier, also supporting an effect of hematocrit on membrane resistance.

To further explore physical interactions between alveolar tissue and erythrocyte membranes, we used a finite-element model to model alveolar capillary CO uptake. Our results showed that, as the number of capillary erythrocytes increases (i.e., increasing hematocrit), endothelial and erythrocyte membranes become more uniformly matched and CO flux increases up to a hematocrit of 40–45% (20). As more alveolar capillaries open to erythrocyte traffic (i.e., increasing blood volume and flow), effective endothelial and erythrocyte surfaces progressively increase and contribute to further elevations in CO flux (24). Above a hematocrit of ~45%, Dl CO or Dm CO per erythrocyte progressively declines as the erythrocytes become crowded and the anatomical match between tissue and erythrocyte membranes becomes less optimal, eventually imposing an upper limit to CO flux (20). Flow-related distortion of erythrocytes can reduce hemodynamic resistances while at the same time reducing Dm CO by making portions of the erythrocyte surface less accessible to diffusion (21). At a given average capillary hematocrit, nonuniform distribution of erythrocytes within a capillary or among separate capillaries could potentially impair overall CO uptake by more than 30% (24).
These simulation data suggest that alveolar-capillary recruitment involves more than opening capillaries or increasing hematocrit but also optimizing erythrocyte distribution to more effectively match erythrocyte and tissue membranes for gas exchange. Simulation also predicts that $DmO_2$ would change with hematocrit independent of changes in $Vc$. The present study provides direct in vivo evidence to corroborate these predictions by showing that postspenectomy reduction of lung diffusing capacity was predominantly due to a reduction of the membrane component, whereas $Vc$ either increased or did not change. Our results complement data from rats (12) showing that increasing hematocrit by isovolemic erythrocyte exchange transfusion enhances peripheral tissue diffusing capacity as well as data from horses (40) that splenectomy impairs peripheral tissue diffusing capacity during exercise.

**Risk-Free Polycythemia**

Although an elevated circulating blood volume increases cardiac preload and raises cardiac output by the Starling mechanism, the associated increase in vascular resistance and cardiac afterload tend to curtail cardiac output. A high hematocrit could cause nonuniform distribution of blood flow and reduce gas-exchange efficiency in the lung and muscle (7, 25). In human subjects, chronic polycythemia caused by primary hematological overproduction or secondary to mountain sickness or severe lung disease is associated with hyperviscosity, which compromises cerebral blood flow and increases the risks of ischemia and thrombosis (16, 44). Athletes who blood dope via autologous transfusion or exogenous erythropoietin administration risk systemic hypertension and sudden death (34). Clinically, a level of hematocrit similar to that observed at maximal exercise in foxhounds or horses (~60%) is considered sufficiently dangerous to require phlebotomy. In contrast, periodic exercise-induced polycythemia in dogs or horses does not incur these risks. The aerobic animal develops polycythemia only for the duration of heightened $O_2$ demand when systemic vascular resistance is normally minimized. When $O_2$ demand subsides, circulating blood volume and hematocrit quickly normalize as the extra erythrocytes return to the relaxing spleen. In contrast, the blood-doping athlete and the polycythemic patient must tolerate an elevated blood volume and hematocrit at all times, regardless of $O_2$ requirements or vascular tone. Thus the evolutionary significance of the splenic reservoir in aerobic species lies not only in the enhancement of pulmonary as well as peripheral (muscle) diffusing capacity by enlarging the hemoglobin pool for $O_2$ uptake and release, and by more effectively matching the membrane surfaces between tissue capillaries and erythrocytes for gas exchange. Further investigation should address whether exercise training or chronic hypoxia enlarges the splenic reservoir, whether a large splenic reservoir improves acclimatization to hypoxia, and whether splenectomy shifts the balance of compensatory mechanisms during chronic hypoxia toward nonhematological sources, such as a greater ventilatory response and/or accelerated growth and remodeling of the alveolar gas exchange units and muscle capillaries.

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**DISCLAIMER**

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