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Rapid intravenous infusion of 20 mL/kg saline alters the distribution of perfusion in healthy supine humans

AC Henderson¹, RC Sá¹, IA Barash³, S Holverda¹, RB Buxton², SR Hopkins^{1,2}, and GK Prisk^{1,2}

¹Department of Medicine, Division of Physiology, University of California, San Diego, La Jolla, CA

²Department of Radiology, University of California, San Diego, La Jolla, CA

³Department of Emergency Medicine, University of California, San Diego, La Jolla, CA

Abstract

Rapid intravenous saline infusion, a model meant to replicate the initial changes leading to pulmonary interstitial edema, increases pulmonary arterial pressure in humans. We hypothesized that this would alter lung perfusion distribution. Six healthy subjects (29±6 years) underwent magnetic resonance imaging to quantify perfusion using arterial spin labeling. Regional proton density was measured using a fast-gradient echo sequence, allowing blood delivered to the slice to be normalized for density and quantified in mL/min/g. Contributions from flow in large conduit vessels were minimized using a flow cut-off value (blood delivered > 35% maximum in mL/min/cm³) in order to obtain an estimate of blood delivered to the capillary bed (perfusion). Images were acquired supine at baseline, after infusion of 20 mL/kg saline, and after a short upright recovery period for a single sagittal slice in the right lung during breath-holds at functional residual capacity. Thoracic fluid content measured by impedance cardiography was elevated post-infusion by up to 13% (p<0.0001). Forced expiratory volume in one second was reduced by 5.1% post-20 mL/kg (p=0.007). Infusion increased perfusion in nondependent lung by up to 16% (6.4±1.6mL/min/g baseline, 7.3±1.8 post, 7.4±1.7 recovery, p=0.03). Including conduit vessels, blood delivered in dependent lung was unchanged post-infusion; however, was increased at recovery (9.4±2.7 mL/min/g baseline, 9.7±2.0 post, 11.3±2.2 recovery, p=0.01). After accounting for changes in conduit vessels, there were no significant changes in perfusion in dependent lung following infusion (1.5±0.5 mL/min/g baseline, 1.5±0.4 post, 1.6±0.5, p=0.72). There were no significant changes in lung density. These data suggest that saline infusion increased perfusion to nondependent lung, consistent with an increase in intravascular pressures. Dependent lung may have been “protected” from increases in perfusion following infusion due to gravitational compression of the pulmonary vasculature.

Keywords

arterial spin labeling; magnetic resonance imaging; pulmonary interstitial edema

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A. Courtney Henderson, Ph.D. University of California, San Diego, Department of Medicine, 9500 Gilman Drive, 0623A La Jolla, CA 92093-0623, (858) 534-2790, (858) 534-4812 (fax), achenderson@ucsd.edu.

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1. INTRODUCTION

Rapid intravenous saline infusion has been used in previous studies to examine the transition from a healthy “dry” lung to an edematous lung (Farney et al., 1977; King et al., 2002; Levinson et al., 1977; Muir et al., 1975). Rapid (within 30 minutes) intravenous infusion of 2 liters of saline in normal human subjects has been shown to increase pulmonary capillary blood volume (Farney et al., 1977), pulmonary arterial pressure (Doyle et al., 1951; Muir et al., 1975), and pulmonary venous pressure (Kumar et al., 2004b). Following intravenous administration of 3 liters of saline, high-resolution computed tomography images show thickening of small airway walls and increased pulmonary arteriole diameter (King et al., 2002).

Numerous studies have helped to form the conceptual framework for the sequence of pulmonary edema formation and the regulation of lung water (Burton, 1951; Charan, 1998; Hogg, 1978; Lai-Fook, 1993; Mellins et al., 1969; Snashall et al., 1977; Staub, 1970; Staub et al., 1967; Warrell et al., 1972). Initially, recruitment and distension of pulmonary vessels occurs. Once vessels are fully distended, fluid leaks from capillaries into the corners of alveoli where it is picked up by the lymphatics and all structures able to eliminate excessive lung fluids. The lymphatics run through the interstitium around extra-alveolar vessels and transport fluid out of the lung. As excess lung fluid continues to accumulate, perivascular and peribronchial cuffing occurs, followed by the development of alveolar wall thickening and alveolar edema.

Excess lung fluid, whether intravascular or extravascular, would be expected to alter the normal distribution of blood flow in the supine human lung, through changes in the pressure difference driving flow or pulmonary vascular resistance (Hogg, 1978), as modeled by the zone model of pulmonary blood flow (Hughes et al., 1968). This model, which describes the gravitational gradient in blood flow as a function of alveolar, pulmonary arterial, and pulmonary venous pressures, predicts the majority of the supine lung to be in zone 3, where venous pressure exceeds alveolar pressure (Hopkins et al., 2007; Hughes et al., 1968; Todd et al., 1978). Under zone 3 flow conditions, blood flow is determined by the arterial-venous pressure difference (West et al., 1965) and it is thought that flow increases down the lung with increasing intravascular pressures due to recruitment and distension of vessels. However, some regions of nondependent supine human lung may have zone 2 characteristics, where vessels are not fully distended and blood flow is driven by the difference between arterial and alveolar pressure. Some of the most dependent supine human lung exhibits zone 4 behavior (Hopkins et al., 2007; Hughes et al., 1968), where flow decreases down the lung despite increasing intravascular pressures. It has been suggested that this occurs because interstitial pressure becomes elevated from gravitational compression of lung tissue and compresses extra-alveolar vessels (Hopkins et al., 2007; Hughes et al., 1968). Other potential mechanisms to explain zone 4 behavior include hypoxic pulmonary vasoconstriction due to low alveolar PO_2 (Pettersson et al., 2006) or local vascular branching structure pattern (Burrowes et al., 2005; Burrowes and Tawhai, 2006).

The aim of this study was to evaluate the effects of rapid saline infusion and an upright posture following infusion on the distribution of pulmonary blood flow. We hypothesized that rapid intravenous infusion of 20 mL/kg saline in normal subjects would alter the distribution of lung perfusion by increasing perfusion to nondependent lung, consistent with that predicted by the zone model; however, whether it would affect dependent lung was unclear. To test this, we acquired perfusion and lung density images using magnetic resonance imaging (MRI) in six supine healthy subjects during breath-holds at functional

residual capacity. Images were acquired at baseline, after infusion of 20 mL/kg saline, and after a short upright recovery period.

2. METHODS

2.1 Subjects

The Human Subjects Research Protection Program of the University of California, San Diego, approved this study. Six healthy subjects (3 female, 3 male, age = 29 ± 6 yrs, height = 175 ± 8 cm, weight = 77 ± 14 kg) participated after giving written informed consent. Subjects underwent screening using respiratory and MRI safety questionnaires prior to testing.

2.2 Protocol Overview

The timeline of the protocol is shown in Figure 1. The total duration of each study, which included consenting and preparing the subject as well as the protocol, was about 5 hours. Baseline spirometry was performed in an upright standing position. A catheter was placed in a peripheral vein in the forearm or hand. The subject was then positioned in the MRI scanner and remained supine throughout baseline imaging, saline infusions, and post-20 mL/kg imaging. Images of perfusion and lung density were collected for a single sagittal slice in the right lung. Data were acquired during short breath-holds at functional residual capacity at baseline (during a period of ~45 minutes). The first infusion of saline (10 mL/kg) occurred over ~15 minutes using a pressure infuser bag (Infu-Surg, Ethox International Inc., Buffalo, NY). After ~15 minutes, the second infusion of saline occurred over ~15 minutes (to give 20 mL/kg total saline infused). Post-20 mL/kg images using the same imaging protocol as baseline were then collected (~45 minutes). The subject then stood up and exited the scanner room. Post-20 mL/kg spirometry was performed in an upright standing position. The subject was allowed to use the restroom if necessary, after which time he/she was returned to the MRI scanner for recovery imaging in the supine posture (~25 minutes). Setting up the subject for MRI took approximately 20 minutes, therefore the time elapsed between the last post-20 mL/kg measurement and the first recovery measurement was ~45 minutes. Recovery measurements using the same imaging protocol as baseline were then collected (~45 minutes). The subject then stood up, exited the scanner room, and spirometry was again performed.

2.3 Pulmonary blood flow imaging

Regional pulmonary blood flow was assessed using 2D arterial spin labeling (ASL) with a flow-sensitive alternating inversion recovery with an extra radiofrequency pulse (FAIRER) imaging sequence and a Half-Fourier Acquisition Single-shot Turbo spin-Echo (HASTE) data collection scheme (Bolar et al., 2006; Mai et al., 1999). This has been recently described in detail (Bolar et al., 2006; Hopkins et al., 2007) and used in a number of studies by our group (Henderson et al., 2006; Hopkins et al., 2007) and is therefore only briefly described here.

Arterial spin labeling exploits the capability of MRI to invert the magnetization of protons (primarily in water molecules) in a spatially selective way using a combination of radiofrequency pulses and spatial magnetic field gradient pulses (Bolar et al., 2006; Mai and Berr, 1999; Mai et al., 1999). By inverting the magnetization of arterial blood, these “tagged” protons in blood act as an endogenous tracer. During each measurement two images of a lung slice are acquired during consecutive breath-holds with the signal of blood prepared differently in the two images. In the first “control” image, an inversion (180°) pulse is applied to the section being imaged (a spatially selective inversion), leaving the arterial blood outside the imaged section undisturbed and with a strong magnetic resonance signal. In the second image, termed the “tag” image, the magnetization of the arterial blood

both inside and outside the imaged section is inverted at the beginning of the experiment with an inversion (180°) pulse applied to the whole lung (a spatially non-selective inversion). Both images are subsequently acquired after a delay chosen to be approximately 80% of one R-R interval. During this delay, blood flows into the imaged slice and there is relaxation of the magnetization. The difference or ASL signal (control – tag) measured for each voxel then reflects the amount of blood delivered during the delay, or inversion time (TI) interval, weighted by a decay factor due to the relaxation of the blood magnetization during that interval (Henderson et al., 2009).

Imaging sequence parameters were as follows: TI = 600–800 msec, TE = 21.3 msec, field of view = 40 cm, slice thickness = 15mm, and collected image matrix size = 256×128 (reconstructed by scanner as 256×256). Therefore, the size of each voxel was $1.56 \times 3.13 \times 15$ mm, or ~ 0.073 cm³. The total scan time was approximately 8–10 seconds. The HASTE imaging sequence had an inter-echo time of 4.5 msec and 72 lines of phase encoding, resulting in a data acquisition time of 324 msec.

2.4 Proton density imaging

Regional proton density was measured using a fast gradient echo sequence described in detail elsewhere (Theilmann et al., 2009). The sequence collects 12 images alternating between two echo times in a single 9-second breath-hold. Six images (even images: 2, 4, 6, ..., 12) were acquired with an echo time of 1.1 msec and six images (odd images: 1, 3, 5, ..., 11) were acquired at an echo time of 1.8 msec. Imaging sequence parameters were TR = 10 msec, flip angle = 10 deg, slice thickness = 15 mm, field of view = 40 cm, receiver bandwidth = 125 kHz, and a full acquisition matrix of 64×64 . Data from the last eight images were fit to a single exponential on a voxel-by-voxel basis to estimate proton density. Assuming that water content is representative of lung density, the proton density measured using this technique is proportional to lung density (Theilmann et al., 2009).

2.5 Data Collection

2.5.1 Pulmonary Function—Routine spirometry was performed at baseline, after post-infusion MRI measurements, and after recovery MRI measurements using an EasyOne spirometer (NDD Medical Technologies, Zurich, Switzerland). Forced expiratory volume in 1 second (FEV₁), forced vital capacity (FVC), and forced expiratory flow over the lung volume interval from 25% to 75% of FVC (FEF_{25–75}) were measured.

2.5.2 MRI—Subjects were imaged using a 1.5 Tesla Signa HDx TwinSpeed MRI system (General Electric Medical Systems, Milwaukee, WI, USA). Two water phantoms doped with gadolinium (Berlex Imaging, Magnevist, 469 mg/mL gadopentetate dimeglumine, 1:5,500 dilution) were placed next to the subject within the field of view for absolute quantification of perfusion and lung density (see below). A torso coil was then placed around the subject's chest for image acquisition. Images of pulmonary blood flow and proton density were acquired by imaging a single sagittal slice (in mid-lung relative to medial-lateral direction). The right lung was chosen to eliminate artifacts from the aorta and heart motion present within the left hemithorax. Proton density images were collected using both the torso coil and the body coil built into the scanner (for reasons described in section on Image Processing).

During the ASL imaging sequence, the subjects inspired air while ten images (5 control images and 5 tag images, therefore 5 ASL difference images) were acquired. Subjects synchronized their breathing with the scanner by taking a normal inspiration and expiration between subsequent image acquisitions. Subjects were trained to breath-hold at functional

residual capacity when image acquisition occurred (single control/tag image acquisition time of less than 1 second). The time between image acquisitions was set to 5 seconds.

2.5.3 Impedance cardiography—Trans-thoracic impedance was used for measurement of thoracic fluid content and cardiac output during a second infusion performed on a separate day in 5 out of 6 subjects using the same infusion protocol and timeline. Unfortunately one of the subjects was unavailable to participate in the second infusion. Exclusion of this subject from the MRI data did not alter the conclusions; therefore the data from this subject was retained. Subjects were instrumented with a tetrapolar impedance cardiograph (BioZ ICG, CardioDynamics, San Diego, CA), which provided measurement of average thoracic fluid content and cardiac output from the impedance signal once per minute, and ECG monitoring.

2.5.4 Metabolic parameters—A facemask (Hans Rudolph, KS, USA) equipped with a non-rebreathing T-valve was fitted to the subject. The outlet of the T-valve was connected to a ~6 m long, large bore low resistance expiratory line, leading out of the scanner room, where oxygen consumption ($\dot{V}_{O_2}$), carbon dioxide production ($\dot{V}_{CO_2}$), total minute ventilation ($\dot{V}_E$), respiratory exchange ratio, respiratory rate, tidal volume, mixed expired oxygen, and mixed expired carbon dioxide were measured using a ParvoMedics Metabolic Measurement System (ParvoMedics, Sandy, UT, USA). The system was calibrated with the 6-meter long tubing in place. Data were averaged over the entire imaging period of each set of measurements (Baseline, Post-20 mL/kg, and Recovery).

2.6 Image Processing

2.6.1 Quantification of regional blood delivered in mL/min/cm³—In order to quantify all blood delivered (comprising both that in large conduit vessels and also that representing true perfusion in the capillary bed) to the imaging slice in mL/min/cm³ lung, a water phantom doped with gadolinium with a T_1 of 1650 msec and T_2 of 976 msec was included in the field of view for calibration. We assumed a T_1 of 1430 msec and T_2 of 117 msec for human pulmonary arterial (mixed venous) blood under normal *in vivo* conditions (hematocrit ~0.4 and oxygen saturation ~75%). Blood delivered was normalized to the mean signal in the phantom, accounting for the actual T_1 and T_2 of the phantom relative to the assumed values for human pulmonary arterial blood (Spees et al., 2001). Blood delivered in units of mL/min/cm³ (averaged over one cardiac cycle) was calculated for each ASL image (Henderson et al., 2009).

2.6.2 Quantification of regional lung density in g/cm³—A proton density image was determined by fitting a single exponential to the images for two different echo times and back-extrapolating to determine the magnetization at time zero on a voxel-by-voxel basis. The resulting proton density image collected using the body coil (which is spatially homogeneous, therefore no coil inhomogeneity correction was needed) was normalized to the signal derived from the water phantom (which is by definition 100% water) to obtain regional lung proton (water) density in units of g H₂O per cm³ lung. This proton density, which reflects protons in both tissue and blood, is subsequently referred to in this manuscript as density (Theilmann et al., 2009). This technique for quantifying regional lung density has been validated, showing a high correlation between measured MRI water content and gravimetric water content with $R^2=0.95$, $p<0.0001$ (Holverda et al., 2011).

2.6.3 Quantification of regional blood delivered in mL/min/g—The proton density images (64×64) were resized to match the resolution of the ASL images (256×256) using bilinear interpolation in MATLAB (The MathWorks, Natick, MA). A mutual information-based technique that included translation and rotation was used to register the two images

(28) using a custom-designed program in MATLAB. Blood delivered to the slice expressed in units of mL/min/g was calculated by dividing the quantified image of blood delivered obtained using ASL (mL/min/cm³) by the quantified density image obtained using the fast gradient echo sequence (g H₂O/cm³ lung) to give blood delivered in milliliters per minute per gram lung (tissue + blood). The ASL and proton density images used in the calculation were both collected using the torso coil; therefore any inhomogeneity in the coil sensitivity profile cancels out upon division of the two images. The resulting image reflects blood delivered in milliliters per minute per gram lung (mL/min/g), without the confounding effects of coil inhomogeneity (Arai et al., 2009; Burnham et al., 2009; Hopkins et al., 2007; Prisk et al., 2007).

6.4 Quantification of regional perfusion in mL/min/g—The arterial spin labeling technique measures blood delivered from outside the tagging band, into the imaging slice, in one RR-interval. This differs from capillary perfusion since it includes blood flow in large conduit vessels. In order to focus more closely on blood flow in the smaller vessels and capillaries and thus provide a better estimate of perfusion, we applied a cut-off value (35% of maximum blood delivered in mL/min/cm³) and assigned voxels in images of blood delivered to one of two data sets: 1) larger conduit vessels (blood delivered > 35% maximum in mL/min/cm³) or 2) “perfusion” comprising smaller vessels and lung tissue (blood delivered < 35% maximum in mL/min/cm³). The 35% cutoff value was chosen based on modeling studies because it is the value at which the ASL signal primarily reflects perfusion without contributions from blood flow in larger conduit vessels (Burrowes and Prisk, unpublished observations). Application of a perfusion cutoff value is expected to reduce the contributions of larger conducting vessels to the results (see Limitations).

2.7 Data Analysis

The mean value for density (g/cm³) and blood delivered (mL/min/g) was calculated for a region of interest encompassing the lung in the sagittal image at all time points. We also evaluated the two components of density, total water content (grams) and lung volume (cm³) at all time points. Total water content for each image was calculated as the product of the mean density, number of voxels within the lung region of interest, and the voxel volume. The lung volume for the slice was calculated as the product of the number of voxels within the lung and the voxel volume. The relative dispersion (standard deviation/mean) was calculated for the same region of interest and used as an index of overall heterogeneity. A higher relative dispersion is representative of a more heterogeneous distribution (Glenny, 1998).

The vertical distributions (distance above the most dependent portion of the lung for each subject) for density and perfusion were plotted in 1 cm increments at baseline, post-20 mL/kg, and recovery. Linear regressions were performed on lung density and perfusion versus height and the slopes were evaluated.

Since distributions of perfusion across vertical distances may not necessarily be best expressed as linear relationships, the sagittal slice image was divided into three gravitationally based regions of interest: nondependent, intermediate, and dependent regions to allow for comparison between regions. The regions were defined to have equal vertical distance based on the maximum anterior to posterior dimension of the lung. Mean density, perfusion, and total water content were calculated for each region (Hopkins et al., 2007; Prisk et al., 2007). In order to assess more local changes in perfusion and lung water content, an additional analysis was performed where the sagittal lung slice was divided into nine regions: three divisions in the anterior-posterior direction (nondependent, intermediate, and dependent lung in the supine posture) and three divisions in the cranial-caudal direction.

2.8 Statistical Analysis

A repeated measures ANOVA (Statview, 5.0.1 SAS Institute, Cary, NC) with one repeated measure was performed to statistically evaluate changes in each major dependent variable at the experimental time points (baseline, post-20 mL/kg, and recovery). A repeated measures ANOVA was performed with two repeated measures to evaluate regional density and perfusion data by gravitational region (3 levels: nondependent, intermediate, dependent region) and state (3 levels: Baseline, Post-20 mL/kg, and Recovery). Dependent variables for this analysis were lung density in units of g/cm^3 of lung and perfusion in units of mL/min/g . Where overall significance occurred, post hoc testing was conducted using Fisher's Protected Least Significant Difference. All data are presented as mean $\pm$ SD. The null-hypothesis (group means are equal, or no effect) was rejected for $p < 0.05$, two tailed.

3. RESULTS

3.1 Spirometry

Table 1 shows the effects of saline infusion on spirometry. FEV_1 decreased by 5.1% (3.92 ± 0.55 to 3.72 ± 0.59 , $p=0.007$) as a result of infusion, but returned toward the baseline value during the recovery period. There were no significant changes in FVC, FEV_1/FVC , or FEF_{25-75} . Since there was a decrease in FEV_1 but no significant change in FEV_1/FVC , one would expect a concomitant reduction in FVC. Forced vital capacity decreased by 4.7%; however, this change did not quite reach the level of statistical significance ($p=0.09$).

3.2 Thoracic fluid content and cardiovascular parameters

Table 2 and Figure 2 show the impedance cardiography data measured during a second infusion on a separate day at baseline, post-20 mL/kg, and during recovery for 5 out of 6 subjects. Thoracic fluid content was increased at all time points following infusion by up to 13% (Figure 2, $p<0.0001$). Systolic blood pressure was elevated at post-20 mL/kg by about 9% compared to baseline and recovery (Table 2, $p=0.019$). Diastolic blood pressure increased slightly (up to 7%) as a result of infusion, but this change did not reach statistical significance ($p=0.086$). Heart rate did not significantly change throughout the study. Stroke volume showed a small (3–5%) but significant reduction at post-20 mL/kg and recovery ($p=0.0015$). Cardiac output was increased at post-20 mL/kg by about 2% on average relative to baseline and 7% relative to recovery; however, this change did not reach the level of statistical significance ($p=0.073$). There were no significant changes in mean arterial oxygen saturation measured by pulse oximetry ($97.7 \pm 1.2\%$ baseline, $96.7 \pm 2.2\%$ post-20 mL/kg, $96.7 \pm 2.3\%$ recovery).

3.3 Metabolic parameters

There were no significant changes in oxygen consumption, carbon dioxide production, total minute ventilation, respiratory exchange ratio, respiratory rate, tidal volume, fraction expired oxygen, or fraction expired carbon dioxide (Table 3).

3.4 Lung Density (g/cm^3)

3.4.1 Overall mean and heterogeneity—Figure 3 shows a typical sagittal lung density image for a subject at baseline. Table 4 includes density data at all time points for a sagittal slice in all 6 subjects. The mean and relative dispersion of density did not significantly change throughout the study.

3.4.2 Gravitational gradient—The vertical distributions of density for the different time points are shown in Figure 4A. Average density (g/cm^3) within an isogravitational plane is shown as a function of distance relative to the most dependent portion of the lung at 1 cm

increments. Standard deviations are shown for baseline and recovery. The standard deviations of the post-20 mL/kg data were comparable and are therefore not shown for clarity. Contrary to our expectations, the lung density vertical distributions were unaltered by infusion. The slope of density versus vertical height determined using linear regression did not significantly change as a result of infusion (Table 4).

3.4.3 Regional analysis—When the lung was divided into three gravitational regions as shown in Figure 3 (Table 5 and Figure 5A), as expected lung density increased from nondependent to dependent regions ($p < 0.0001$). Mean density for the three gravitational regions did not significantly change with infusion, in keeping with the vertical distribution data above. When the nine-region analysis was performed, there were no significant differences in density following infusion in any region.

3.5 Total lung water content (g)

There were no significant changes in the mean, standard deviation, or relative dispersion of total lung water content for the slice, lung thirds (nondependent, intermediate, dependent), or the nine region analysis. There were also no significant changes in lung volume for the slice with infusion.

3.6 Blood delivered (mL/min/g)

3.6.1 Overall mean and heterogeneity—Typical sagittal slice images of blood delivered (mL/min/g) at baseline, post-20 mL/kg, and recovery are shown in Figure 6 (top row). Table 4 includes data for blood delivered to the imaging slice at all time points for 6 subjects. The mean amount of blood delivered was not significantly different post-20 mL/kg; however, it was increased at recovery ($p = 0.01$). The relative dispersion of blood delivered, a measure of global heterogeneity (Arai et al., 2009; Burnham et al., 2009; Glenny, 1998; Henderson et al., 2006; Henderson et al., 2009; Hopkins et al., 2007; Prisk et al., 2007), did not significantly change throughout the study.

3.6.2 Gravitational gradient—The vertical distributions of blood delivered for the different time points are shown in Figure 4B. Average blood delivered (mL/min/g) within an isogravitational plane is shown as a function of distance relative to the most dependent portion of the lung at 1 cm increments. Standard deviations are only shown for baseline and recovery data for clarity but were similar at post-20 mL/kg. Blood delivered in the nondependent region for height values > 10 cm is slightly greater after infusion. The blood delivered was increased at recovery in dependent lung. The slope of blood delivered versus vertical height determined using linear regression was -0.030 ± 0.006 mL/min/g/cm at baseline and -0.018 ± 0.015 mL/min/g/cm at post-20 mL/kg ($\sim 40\%$ change), although this change was of borderline statistical significance ($p = 0.06$) (Table 4). The slope of blood delivered versus vertical height at recovery was comparable to baseline.

3.6.3 Regional analysis—When the lung was divided into three gravitational regions (Table 5 and Figure 5B), the blood delivered to the intermediate and dependent regions was higher compared to the nondependent region for all time points ($p < 0.001$). Mean blood delivered to the nondependent region was increased at post-20 mL/kg and recovery by 28% and 31% respectively relative to baseline ($p = 0.0007$). The blood delivered to the intermediate region was not significantly different post-infusion. The blood delivered to dependent lung was not significantly different from baseline at post-20 mL/kg; however, it was increased at recovery ($p = 0.01$).

When the nine-region analysis was performed, there were no significant differences in blood delivered in the cranio-caudal direction. The nondependent and dependent regions that were

most cranial only contained a small number of voxels (~5% of voxels within lung in the slice) due to the shape of the lung, and therefore were not included in the analysis. We found that the blood delivered to the nondependent regions was increased at post-20 mL/kg and recovery relative to baseline ($p < 0.05$). Blood delivered to the dependent regions was elevated at recovery ($p = 0.006$). The nine-region analysis results were consistent with the results for blood delivered when the lung was divided into thirds and did not show differences in the cranio-caudal direction; therefore we only report the results from dividing the lung into thirds (nondependent, intermediate, and dependent) here for simplicity.

3.7 Perfusion (mL/min/g)

Typical sagittal slice images of perfusion (mL/min/g), which excludes larger conduit vessels, collected at baseline, post-20 mL/kg, and recovery are shown in Figure 6 (bottom row). Black regions within the lung represent regions thought to represent contributions from flow in large conduit vessels and were excluded from perfusion results. The mean and relative dispersion of perfusion, defined as voxels containing smaller vessels and lung tissue (blood delivered $< 35\%$ of maximum in mL/min/cm³), for the slice did not significantly change following infusion (Table 4). Mean perfusion in the nondependent region was increased at post-20 mL/kg and recovery by 14% and 16% respectively relative to baseline (Table 5 and Figure 7, $p = 0.03$). There were no significant changes in perfusion in the intermediate ($p = 0.90$) or dependent ($p = 0.36$) regions (Table 5 and Figure 7). The standard deviation and relative dispersion of perfusion did not change in any of the three regions as a result of infusion.

3.8 Large conduit vessels

The number of voxels containing large conduit vessels (blood delivered $> 35\%$ of maximum in mL/min/cm³) for the entire lung slice was increased at recovery relative to baseline by 49% (Table 6, $p = 0.047$). When the lung was divided into thirds, the number of voxels containing large conduit vessels did not change as a result of infusion for the nondependent region ($p = 0.07$) or intermediate region ($p = 0.21$). In the dependent region, however, the number of voxels containing larger vessels was elevated at recovery relative to baseline by 60% ($p = 0.01$).

4. DISCUSSION

The principal findings of this study are that: 1) mean perfusion in nondependent lung increased with infusion of 20 mL/kg saline and remained elevated at recovery (Table 5 and Figure 7), 2) there was no significant change in perfusion in intermediate lung, and 3) interestingly, blood delivered in dependent lung was not elevated following infusion of 20 mL/kg saline but was increased at recovery after a short upright recovery period. After removal of large conduit vessels, there were no significant changes in perfusion in dependent lung following infusion (at post-20 mL/kg or recovery). These data suggest that in dependent lung, compression of lung tissue and high interstitial pressures (zone 4) may “protect” pulmonary vasculature in dependent lung from increases in blood flow with volume overload, since neither blood delivered nor perfusion was significantly elevated at post-20 mL/kg. Despite these alterations in perfusion, there were no statistically significant changes in lung density or water content measured using MRI, although there were changes in thoracic fluid content measured using impedance cardiography. There were also no significant changes in any metabolic parameters, therefore saline infusion did not induce any changes in metabolism, consistent with previous results (Prisk et al., 2010).

4.1 Changes in nondependent lung while supine

There was an increase in blood delivered and perfusion to nondependent lung, which is consistent with a previous study using a similar intravenous saline infusion protocol. Muir et al. (Muir et al., 1975) studied the cardiovascular and pulmonary effects of intravenous infusion of two liters of saline over 20 minutes. Regional perfusion, evaluated by imaging xenon-133 in horizontal slices using a gamma camera, showed an increase in blood flow to the nondependent lung apices that persisted into the recovery period 20 minutes later.

Studies have shown that pulmonary arterial and venous pressures, both of which are key determinants of pulmonary blood flow, increase with saline infusion. After infusion of 2 liters of saline over 20 minutes, Muir et al. found that pulmonary arterial pressure nearly doubled, increasing from 7.5 to 14.7 mmHg (Muir et al., 1975). In a study by Kumar et al., infusion of 3 liters of saline over 3 hours raised pulmonary wedge pressure, an estimate of pulmonary venous pressure, by 78% from 9.7 mmHg to 15.3 mmHg (Kumar et al., 2004b). If infusion increased pulmonary arterial and venous pressures, this may tend to increase blood flow in nondependent lung, possibly due to a transition from zone 2 to zone 3 flow conditions with distension of capillaries in the supine human (Hopkins et al., 2007; Todd et al., 1978).

4.2 Changes in dependent lung while supine

Blood delivered in dependent lung was unchanged shortly after infusion of 20 mL/kg saline. Perfusion, or blood delivered with large conduit vessels removed, also was not significantly different at post-20 mL/kg relative to baseline. Vessels in dependent supine lung under zone 4 conditions (Hopkins et al., 2007) would be expected to be compressed due to gravitational compression of lung tissue, perhaps creating a protective effect from increases in blood flow following infusion. Our results suggest that both large conduit vessels and capillaries were “protected” from such increases in flow following infusion, since neither blood delivered nor perfusion was elevated at post-20 mL/kg.

Studies using xenon-133 (Hughes et al., 1968) showed that blood flow is reduced in dependent regions in the normal human lung for a given arterial-venous pressure difference, and it was suggested that vessels are compressed since alveolar expansion is minimal and interstitial pressure is high. Under such conditions the caliber of extra-alveolar vessels would be narrowed, and this effect is thought to be least at high lung volumes. A recent study by our group, however, showed using magnetic resonance imaging that perfusion in mL/min per gram of lung tissue was remarkably constant in dependent lung, irrespective of lung volume (Hopkins et al., 2010). Nevertheless, this mechanism may explain our findings since gravitational compression of lung tissue and interstitial pressure would be expected to be greater in dependent lung following saline infusion, perhaps preventing an increase in blood flow despite increased intravascular pressures.

A previous study by our group found that infusion of 20 mL/kg saline reduced expiratory flow but did not significantly impact gas exchange, suggesting that hypoxic pulmonary vasoconstriction may have occurred in regions of reduced ventilation and low alveolar PO_2 (Prisk et al., 2010). Our finding of a small reduction in FEV_1 at post-20 mL/kg suggests the presence of only subtle perivascular and peribronchial cuffing post-infusion, without any alveolar flooding that could lead to hypoxic regions and stimulate hypoxic pulmonary vasoconstriction, since there were no detectable changes in lung density or total lung water content. There also was not a change in the relative dispersion, an index of global heterogeneity, of blood delivered following infusion for the whole slice, lung thirds, or any of the nine regions, which would be expected to change if significant hypoxic pulmonary vasoconstriction occurred (Hopkins et al., 2005).

4.3 Changes in dependent lung following an upright period

Blood delivered in dependent lung was increased at recovery, which was collected while supine after a short upright period. Interestingly, this was no longer significantly different when large conduit vessels were removed. Furthermore, there was an increase in the number of voxels containing large conduit vessels in dependent lung at recovery, suggesting expansion of the larger vessels. These data suggest that the changes in blood flow in dependent lung present at recovery are primarily due to changes occurring in the large conduit vessels.

When the subject was supine in the scanner during imaging at baseline and post-20 mL/kg, posterior lung was dependent. While the subject was upright and walking around between post-20 mL/kg and recovery imaging, caudal lung was dependent. One would expect a redistribution of blood volume and changes in the distribution of perfusion during this upright period. Our results suggest that during post-20 mL/kg imaging, the pulmonary vasculature (large conduit vessels and capillaries) in posterior dependent lung is “protected” from increases in perfusion, perhaps due to gravitational compression of lung tissue and high interstitial pressures (zone 4). When the subject was upright and walking around, this “protection” was no longer present in most of the posterior lung since it was no longer dependent. As a result, blood flow may have increased in posterior lung and expanded large conduit vessels while upright. Once the subject was supine again at recovery, our data suggest that the large conduit vessels remained expanded.

4.4 Changes in pulmonary function and impedance cardiography measures

Our results show that thoracic fluid content was elevated following infusion and this persisted into the recovery period (Figure 2). The magnitude and duration of the changes were similar to a previous study by our group using a similar intravenous saline infusion protocol (Prisk et al., 2010). Other previous studies utilizing rapid saline infusion have shown fluid increases inside (Farney et al., 1977) and surrounding pulmonary vasculature (King et al., 2002), consistent with our finding of increased thoracic fluid content. An increase in pulmonary capillary blood volume and fluid accumulation would be expected to increase thoracic fluid content; therefore our findings are consistent with the results of these studies.

Peribronchial cuffing has been hypothesized to result in airway obstruction (Cutillo, 1995; Pellegrino et al., 2003). Indeed, we found that rapid infusion of 20 mL/kg of saline reduced forced expiratory flow as indicated by FEV₁; however, there were no significant changes in FVC or FEV₁/FVC. Since there was a decrease in FEV₁ but no significant change in FEV₁/FVC, one would expect a concomitant reduction in FVC. However, forced vital capacity decreased by 4.7%, but this change did not reach the level of statistical significance ($p=0.09$). Reduced expiratory flows were also evident following intravenous saline infusion in a previous study by our group (Prisk et al., 2010) and other previous studies (Cutillo, 1995; Pellegrino et al., 2003; Robertson et al., 2004), without any significant change in diffusing capacity of the lung (Robertson et al., 2004), suggesting the presence of peribronchial cuffing without significant fluid accumulation in the alveoli. Expiratory flow returned almost to baseline values after recovery measurements were acquired, suggesting that any peribronchial cuffing resulting from infusion was no longer significantly obstructing airways. It has been suggested that perivascular cuffing may occur simultaneously with peribronchial cuffing (Burton, 1951; Charan, 1998; Hogg, 1978; Lai-Fook, 1993; Mellins et al., 1969; Snashall et al., 1977; Staub, 1970; Staub et al., 1967; Warrell et al., 1972). Our results for spirometry suggest that peribronchial and perivascular cuffing may have been present during post-20 mL/kg measurements but were resolved at recovery.

Our impedance cardiography data show that infusion increased systolic blood pressure and decreased stroke volume; however, there were no significant changes in diastolic blood pressure (Table 2). Although there was a slight trend for changes in heart rate and cardiac output as a result of infusion, this did not reach the level of statistical significance ($p = 0.27$ for heart rate, $p = 0.073$ for cardiac output). It should be noted that stroke volume decreased by only 3–5%, and although the changes were statistically significant, they may not have physiological relevance. Previous studies have shown varied results regarding the effects of intravenous saline infusion on cardiovascular parameters such as stroke volume, heart rate, and cardiac output in normal humans, perhaps because the changes, even when significant, are often small (5–10%) and depend on the volume of saline infused as well as the timeline of the infusion and data acquisition (Doyle et al., 1951; Guazzi et al., 1999; Guazzi et al., 2004; Kumar et al., 2004a; Muir et al., 1975; Prisk et al., 2010).

4.5 Lung density

Despite the effects of infusion on perfusion, thoracic fluid content, and FEV₁, there was no effect of saline infusion on our measurements of lung density or total water content for a sagittal slice (Table 4). Although perfusion was increased in nondependent lung post-infusion, lung density or total lung water in nondependent lung did not significantly increase. It is possible that although there may have been subtle changes in perfusion in nondependent lung as well as perivascular and peribronchial cuffing throughout the lung (as would be suggested by the decreased FEV₁ post-20 mL/kg), there was not a detectable increase in lung density or overall lung water using MRI.

The increase in thoracic fluid content suggests an increase in fluid up to 13%, although this could have occurred anywhere in the thorax (for example in the chest wall, mediastinum, or large capacitance vessels in the abdomen). To determine whether there was fluid accumulation in the chest wall, we evaluated a region of interest in each density image that included everything within the thoracic cage but outside the lung. We calculated the mean density and total grams of water in the chest wall at baseline, post-20 mL/kg, and recovery. We found that neither density nor total grams of water in the chest wall were significantly different as a result of saline infusion.

Comparison of thoracic fluid content data to MRI measures of lung water showed that thoracic fluid content did not significantly correlate with mean lung density for the imaged sagittal slice ($p = 0.11$); however, it did significantly correlate with the total grams of water for the imaged slice ($p = 0.04$), although the correlation coefficient was low ($R^2 = 0.21$). Impedance cardiography may be more sensitive to small changes in lung water content, since it measures the impedance signal from the entire lung, thorax and likely some of the abdomen. We only imaged a single slice, therefore it may be more difficult to detect small changes in lung water as would occur during subtle changes in perfusion in nondependent lung and perivascular or peribronchial cuffing post-infusion. There was some degree of variability in the lung density data for a given subject. In order to detect a significant difference in mean lung density post-infusion, lung density would have needed to increase by at least 13% given the variability in our data set. These data suggest that the changes in perfusion and lung water resulting from this infusion protocol were simply not enough to be able to detect a statistically significant difference using our MRI measurements of lung density for a single slice.

4.6 Technical Limitations

ASL techniques have been widely used to determine regional blood flow in other organ systems, such as the brain (Buxton, 2002). The technique has been validated in tube-flow models (Andersen et al., 2000), heart (Poncelet et al., 1999), brain (Walsh et al., 1994), and

skeletal muscle (Raynaud et al., 2001). However, there are some limitations to the technique that should be considered. ASL provides an image map of all tagged protons that move into the imaging slice during the delay between tagging and image acquisition. The tag is triggered from the ECG signal, and the time between the tag and image acquisition is set to be 80% of the R-R interval in order to fully capture one systole of blood flow. When the blood delivered is quantified in mL/min/g, the measured signal in the ASL difference image is divided by the RR-interval, and therefore it essentially represents the average flow during one systole (Henderson et al., 2009).

We only imaged a single sagittal slice in this study, which may or may not be representative of the entire lung. Typically each lung comprises 4–6 15mm sagittal slices with 0 cm spacing in between the slices. The lung region of interest in the images for this study on average contained about 6,200 voxels with a voxel volume of 0.0366 cm^3 ($40 \text{ cm}/256 \text{ voxels} * 40 \text{ cm}/256 \text{ voxels} * 1.5 \text{ cm}$), or an average volume of lung in the slice of 230 mL. Functional residual capacity for an average male is 3 liters; therefore we are imaging about 8% of the lung in terms of volume. We performed our imaging in the sagittal plane deliberately, recognizing that there might well be differences in dependent and nondependent lung. However, there is little to suggest that in these otherwise normal subjects, infusion in the supine posture would be greatly different in different slices.

The ASL images are all collected during breath holds at functional residual capacity. A recent study by our group found that blood delivered in mL/min per gram of lung tissue in dependent and intermediate lung was constant at different lung volumes (Hopkins et al., 2010). Blood delivered in nondependent lung was reduced at total lung capacity, but was not significantly different between residual volume and functional residual capacity. Therefore, we expect that changes in blood flow during normal tidal breathing near functional residual capacity would likely be minimal.

In order to focus more closely on blood flow in smaller vessels and capillaries and thus provide a better estimate of perfusion, we applied a cut-off value (35% of maximum blood delivered in $\text{mL}/\text{min}/\text{cm}^3$) and assigned voxels in images of blood delivered to being large conduit vessels or “perfusion” comprising smaller vessels and lung tissue. The 35% cutoff value was chosen based on modeling studies; however, this method has not been validated. It is possible that when applying this cutoff, some voxels may not be assigned to the proper data set. However, based on the images, it appears that the locations of voxels suspected to contain primarily large conduit vessels using this technique are consistent with where larger vessels would occur based on the anatomy of the pulmonary vasculature (Figure 6).

In order to determine whether the distribution of perfusion was altered at smaller scales by graded saline infusion, we would need to register the baseline, post-20 mL/kg, and recovery perfusion images and subtract them on a voxel-by-voxel basis to create a difference map. However, because the images are collected during different breath holds, widely separated in time (some after removal and return to the scanner), it is difficult to register the images with absolute confidence such that every voxel in the lung perfectly corresponds. A related question of interest is whether peak flow in a particular small region (containing multiple voxels) was elevated at post-20 mL/kg or recovery relative to the rest of the lung. This particular question could not be answered with certainty since we imposed a cutoff perfusion value to minimize the contributions from voxels containing large conduit vessels.

The fast gradient echo imaging sequence used in this study to measure lung water has been validated in a previous study by our group (Holwerda et al., 2011). The methodology measures total proton density, which would be expected to reflect total lung water (including

water in the blood and tissues). Therefore, it cannot distinguish between intravascular water and extravascular water.

4.7 Summary

In summary, the results of this study show that rapid intravenous infusion of 20 mL/kg saline: 1) increased perfusion in nondependent lung, 2) increased thoracic fluid content, and 3) reduced FEV₁. Before removal of large conduit vessels, blood delivered in dependent lung was unchanged at post-20 mL/kg; however, was increased at recovery. After removal of large conduit vessels, there were no significant changes in perfusion in dependent lung following infusion. Despite these physiological effects of saline infusion, there were no significant changes in lung density or total water content for a sagittal slice in the right lung. These data suggest that rapid intravenous saline infusion increased perfusion to nondependent lung, consistent with an increase in pulmonary intravascular pressures. Dependent lung may have been “protected” from increases in perfusion immediately following infusion due to gravitational compression of the pulmonary vasculature. Infusion of 20 mL/kg saline may have resulted in subtle peribronchial cuffing that compressed small airways; perhaps explaining the small but significant reduction in forced expiratory flow at post-20 mL/kg. Our spirometry results suggest that subtle peribronchial cuffing may have been present during post-20 mL/kg measurements but was resolved during recovery measurements.

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Figure 1.

Protocol timeline. The human figure is used to denote when the subject was upright or in the supine posture. MRI measurements were made during time segments denoted as Baseline, Post-20 mL/kg, and Recovery. Metabolic parameters were measured every 10–12 seconds throughout the protocol. Impedance cardiography was measured once per minute throughout a second infusion protocol on a separate day in 5 out of 6 subjects.

Figure 2.

Thoracic fluid content (shown in arbitrary units) as measured by impedance cardiography at baseline, post-20 mL/kg saline, and recovery. Thoracic fluid content was elevated post-infusion and at recovery ($p < 0.0001$).

*Significantly different from baseline, $p = 0.0001$

Figure 3. Typical density image (g/cm³) collected at baseline with the lung thirds shown (nondependent, intermediate, and dependent regions).

Figure 4.

Mean (A) density (g/cm^3) and (B) blood delivered ($\text{mL}/\text{min}/\text{g}$) shown as a function of distance from the most dependent portion of the lung for a sagittal lung slice in 6 subjects. The figure was constructed so that vertical height increases up along the y-axis. Data were averaged for voxels lying within the same gravitational plane. Standard deviations are shown for baseline and recovery. Note that density increases from the gravitationally nondependent to the dependent regions, but is unaltered by saline infusion. Blood delivered also increases down the lung (consistent with zone 3), and then decreases in the most dependent lung region (consistent with zone 4).

A)

B)

Figure 5.

Mean (A) density and (B) blood delivered data for the lung divided into three gravitationally-based regions of interest: nondependent, intermediate, and dependent. Data are for a single sagittal slice in the right lung, averaged for 6 subjects, divided horizontally into three regions of interest with equal vertical height based on the maximum anterior-posterior dimension of the lung. Lung density did not significantly change with infusion for any of the three lung regions. Blood delivered was higher in nondependent lung at post-20 mL/kg and recovery when compared to baseline, but did not significantly change in the intermediate region. Blood delivered to dependent lung was not significantly different at post-20 mL/kg compared to baseline; however, it was increased at recovery.

* Significantly different from baseline, $p < 0.05$

[†] Significantly different from two other time points, $p < 0.05$

Figure 6.

Typical images of blood delivered (mL/min/g) (top row) and perfusion (mL/min/g) (bottom row) for a healthy subject at baseline. Note that the perfusion image is equivalent to the blood delivered image with larger conduit vessels removed (lung regions shown as red in top row of images become black in bottom row of images). Images for baseline (left column), post-20 mL/kg (middle column), and recovery (right column) are shown for comparison.

Figure 7.

Mean perfusion data for the lung divided into three gravitationally-based regions of interest: nondependent, intermediate, and dependent (Figure 4B with large conduit vessels removed). Perfusion was higher in nondependent lung at post-20 mL/kg and recovery when compared to baseline, but did not significantly change in the intermediate or dependent regions.

* Significantly different from baseline, $p < 0.05$

Table 1

Effects of infusion on spirometry (n=5)

	Baseline	Post-20 mL/kg	Recovery	p-value
FEV ₁ (L)	3.92±0.55	3.72±0.59 [†]	3.85±0.53	0.007
FEV ₁ (% predicted)	104±12	98±13 [†]	102±10	0.009
FVC (L)	4.72±0.71	4.50±0.69	4.67±0.67	0.09
FVC (% predicted)	103±10	98±12	102±10	0.08
FEV ₁ /FVC	0.83±0.04	0.83±0.07	0.83±0.03	0.92
FEV ₁ /FVC (% predicted)	100±3	99±8	99±4	0.93
FEF ₂₅₋₇₅	3.90±0.49	3.71±0.87	3.85±0.41	0.52
FEF ₂₅₋₇₅ (% predicted)	101±16	95±20	99±13	0.46

[†] Significantly different from two other time points, p<0.05

Table 2

Cardiovascular parameters at Baseline, Post-20 mL/kg, and Recovery (n=5)

	Baseline	Post-20 mL/kg	Recovery	p-value
Systolic BP (mm Hg)	116±6	127±9 [*]	120±8	0.019
Diastolic BP (mm Hg)	74±6	79±7	75±6	0.086
Heart rate (beats/min)	62±9	66±7	64±9	0.27
Stroke volume (mL)	92±23	89±24 [†]	87±24 [†]	0.0015
Cardiac output (L/min)	5.7±1.1	5.8±1.2	5.4±1.1	0.073

^{*} Significantly different from baseline, p<0.05

[†] Significantly different from two other time points, p<0.05

Table 3

Metabolic parameters at Baseline, Post-20 mL/kg, and Recovery (n=6)

	Baseline	Post-20 mL/kg	Recovery	p-value
$\dot{V}_{O_2}$ (mL/min)	285±33	319±55	297±77	0.23
$\dot{V}_{CO_2}$ (mL/min)	264±45	274±55	267±78	0.82
$\dot{V}_E$ (L/min)	8.6±1.3	9.0±1.7	9.0±2.4	0.62
RER	0.94±0.09	0.87±0.08	0.92±0.07	0.32
f (breaths/min)	10.9±1.5	10.5±1.6	11.0±2.1	0.66
V_t (L)	0.84±0.19	0.91±0.19	0.90±0.29	0.33
FEO ₂	17.0±0.3	16.8±0.4	17.0±0.4	0.14
FECO ₂	3.7±0.3	3.7±0.4	3.6±0.4	0.13

$\dot{V}_{O_2}$, oxygen consumption; $\dot{V}_{CO_2}$, carbon dioxide production; $\dot{V}_E$, total minute ventilation; RER, respiratory exchange ratio; f, respiratory rate; V_t , tidal volume; FEO₂, fraction expired oxygen; FECO₂, fraction expired carbon dioxide

Table 4

Density, blood delivered, and perfusion data for whole lung within sagittal slice at Baseline, Post-20 mL/kg, and Recovery (n=6)

	Baseline	Post-20 mL/kg	Recovery	p-value
Mean density (g/cm ³)	0.21±0.03	0.21±0.02	0.21±0.03	0.85
RD density	0.30±0.04	0.29±0.02	0.29±0.05	0.84
Density Slope (g/cm ⁴)	-0.041±0.005	-0.039±0.010	-0.035±0.008	0.27
Mean blood delivered (mL/min/g)	8.6±2.2	9.3±1.7	10.3±2.1 *	0.01
RD blood delivered	0.91±0.17	0.92±0.11	0.99±0.18	0.49
Blood delivered slope (mL/min/g/cm)	-0.030±0.006	-0.018±0.015	-0.032±0.008	0.06
Mean perfusion (mL/min/g)	1.5±0.5	1.5±0.4	1.6±0.5	0.72
RD perfusion	0.76±0.17	0.75±0.09	0.75±0.12	0.95

* Significantly different from baseline, p<0.05

Table 5

Regional density, blood delivered, and perfusion data at Baseline, Post-20 mL/kg, and Recovery (n=6)

DENSITY (g/cm ³)	Baseline	Post-20 mL/kg	Recovery	p time
Nondependent	0.17±0.02 [#]	0.16±0.02 [#]	0.17±0.02 [#]	0.79
Intermediate	0.21±0.02 [#]	0.21±0.02 [#]	0.22±0.02 [#]	0.93
Dependent	0.24±0.03 [#]	0.23±0.02 [#]	0.24±0.04 [#]	0.82
p region	<0.0001	<0.0001	<0.0001	

BLOOD DELIVERED (mL/min/g)	Baseline	Post-20 mL/kg	Recovery	p time
Nondependent	6.4±1.4 [#]	8.2±1.5 ^{*#}	8.4±1.7 ^{*#}	0.0007
Intermediate	10.1±2.2	10.7±1.8	11.6±2.3	0.08
Dependent	9.4±2.7	9.7±2.0	11.3±2.2 [†]	0.01
P region	<0.0001	0.0007	0.0001	

PERFUSION (mL/min/g)	Baseline	Post-20 mL/kg	Recovery	p time
Nondependent	6.4±1.6 [#]	7.3±1.8 [*]	7.4±1.7 [*]	0.03
Intermediate	7.3±1.8 [#]	7.5±1.8	7.3±2.1	0.90
Dependent	7.8±1.9 [#]	7.9±2.0	8.5±2.1 [#]	0.36
p region	0.0002	0.42	0.006	

p time, ANOVA p-value for the overall effect of infusions and recovery

p region, ANOVA p-value for the overall effect of lung region

^{*} Significantly different from baseline, p<0.05[†] Significantly different from two other time points, p<0.05[#] Significantly different from the other 2 regions, p<0.05

Table 6

Contributions from large conduit vessels expressed as number of voxels and percentage of total number of voxels for region of interest (n=6)

NUMBER OF VOXELS	Baseline	Post-20 mL/kg	Recovery	p time
Lung slice	409±159 6.6%	483±296 7.5%	611±225 [*] 10.4%	0.047
Nondependent	31±15 [#] 2.0%	51±49 [#] 3.0%	60±28 [#] 4.1%	0.07
Intermediate	213±53 8.7%	267±168 11%	288±92 13%	0.21
Dependent	165±113 7.3%	165±132 7.1%	264±134 [*] 12%	0.01
p region	0.001	0.01	0.0004	

p time, ANOVA p-value for the overall effect of infusions and recovery

p region, ANOVA p-value for the overall effect of lung region

^{*} Significantly different from baseline, p<0.05

[†] Significantly different from two other time points, p<0.05

[#] Significantly different from the other 2 regions, p<0.05