Lung ultrasound of the dependent lung detects real-time changes in total lung volume in the preterm lamb – Online data supplement

Arun Sett, Kelly R Kenna, Rebecca J Sutton, Elizabeth J Perkins, Magdy Sourial, Jack D Chapman, Susan M Donath, Arun Sasi, Sheryle R Rogerson, Brett J Manley, Peter G Davis, Prue M Pereira-Fantini, David G Tingay

Abbreviations

cm H_20	Centimeters of water
DICOM	Digital Imaging and Communications in Medicine
EIT	Electrical impedance tomography
FiO_2	Fraction of inspired oxygen
hr	Hour
kg	Kilogram
L/min	Liters per minute
LUS	Lung ultrasound
mg	Milligram
ml	Milliliters
PEEP	Positive end expiratory pressure
PIP	Peak inspiratory pressure
Ti	Inspiratory time
TTV	Targeted tidal volume
μg	Microgram

Supplementary methods:

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This was a sub-study of a larger group of studies aiming to determine the impact of different pressure, flow and tidal volume strategies in the delivery room on lung injury. The study was approved by the Murdoch Children's Research Institute Animal Ethics Committee, Melbourne, Australia in accordance with National Health and Medical Research Council (Australia) guidelines and is reported as per the ARRIVE guidelines (1).

Animal preparation

Preterm lambs of 124–128 days gestation (term 140 days) were delivered from anaesthetized (isoflurane [0.5-1%], fentanyl [2-2.5 μg/kg/hr], ketamine [5 mg/kg/hr] and midazolam [1-1.5 mg/kg/hr]), betamethasone treated Border-Leicester/Merino ewes via cesarian section. Lambs were exteriorized and a saline filled glove was placed over the fetal head to prevent lung liquid loss. A carotid artery and a jugular vein catheter were inserted to monitor blood gas status and administer fluids as required. A custom built 32-electrode electrical impedance tomography (EIT) belt (Swisstom AG, Landquart, Switzerland) (2, 3) was fitted around the chest. After intubation with a 4.0 mm cuffed endotracheal tube and passive lung liquid drainage, ventilation was commenced on placental support using the randomly assigned ventilation protocol as part of the primary studies. Lambs were ventilated with using a pressure-controlled, time-cycled SLE5000 ventilator. Ventilation parameters consisted of positive end expiratory pressure (PEEP); 8 centimeters of water (cm H_2O), maximal peak inspiratory pressure (P_{max}); 30 - 50 cm H_2O titrated to achieved tidal volume and lung compliance, targeted tidal volume (TTV); 3 – 7 ml/kg, rate; 60 breaths per minute, inspiratory time (Ti); 0.45 seconds, FiO_2; 0.21, bias flow; 4 – 8

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L/min. All lambs received a dynamic PEEP open lung manoeuver prior to initiation of ventilation (2). After 15 minutes ventilation was ceased, the endotracheal tube was clamped and lambs were maintained on placental support for 30 minutes whilst apneic. At conclusion, the lung was opened to atmosphere for 2-4 minutes and the static PV relationship of the respiratory system was mapped using a 200 mL calibrated glass syringe with pre-defined pressure increments from atmosphere (0 cm H_2O) to maximal inflation pressure (35 cm H_2O) (2). The lung was held at each pressure step until volume stability was achieved or 30 seconds, whichever was first. Opening and closing pressure was identified as the lower inflection point on the inflation limb and upper inflection point on the deflation limb respectively. On completion, the umbilical cord was clamped and a lethal dose of sodium pentobarbitone (100 mg/kg) was administered.

Lung ultrasound (LUS)

LUS was performed simultaneously with mapping of the PV relationship of the respiratory system using a Logiq E ultrasound system (GE Healthcare, Wauwatosa, WI, USA) with a 12-megahertz broadband high-frequency linear transducer. Depth was set to 2.5 cm and the focal zone positioned at the pleural line. Gain was set to 40 decibels and not adjusted between animals. Filters were deactivated. Images were acquired from the right lower lateral position. The transducer was orientated longitudinally to capture 3 rib spaces. The transducer was adjusted to be perpendicular to the skin surface as indicated by maximal pleural line intensity and rib shadowing. Three second videos at each pressure increment of 0, 5, 10, 15, 20, 30 and 35 cm H_2O during the inflation and deflation series were acquired. All LUS images were stored under a unique study number with no details of the measured

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pressures or volumes. Still LUS images were randomized using a computer-generated random number sequence and blindly reported by two researchers (S.R.; 10 years of LUS experience and A.S.; 1 year of LUS experience. Images were exported in Digital Imaging and Communications in Medicine (DICOM) format and converted to mp4 format for reporting. For disagreements, consensus was reached through blinded reporting of the images by A.S. Worst aeration patterns were considered representative (4).

LUS scoring system

An expanded scoring system was developed for this experiment. The system is based on previously validated scoring systems (5, 6). Aeration patterns ranged from 0 – 5, with 5 indicating the best aeration. The purpose of expanding the scoring system is to better quantify the proportion of atelectatic lung present in each image. Score 0 represents complete loss of lung aeration; in this state true lung tissue can be imaged and has a similar sonographic appearance to liver tissue. Atelectatic lung can be seen throughout the field of the image Score 1 represents partially atelectatic lung; the speckling appearance represents air bronchograms. Score 1 is defined by greater than 50% of the depth of the lung image demonstrating a speckling pattern, representing proportionally less atelectasis than Score 0. Score 2 represents improved aeration of atelectatic lung; less than 50% of the depth of the lung field demonstrating speckling. In scores 0 – 2 the pleural line is not established. Score 3 is defined by an established but thickened pleural line and dense coalescing B-lines. This score is analogous to a Type 1 lung pattern (5-7) in simplified scoring systems and a score 2 in the semi-quantitative scoring system (8, 9). Score 4 is defined by a bright pleural line with > 3 well separated B-lines; this score is analogous to a Type 2

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lung pattern (5-7) and a score 1 in the semiquantitative scoring system (8, 9). Score 5 is defined by a bright thin pleural line, numerous A-lines and less than 3 B-lines; this score is analogous to a Type 3 lung pattern (5-7) and a score 0 in the semiquantitative scoring system (8, 9)

Electrical impedance tomography

EIT images were continuously sampled at 48 frames/second throughout mapping of the static PV relationship of the respiratory system. Data were reconstructed using an anatomically correct finite element model of the lamb thorax filtered to the respiratory domain (10). The time-course EIT signal for the whole lung was calibrated against the volume changes measured by the super syringe (2), and the lung volumes of the right whole lung, dorsal, central and ventral regions were determined from weighting the pixel distribution of each region to the calibrated whole lung volumes (11, 12).

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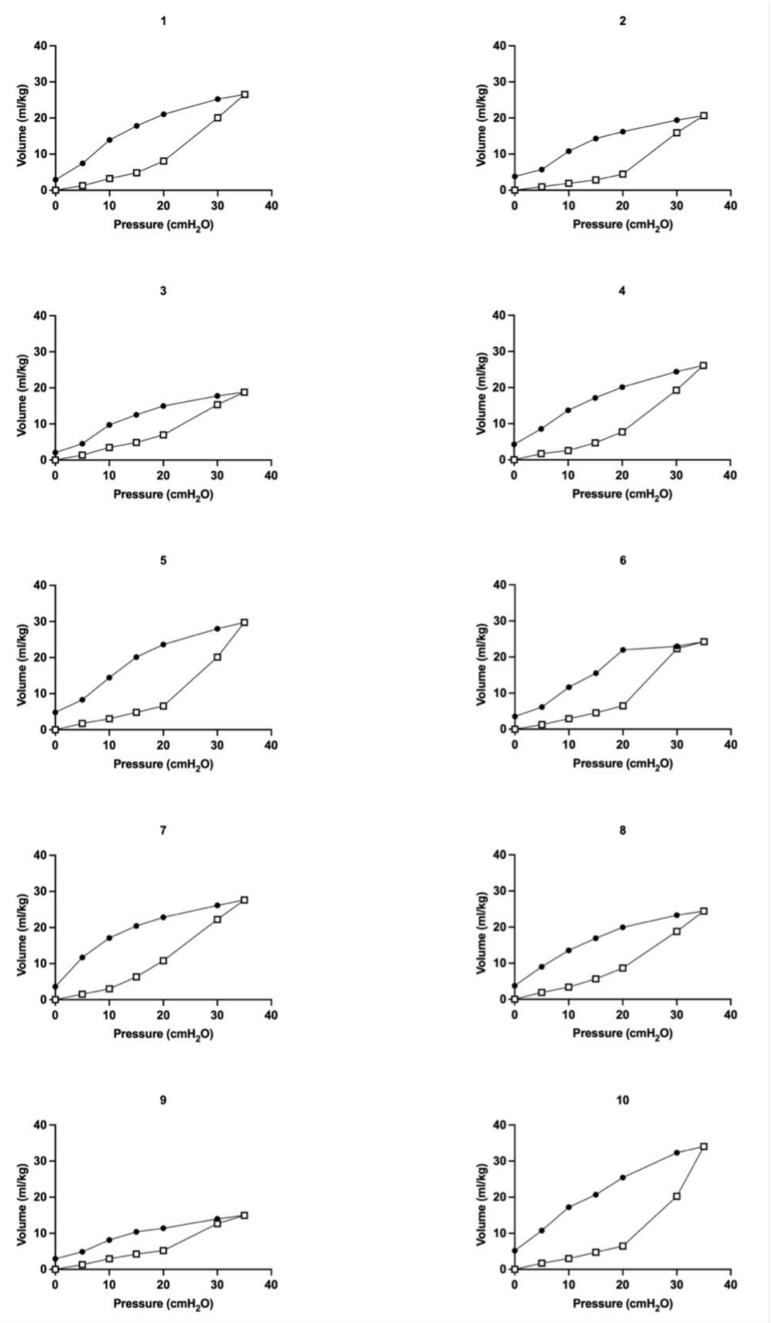
Supplementary figure legends



Supplementary Figure E1A:

Individual PV curves for lambs 1 to 10. Hysteresis is seen in all lambs. Opening and closing pressure occurs at 20 and 10 cm H_2O respectively in all lambs except lamb 6. Open squares; inflation series. Closed circles; deflation series.

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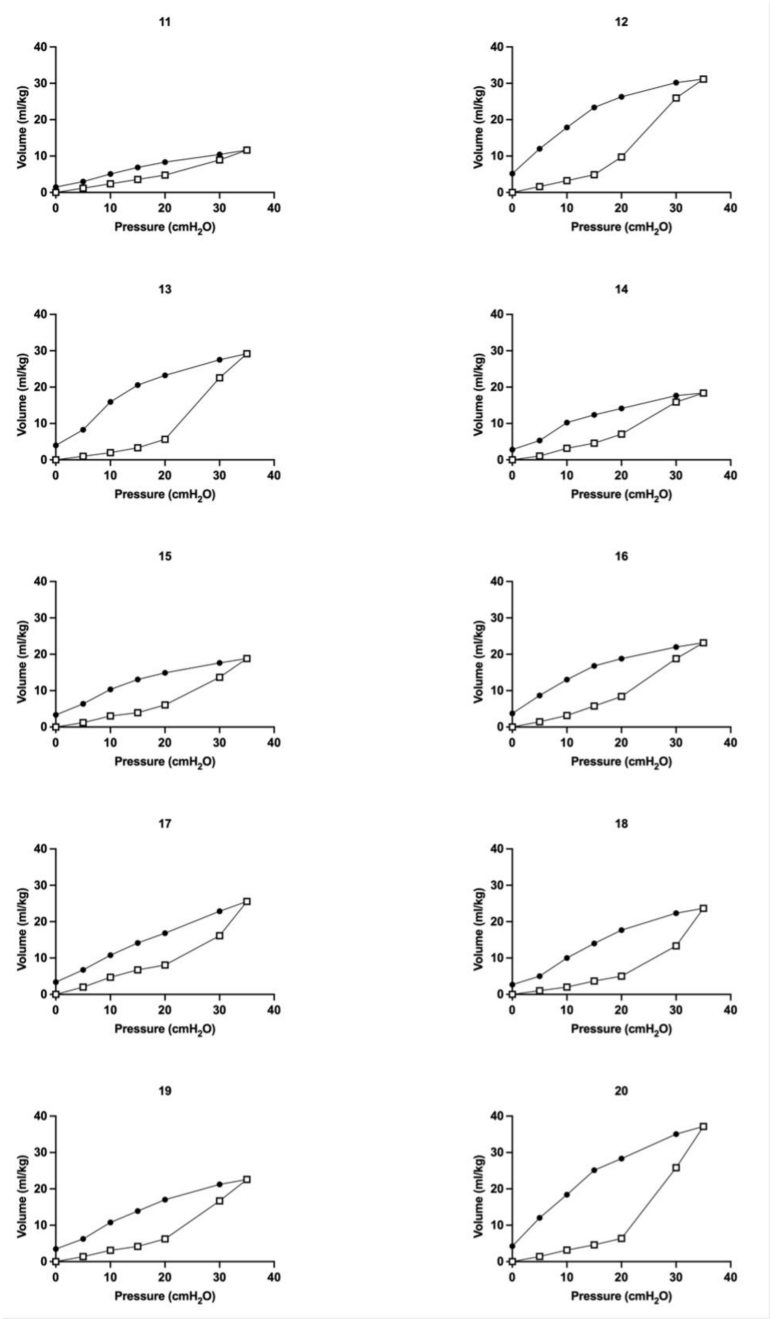
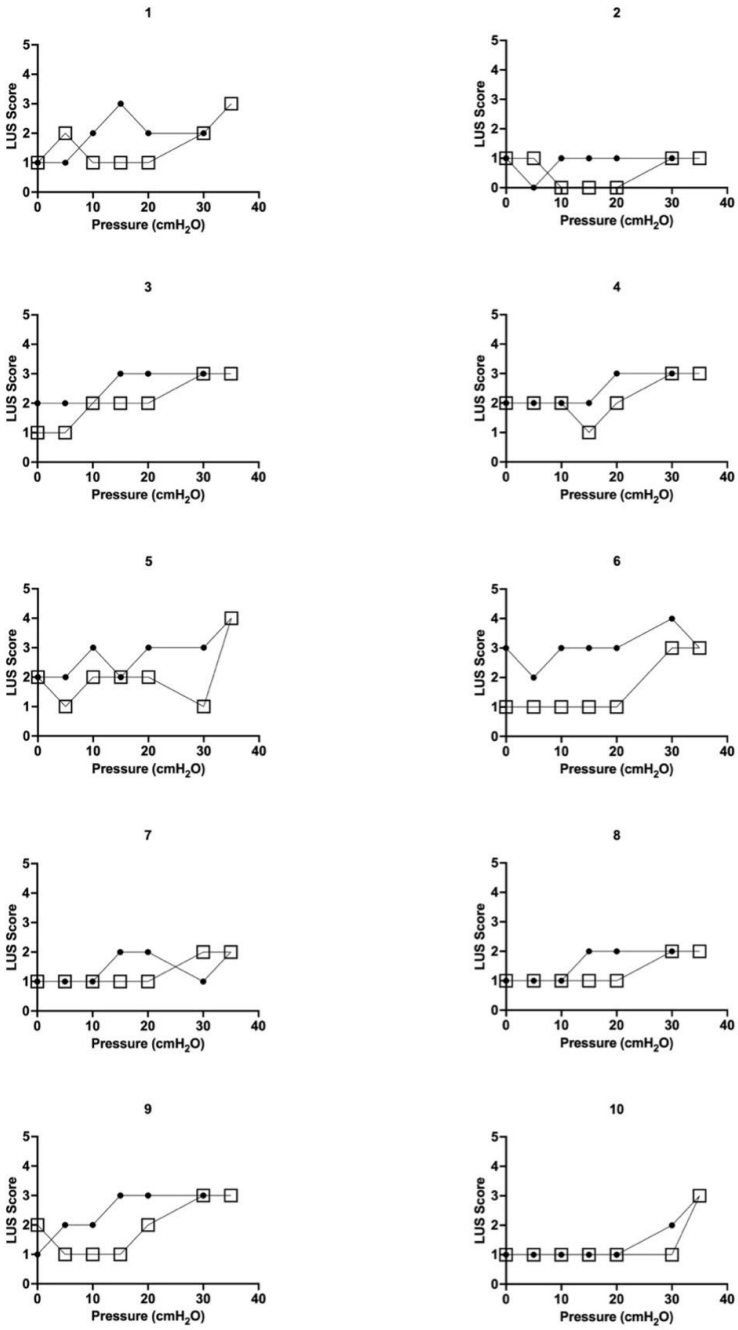


Supplementary Figure E1B:

Individual PV curves for lambs 11 to 20. Hysteresis is seen in all lambs. Opening and closing pressure occurs at 20 and 10 cm H_2O respectively in all lambs. Open squares; inflation series. Closed circles; deflation series



Supplementary Figure E2A:

Individual LUS scores for lambs 1 to 10. Hysteresis is seen in all lambs. Open squares; inflation series. Closed circles; deflation series

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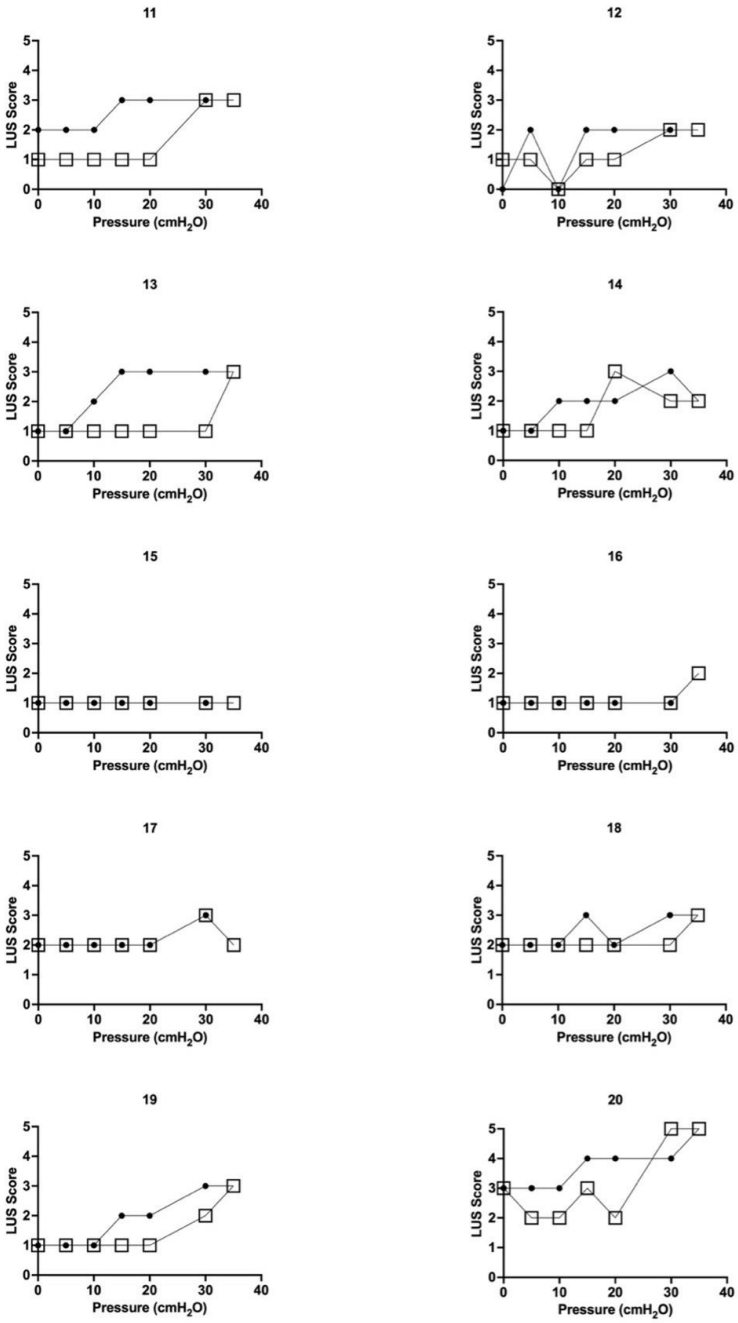

Supplementary Figure E2B:

Individual LUS scores for lambs 11 to 20. Hysteresis is seen in all lambs except 15, 16 and 17. Open squares; inflation series. Closed circles; deflation series.

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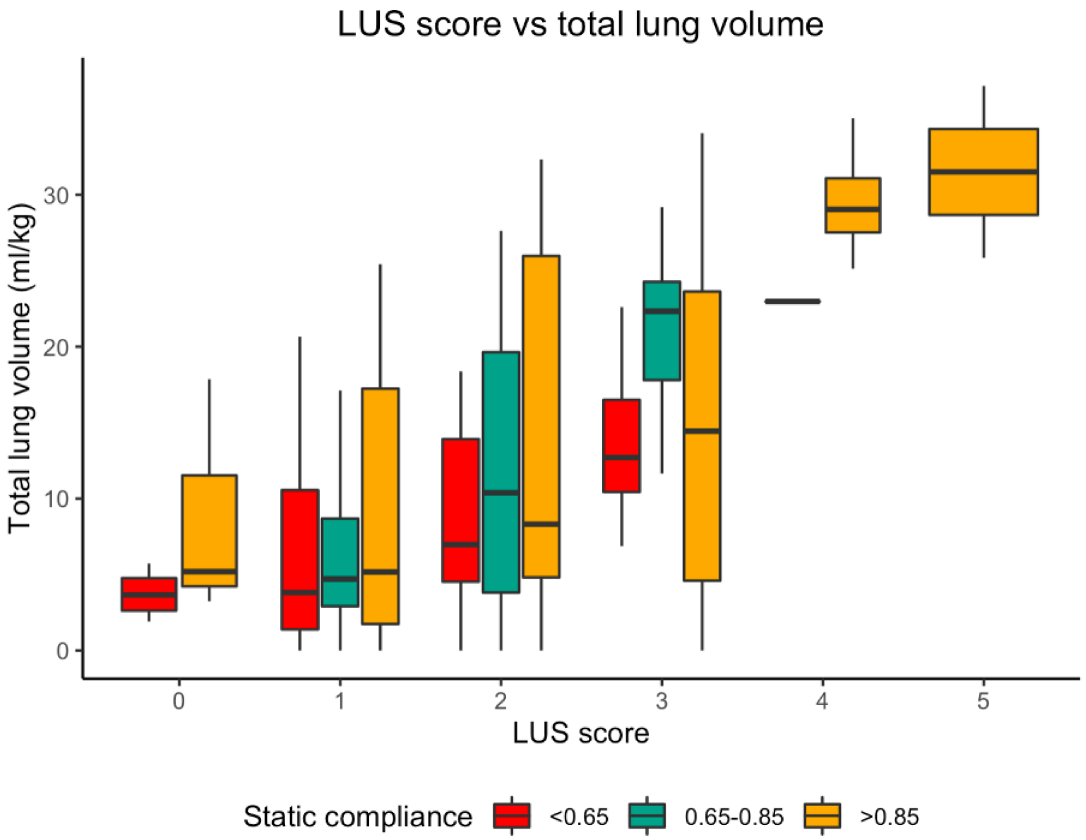

Supplementary Figure E3:

Distribution of LUS scores by static respiratory system compliance. Red: static respiratory system compliance <0.65 ml/kg/cm H_2O, Green: 0.65–0.85 ml/kg/cm H_2O, Yellow: >0.85 ml/kg/cm H_2O

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			Pressure (cm H_2O)											
			Inflation Series							Deflation Series				
			0	5	10	15	20	30	35	30	20	15	10	5
Pressure (cm H_2O)	Inflation Series	5	ns											
		10	ns	ns										
		15	ns	ns	ns									
		20	ns	ns	ns	ns								
		30	ns	ns	ns	ns	ns							
		35	0.01	0.02	0.01	<0.01	0.03	ns						
	Deflation Series	30	0.02	0.04	0.02	0.02	0.02	ns	ns					
		20	0.04	ns	0.03	0.03	ns	ns	ns	ns				
		15	ns	0.05	0.05	0.03	ns	ns	ns	ns	ns			
		10	ns	ns	ns	ns	ns	ns	0.03	0.03	ns	ns		
		5	ns	ns	ns	ns	ns	ns	0.01	0.02	0.02	0.05	ns	
		0	ns	ns	ns	ns	ns	ns	0.02	0.02	ns	ns	ns	ns

Supplementary Table E1: Results for Wilcoxon signed rank sum test with Bonferri correction. Significant results are highlighted in blue. Results approaching significance are highlighted in orange, ns; not significant. Green dashed line; opening pressure, red dashed line; closing pressure.

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