## online supplement 1 - Methods unabriged

The study conformed to the regulations of the Australian Code for the care and use of animals for scientific purposes (1) and was approved by the Animal Ethics Committees of Murdoch University (R2588/13) and of the University of Western Australia (RA/3/900/77). Studies were performed at Murdoch University Veterinary Hospital.

### Preparation of animals, anesthesia, and ventilation

Five female pigs (Large White x Landrace x Duroc breed) with a median (IQR) weight of 29.3 (29.0 to 30.6) kg were included in this study.

Anesthesia was induced with a combination of intramuscular zolazepam, tiletamine and xylazine (2 mg/kg each) (Zoletil ®, Virbac, Milperra NSW, Australia and Xylazil 100, Ilium, Troy Laboratories, Glendenning NSW, Australia). The trachea was intubated and anesthesia was maintained with total intravenous anesthesia: thiopentone 9 mg/kg/h (Thiopentone, Troy Laboratories, Glendenning NSW, Australia); 15 mg/kg, morphine 0.1 to 0.2 mg/kg/h, and ketamine 0.4 to 0.8 mg/kg/h. One gram of intravenous vancomycin (Hospira, Melbourne VIC, Australia) was administered over 30 min. Pancuronium bromide (AstraZeneca, North Ryde NSW, Australia) was administered as a neuromuscular blocking agent with an intravenous bolus (0.1 mg/kg) followed by an intravenous infusion (0.1 mg/kg/h). The adequacy of paralysis was assessed with a peripheral nerve stimulator and a train of four stimulus pattern.

The pigs were mechanically ventilated (Babylog VN500, Draeger, Lübeck, Germany) using the following settings: volume guaranteed pressure-controlled continuous mandatory ventilation (PC-CMV/VG), FiO2 0.6, inspiration time adjusted to obtain inspiration to expiration ratio = 1:1.5, inspiratory flow 30 L/min, tidal volume 8 mL/kg. The initial PEEP setting was 5 cmH2O and altered according to the experimental protocol (see below). The maximal airway pressure alarm was set above inspiratory airway pressure throughout the experiment to allow full delivery of the set tidal volume. The initial respiratory rate was adjusted to maintain an end-tidal CO2 of 35 - 45 mmHg. Subsequently, PEEP was the only ventilation setting altered throughout the remainder of the protocol. The cuff pressure was adjusted to 5 cmH2O above inspiratory pressure to minimize gas leak.

The pigs were euthanized by intravenous injection of pentobarbitone (160 mg/kg, 325 mg/mL Lethabarb, Jurox, Rutherford, NSW) at the end of the experiment.

### Respiratory mechanics and lung volumes

Esophageal pressure was recorded using a thin-walled latex balloon (10 cm long) sealed over one end of a polyethylene catheter (Cardinal Health, Hoechberg, Germany) connected to a pressure transducer. Following gastric insertion, the catheter was retracted stepwise until the optimal position in the esophagus was confirmed from the pressure trace and ultimately using CT-guidance.

Airway pressure was transduced at the proximal end of the endotracheal tube. End-inspiratory (EI) and end-expiratory (EE) pressures were obtained after a pause of 3 seconds. The static elastances of the respiratory system (*E*rs), chest wall (*EW*) and lung (*E*L) and the transpulmonary pressures were derived as described previously (2, 3).

Arterial oxygen tension, oxygen saturation, carbon dioxide tension, hemoglobin concentration, mixed venous oxygen tension, and oxygen saturation were measured with a blood gas analyzer immediately following blood collection (Rapidlab 1200, Siemens, Leverkusen, Germany). PaO2 over fractional inspiratory oxygen concentration (P/F ratio), shunt and dead-space fraction were calculated using standard formulae (3).

### Hemodynamic parameters

The animals remained supine throughout the study. Mean arterial blood pressure was measured at the femoral artery. Cardiac output was measured by transpulmonary thermodilution (PiCCO, Pulsion Medical System, Feldkirchen, Germany) with ice-cold saline injected into the internal jugular vein (4). All hemodynamic pressures and IAP were zeroed at the mid-axillary line at the level of the sternum and measured during end-expiration (5). Pressure was sampled and stored continuously using Powerlab and LabChart (v7.0; ADI Instruments, Bella Vista, Australia). Data were analyzed *post-hoc* in LabChart.

Pigs were stabilized hemodynamically with 4 % succinylated gelatin solution (500 mL over the first 30 min followed by 1 mL/kg/h, Gelofusine®, B. Braun, Bella Vista NSW, Australia). Noradrenalin infusion (3 mg/50 mL) was administered if required to maintain a mean arterial pressure ≥ 70 mmHg.

### Intra-abdominal pressure generation and measurement

A large bore orogastric tube was inserted to allow continuous gastric drainage. Intra-abdominal hypertension of 27 cmH2O (20 mmHg) was created by the insufflation of air into the peritoneal cavity through an air-tight catheter. A three-way tap connected to a transducer allowed direct measurement of IAP.

### Experimental protocol

Measurements, including a CT scan, were performed initially at baseline IAP (abdomen not inflated) and again after peritoneal inflation of air to an IAP of 27 cmH2O (20 mmHg). The initial PEEP level was set at 5 cmH2O. PEEP was incremented subsequently to 12, 17, 22, and 27 cmH2O (“ascending”). These PEEP levels corresponded to 19, 44, 63, 81, and 100 % of IAP, respectively. PEEP levels were then decreased (“descending”) to ascertain an optimal “deflation” PEEP. Hysteresis was assessed by comparing measurements obtained at ascending and descending PEEP levels. Recruitment maneuvers were not used. CT images and physiological measurements (see below) were obtained five minutes after a stablization period. Ventilation settings were kept constant except for PEEP.

### Lung injury

The experimental protocol was carried out first with healthy lungs, after which lung injury was created by injecting oleic acid (≥ 99 %, Sigma-Aldrich, Steinheim, Germany) into the internal jugular vein (3): an initial bolus of 0.04 mL/kg oleic acid was followed by a further bolus of 0.01 mL/kg every 10 min until a P/F ratio of 200-300 mmHg was established.

### Computed tomography image acquisition and analysis

A whole-lung helical CT scan (Siemens Somatom Emotion 16, Erlangen, Germany) was performed during an inspiratory pause followed by a repeat scan during an expiratory pause (each about 20 seconds) (6). The scan parameters were standardized to 130 kV, 110 effective mA, and 1.0 pitch. All scan acquisitions were reconstructed using 3 mm slice thickness, with a 3 mm slice interval, and a lung kernel (B90s) at each tested PEEP level.

Image analyses was performed with Maluna® software (MALUNA 3.17, Peter Herrmann, Department of Anesthesiology, Emergency and Intensive Care Medicine, University of Göttingen, Göttingen, Germany). Lungs images were manually outlined and big vessels and airways were excluded in a blinded fashion. Lung volumes were calculated and three segments along the dorso-ventral axis were performed automatically. Left and right lungs were analyzed separately, but combined in graphs and tables. Based on the degree of aeration/density of lung tissue, four aeration compartments were computed: overdistended tissue (-1,000 to -901 Hounsfield units [HU]), normally aerated tissue (-900 to -501 HU), poorly aerated tissue (-500 to -101 HU), and non-aerated (atelectatic) tissue (-100 to 200 HU) (7). Tissue mass was calculated as previously described (8).

CT lung volumes were analyzed further in Excel (v 16 for Mac, Microsoft, Redmond, Washington, USA). Three different equations were applied to the data as outlined below.

1. The Venegas equation (V = a + [b/(1+e –(P-c)/d)]) describes the characteristic sigmoid shape of pulmonary (9) and other P-V curves (10). In the tested situation, V represented CT measured lung volume, P represented PEEP, and a, b, c, and d represented fitting parameters. In the original Venegas equation, a and b approximate the residual volume (lower asymptote volume) and the vital capacity (upper asymptote volume) respectively (9). The upper and lower inflections points are defined as P=c+/-2d (9).
2. An exponential equation, V = e + f \*Ln (P-g) where V represented CT measured lung volume, P represented PEEP, and e, f, g represented fitting parameters.
3. A linear equation, V= h + i \* P, where V represented CT measured lung volume, P represented PEEP, and h and i represented fitting parameters.

The accuracy of each equation was assessed for describing the effect of different PEEP levels on CT volumes. Fitting parameters that best described the measured pressure-volume curve were found for each corresponding pressure-volume data set using the Excel “Solver” function. The best fit was defined as a curve resulting in the smallest root mean square between the measured and calculated pressure-volume points.

### Statistics

A linear mixed model was applied to assess the effect of factors (IAH, lung injury, ascending vs descending PEEP) and covariates (PEEP) on different variables using SPSS (v25, IBM, St Leonards NSW, Australia). This approach accounted for the correlation between the repeated measures on each pig. Main effect was used for analysis of respiratory and hemodynamic outcomes. Main effect plus an interaction with lung segments (PEEP and lung injury) was used for CT measured lung volumes. Laterality (left/right) was included as a fixed factor in the linear mixed model. Differences between pigs were accounted for as a random effect. Missing values were imputed based on the average relative differences between any pig with missing data and the other animals. Linear regression was performed to assess for correlations. A p-value of <0.05 was considered statistically significant. For descriptive statistics, median (IQR) is reported.

### References

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