

ONLINE SUPPLEMENTARY APPENDIX

Regional Ventilation and Perfusion During Flow-Controlled Ventilation in Experimental Lung Injury: Assessment from Dual-Energy Computed Tomography

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Anaesthetic procedures and instrumentation

The animals were bred at a local farm according to standard animal housing procedures until transported in straw-bedded boxes to the laboratory. Anaesthesia was induced by intramuscular injection of tiletamine/zolazepam 6 mg/kg (Zoletil®, Boehringer Ingelheim, Copenhagen, Denmark) and xylazine 2.2 mg/kg (Rompun® vet, Elanco, Ballerup, Denmark). The animals were placed in the supine position, and an auricular vein was cannulated. A bolus of fentanyl (Pharmalink, Spånga, Sweden) 5–10 µg/kg was administered intravenously (iv) before a tracheostomy was performed and the airway secured with a tube (Hi-Contour, Mallinckrodt Medical, Athlone, Ireland) with 8.0 mm inner diameter. Anaesthesia was then maintained with iv ketamine 30 mg/kg/h (Ketaminol® vet., MSD Animal Health, Stockholm, Sweden), midazolam (Dormaquin vet, Dechra Veterinary Products AB, Lund, Sweden) 0.1–0.2 mg/kg/h and fentanyl 4 µg/kg/h in buffered glucose 2.5% (Rehydrex; Fresenius Kabi, Uppsala, Sweden). Intravenous rocuronium at 5 mg/kg/h (B. Braun, Danderyd, Sweden) was added for continuous muscle relaxation after adequate depth of anaesthesia was ensured by absence of reaction to pain stimulus between the front hooves. Ringer's acetate (Baxter, Kista, Sweden) was infused iv at a rate of 10 mL/kg/h during the experiment. The animals

were kept under deep anaesthesia throughout the protocol and finally euthanized with iv injection of 100 mmol potassium chloride (Addex®-Kaliumklorid, Fresenius Kabi, Uppsala, Sweden).

Before instrumentation, Volume-Controlled Ventilation (VCV; Servo-i, Maquet, Solna, Sweden) was initiated with the following settings: tidal volume (VT) 6–8 mL/kg, positive end-expiratory pressure (PEEP) 5 cm H₂O, fraction of inspired oxygen (FiO₂) 0.3, inspiration to expiration (I:E) ratio 1:2, and respiratory rate (RR) set for a minute volume (MV) targeting normocapnia. The right jugular vein was surgically accessed and cannulated with a 9 French (Fr) pulmonary artery (PA) catheter introducer (Merit Medical, South Jordan, UT, USA) and a 7 Fr central venous catheter (Arrow Medical, Kington, UK) for measurement of central venous pressure (CVP) and administration of medications. Through the PA introducer, a floating tip PA catheter (Swan-Ganz thermodilution SP5107H, 7F, 110 cm; Baxter, Irvine, CA, USA) was positioned in the PA with guidance from pressure recordings on a bedside monitor (Philips IntelliVue X2 and Philips MP30, Philips Medical Systems Nederland BV, Best, The Netherlands) for cardiac output (CO) measurement and mixed venous blood sampling. For invasive blood pressure recording and arterial blood sampling, a 5 Fr catheter was inserted into the left common carotid artery after surgical cutdown. A urinary catheter was surgically introduced into the bladder to monitor urinary output.

A belt with 32 electrodes (size XXS, Timpel, São Paulo, Brazil) for acquiring electric impedance tomography (EIT) data was applied immediately caudal to the joint of the front legs and connected to the EIT machine (Enlight 1800, Timpel, São Paulo, Brazil). An esophageal balloon catheter (Erich Jaeger, GmbH, Höchberg, Germany) to monitor

esophageal pressure (Pes) was positioned in the distal third of the esophagus according to the method described by Baydur et al. (1).

Physiological data acquisition

Air flow was recorded from a Fleisch pneumotachograph (Laminar Flow Element type PT, Special Instruments GmbH, Nördlingen, Germany) mounted on the endotracheal tube and connected to a differential pressure transducer (Diff-Cap Pressure Transducer, Special Instruments GmbH, Nördlingen, Germany); airway pressure (Paw) and esophageal pressure (Pes) from a balloon catheter were measured via dedicated pressure transducers (Digimaclic Pressure Transducers, Special Instruments GmbH, Nördlingen, Germany).

The respiratory signals were acquired via a data acquisition system (PowerLab PL 16/35, ADInstruments, Dunedin, New Zealand) controlled by the LabChart software (ver. 7.0, ADInstruments, Dunedin, New Zealand). The inspiratory and expiratory airway volumes were obtained via flow integration.

With pressure sensors calibrated to atmospheric pressure, blood pressures were recorded at mid-thoracic level. Cardiac output was determined by standard PA thermodilution as the mean of three bolus injections of 10 mL iced saline administered at random points within the respiratory cycle. Transpulmonary pressure at end-inspiration and end-expiration was calculated as $P_{aw} - P_{es}$ with P_{aw} and P_{es} values being the mean from two breaths. Mean airway pressure was calculated from two consecutive representative breaths by dividing the area under the pressure–time curve by the duration of the two breaths. Static compliance was measured in VCV 1:1 and corrected compliance was calculated for FCV according to the ventilator manufacturer algorithm (Evone® ventilator; Ventinova Medical BV, Eindhoven, The Netherlands). Blood gases were analyzed (ABL 800 Flex, Radiometer, Copenhagen,

Denmark) with the haemoglobin saturation curve corrected for porcine haemoglobin. Shunt fraction was calculated according to the Berggren equation (2).

Induction of lung injury

Acute lung injury (ALI) was induced by whole lung lavage with body-tempered physiological saline in sequential aliquots of 30 ml/kg (n=2) or iv injected oleic acid (OA; 0.1 ml/kg in aliquots, n=9) targeting a moderate injury, defined as normoxemia on FiO₂ 0.5. During the induction of ALI, the animals were ventilated according to the initial settings (VCV I:E 1:2, VT 6–8 mL/kg, PEEP 5 cm H₂O, normocapnia) and to allow for the injury to stabilize the animals were left at rest for one hour after the last lavage or OA injection.

Computed tomography image acquisition

With the EIT belt removed to avoid artifacts during radiographic exposure, dual-energy computed tomography (DECT) scanning was performed using a second-generation dual-source CT scanner (SOMATOM Definition Flash, Siemens Healthineers, Erlangen, Germany). Simultaneous acquisitions were obtained at 80 and Sn140 kVp, where the high-energy tube was equipped with a tin filter (Sn) to improve spectral separation. The detector configuration was 2 × 16 × 0.6 mm, providing a 38.4 mm z-axis coverage. Guided by topograms obtained during end-inspiratory and end-expiratory breath-holds, the scanner table was fixed at a position where the imaged transverse section was located between the heart and the diaphragm. Dynamic scanning with zero pitch (stationary table acquisition) during each ventilation mode lasted 30 s, and reconstructed images were generated at 380 ms intervals. During each acquisition, 22 mL of iomeprol (400 mg I/mL; Iomeron®, Bracco Imaging SpA, Milan, Italy) was administered iv over 15 s following an initial delay of 2 s.

Transverse 5-mm-thick images from both DECT energy levels were reconstructed on the scanner workstation using the Qr40f quantitative soft-tissue reconstruction kernel. Images were reconstructed using a 210 - 244 mm reconstruction diameter (field of view) with a 512×512 matrix, resulting in an in-plane pixel size ranging from 0.411×0.411 to 0.477×0.477 mm. Single detector collimation was 0.6 mm with a total collimation width of 19.2 mm. Tube current was 281 mA (80 mAs) for the 80 kVp tube and 109 mA for the Sn140 kVp tube. To enable volumetric characterization of tidal excursion at higher temporal resolution, an additional dynamic single-energy CT (SECT) acquisition at 100 kVp was performed after each DECT acquisition. Dynamic images with zero pitch were reconstructed at 50 ms intervals as 5-mm-thick transverse images using the Br59f body reconstruction kernel, optimized for thoracic imaging. Images were reconstructed with a 512×512 matrix, a reconstruction diameter between 206 and 262 mm (0.402×0.402 to 0.512×0.512 mm in-plane pixel size), 1.2 mm detector collimation with a 38.4 mm total collimation width. Tube current was 246 mA.

For all acquisitions (DECT and SECT), images were reconstructed using filtered back projection (FBP). Iterative reconstruction, iMAR (Iterative Metal Artifact Reduction), beam hardening correction, and tube current modulation were disabled.

Reconstruction of iodine distribution images

Voxel-wise three-material decomposition was performed in syngo.CT (version VB20A, Siemens Healthineers, Erlangen, Germany) on paired 80 kVp and Sn140 kVp DECT images using iodine, air, and water as basis materials. The decomposition was performed using the

DriveFlash 80/Sn140 kV preset with fixed attenuation values of –1000 Hounsfield units (HU) for air and 0 HU for water for both low- and high-energy images. The relative contrast medium parameter was set to 3.01, and iodine beam hardening correction was enabled. The resulting iodine distribution images were expressed in HU and used for the subsequent processing described in the main manuscript.

Supplementary table S1

Table S1. Hemodynamic, blood gas, and respiratory mechanics variables during the different ventilation modes.

Ventilation mode ^a :	Baseline, VCV 1:1		VCV 1:1		FCV-MV		FCV-CO ₂		Control, VCV 1:1	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR
HR (min ⁻¹)	80	[75-88]	108	[87-134]	103	[88-125]	102	[87-131]	106	[86-122]
MAP (mmHg)	79	[67-85]	79	[72-97]	79	[73-94]	88	[72-98]	87	[75-98]
MPAP (mmHg)	21	[19-25]	26	[25-27]	27	[22-29]	25	[24-26]	24	[20-25]
CVP (mmHg)	11	[9-12]	10	[8-12]	10	[9-11]	10	[9-12]	10	[9-12]
PAOP (mmHg)	11	[11-14]	12	[9-12]	12	[10-12]	11	[10-12]	12	[10-12]
CO (L·min ⁻¹)	2.9	[2.6-3.4]	3.6	[3.3-5.4]	3.8	[3.2-5.9]	4.0	[3.2-5.4]	3.9	[3.2-5.2]
Shunt (%)	5.7	[4.3-6.9]	8.6	[6.0-14.0]	7.2	[6.5-16.3]	7.8	[4.7-15.0]	10.3	[4.6-12.7]
SpO ₂ (%)	100	[99-100]	97	[95-100]	96	[95-99]	98	[95-100]	96	[92-100]
P _a O ₂ (kPa)	16.2	[15.1-18.4]	14.3	[12.3-16.6]	14.6	[12.8-16.7]	15.5	[12.7-17.0]	14.6	[13.1-16.0]
P _a CO ₂ (kPa)	5.63	[5.49-5.87]	6.01	[5.1-7.07]	6.75	[5.34-7.55]	6.08	[5.25-6.99]	5.82	[4.86-6.76]
EtCO ₂ (kPa)	5.7	[5.5-6.2]	5.7	[5.2-7.7]	5.8	[5.4-7.3]	5.8	[5.2-7.4]	5.8	[5.0-7.4]
P _{Peak} (cmH ₂ O)	17	[17-19]	25	[22.5-26.5]	23	[20-25]	25	[22-29]	24	[22-26]
P _{Mean} (cmH ₂ O)	8.8	[8.7-9.5]	13.7	[11.5-15.3]	14.4	[13.0-16.6]	15.7	[13.4-17.0]	13.4	[11.5-14.5]
PEEP (cmH ₂ O)	5	[5-6]	9	[6-10]	8	[5-10]	8	[5-9]	9	[6-10]
V _t (mL)	231	[211-299]	240	[198-296]	234	[204-296]	243	[205-347]	240	[199-296]
RR (min ⁻¹)	32	[20-37]	33	[19-39]	36	[19.5-39]	37	[22-38.5]	33	[19-39]
MV (L·min ⁻¹)	7.5	[6.0-8.3]	7.9	[5.7-9.2]	7.7	[6.2-8.7]	7.9	[7.2-8.9]	8.0	[5.8-9.3]
Peak flow _{insp} (L·s ⁻¹)	0.28	[0.24-0.32]	0.27	[0.23-0.32]	0.24	[0.22-0.28]	0.29	[0.24-0.30]	0.28	[0.25-0.32]
Peak flow _{exp} (L·s ⁻¹)	-0.61	[-0.68--0.55]	-0.53	[-0.74--0.50]	-0.26	[-0.28--0.20]	-0.28	[-0.28--0.23]	-0.51	[-0.71--0.44]
C _{dyn} (L·cmH ₂ O ⁻¹)	22.0	[20.0-27.0]	16.4	[14.1-18.6]	15	[12.5-16.5]	15.0	[12.0-16.5]	17.5	[14.8-21.0]
C _{stat} (L·cmH ₂ O ⁻¹) ^b	25.0	[24.0-29.0]	21.0	[18.4-23.5]					22.0	[19.4-24.0]
C _{corr} (L·cmH ₂ O ⁻¹) ^c					22.0	[19.1-23.1]	20.3	[18.1-22.0]		

^a–Ventilation modes: Baseline, VCV 1:1, before induction of acute lung injury (ALI); VCV 1:1, first volume-controlled ventilation period performed in the computed tomography (CT) laboratory after induction of ALI; FCV-MV, flow-controlled ventilation in the CT laboratory with minute ventilation matched to VCV; FCV-CO₂, flow-controlled ventilation in the CT laboratory with end-tidal carbon dioxide (EtCO₂) matched to VCV; Control, VCV 1:1, volume-controlled ventilation period performed in the CT laboratory after intervention sequence.

^b–C_{stat}, static respiratory system compliance, calculated only during VCV

^c–C_{corr}, corrected respiratory system compliance (see Appendix), calculated only during FCV.

Abbreviations: VCV, volume-controlled ventilation; FCV, flow-controlled ventilation; IQR, Inter Quartile Range; HR, heart rate; MAP, mean arterial pressure; MPAP, mean pulmonary arterial pressure; CVP, central venous pressure; PAOP, pulmonary artery occlusion pressure; CO, cardiac output; Shunt, intrapulmonary shunt fraction; SpO₂, peripheral oxygen saturation; P_aO₂, arterial partial pressure of oxygen; P_aCO₂, arterial partial pressure of carbon dioxide; EtCO₂, end-tidal carbon dioxide; P_{Peak}, peak airway pressure; P_{Mean}, mean airway pressure; PEEP, positive end-expiratory pressure; V_t, tidal volume; RR, respiratory rate; MV, minute ventilation; Peak flow_{insp}, peak inspiratory flow; Peak flow_{exp}, peak expiratory flow; C_{dyn}, dynamic respiratory system compliance; C_{stat}, static respiratory system compliance, C_{corr}, corrected respiratory system compliance.

Supplementary figure S1

Figure S1a. PEEP-titration at baseline VCV 1:1

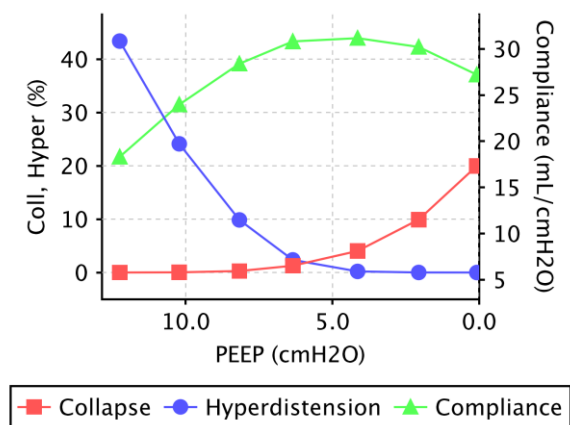


Figure S1a. Example of positive end-expiratory pressure (PEEP) titration during baseline VCV 1:1 before induction of acute lung injury. PEEP was titrated using the Overdistension–Collapse (OD–CL) method based on electrical impedance tomography (EIT) during a stepwise decremental PEEP maneuver.

Figure S1b. PEEP-titration at FCV-MV

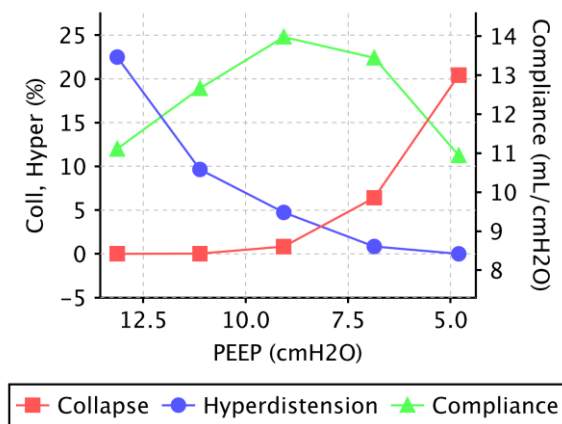


Figure S1b. Example of PEEP-titration during FCV-MV after induction of ALI according to the OD–CL method by EIT during a stepwise decremental PEEP maneuver.

Supplementary figure S2

Figure S2. Mean iodine concentration over time in the aorta

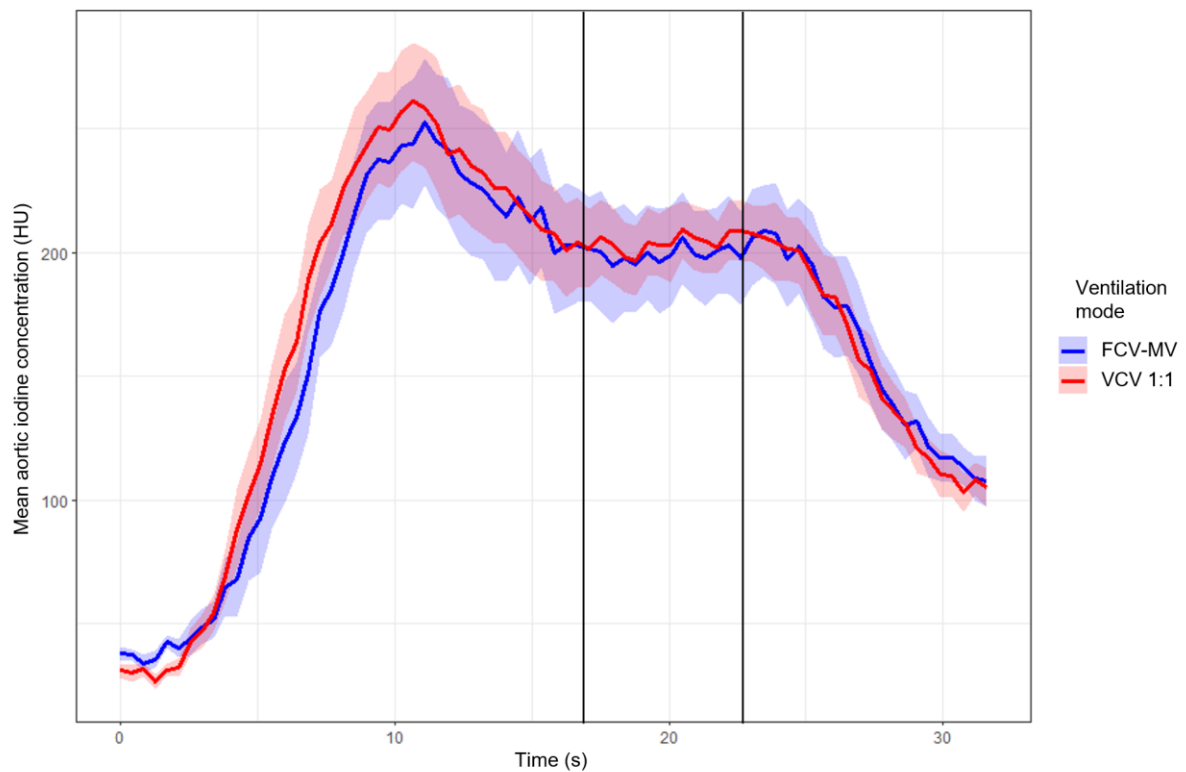


Figure S2. Mean (± 1 standard deviation, shaded regions) aortic iodine concentration (HU) over time following contrast administration. The interval between the black vertical lines (approximately 20 s after start of image acquisition) was considered to represent optimal pulmonary parenchymal enhancement and was used for selecting images for subsequent quantitative analysis.

Supplementary figure S3

Figure S3. Example of Sn140 kVp image and iodine distribution image

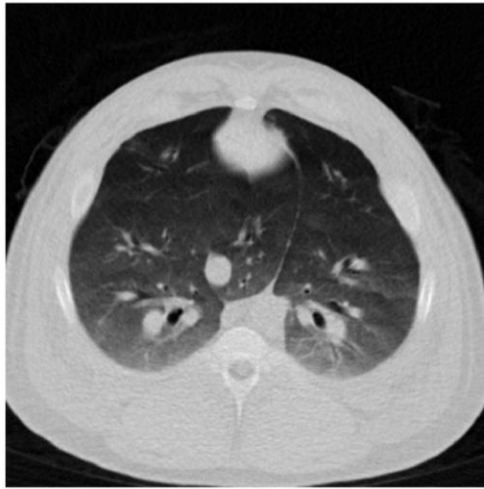
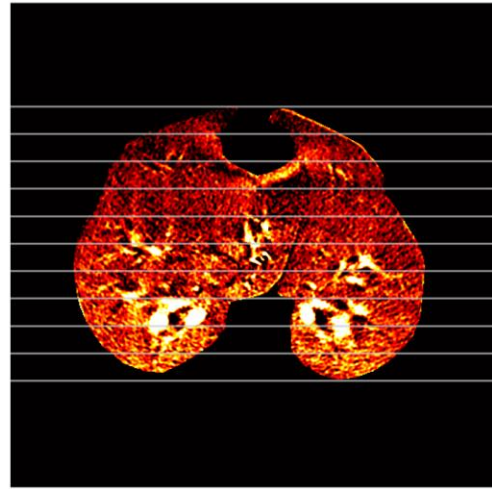


Image acquired at higher energy spectrum (Sn140 kVp)



Iodine distribution image delineated with binary mask and 10 equally distributed ventro-dorsal regions outlined

Figure S3. Representative Sn140 kVp CT image and corresponding iodine distribution image showing the manually segmented binary lung mask and the ten equally sized horizontal ventro-dorsal regions used for regional analysis.

Supplementary figure S4

Video S4. Ten-second video clip showing a representative dynamic single-energy CT acquisition sequence, illustrating the difference in regional gas emptying patterns between VCV 1:1 (left) and FCV-MV (right, labeled "FCV 1" in the video).

References

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2. Peyton PJ, Robinson GJ, McCall PR, Thompson B. Noninvasive measurement of intrapulmonary shunting. *J Cardiothorac Vasc Anesth.* 2004;18(1):47-52.