ONLINE DATA SUPPLEMENT

Bubble continuous positive airway pressure enhances lung volume and gas exchange in preterm lambs

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DETAILED METHODS

Investigations were approved by the animal ethics committee of the Western Australia Department of Agriculture and Cincinnati Children's Hospital Medical Centre. Animals were in a flock of sheep quarantined for foot rot, although there was no evidence of foot rot in any of the animals that we used.

Animals, Delivery and Postnatal Care

We used a previously described ovine model of lung disease in moderately preterm lambs treated with CPAP via tracheal tube (1). Labor was induced at 131-132 d gestation in date-mated Merino ewes carrying twins with 20 mg IV Epostane (Sanobi-Synthelabo, PA, USA) and 0.5 mg/kg IM Betamethasone (Celestone Chronodose; Schering Plough, NSW, Australia). Lambs were delivered alive 40 h later by hysterotomy immediately following penetrating captive bolt euthanasia to the pregnant ewe. Lambs (n=5) randomized to no ventilation were killed (50 mg/kg pentobarbital IV) directly after delivery. The remaining lambs were dried, intubated with a 4.5 mm internal diameter, cuffed tracheal tube (Portex Ltd, UK) and treated with continuous positive airway pressure (CPAP) using heated, humidified 100% oxygen. Treated lambs were quasi-randomized to three groups: constant pressure CPAP (Bournes BP200, USA) with a bias flow of 8 L/min (n=12); or bubble CPAP using the same disposable circuit (Fisher & Paykel Healthcare, Auckland, NZ) and a bias flow of 8 L/min (n=12) or 12 L/min (n=10). The depth of underwater seal in the Bubble CPAP circuits was adjusted to achieve similar mean pressures at the airway opening to those obtained with the constant pressure CPAP system measured using a pressure transducer encased within a neonatal respiratory monitor (Florian, Acutronics, Ch). The unsedated, spontaneously breathing lambs were positioned prone, covered with plastic wrap and kept warm on an infant radiant warmer (Fisher & Paykel Healthcare, NZ). Blood obtained from a catheter inserted 20 cm through an umbilical artery into the aorta, was used for blood gas and pH measurements at regular intervals throughout the study (15, 30, 45, 60, 90, 120, 150 min).

Physiological Measurements

Physiological differences between the two treatments were assessed at 2.5 h after attainment of clinical stability (2) using an infant lung function system (Exhalyser, EcoMedics, Duernten, CH). The system incorporated an ultrasonic flowmeter to measure flow and molar mass, a mainstream infrared CO_2 analyser (Duet $EtCO_2$, Welch-Allyn OEM Technologies, OR, USA) and sidestream laser diode O_2 sensor with visible spectrum absorption spectroscopy analysis (Oxygraf, CA, USA). Tidal flow parameters were determined from 30 s data epochs recorded during regular quiet breathing using a sampling frequency of 200 Hz. Peak and trough CO_2 and O_2 were manually determined for each breath during the 30 s interval to determine average volume of CO_2 removal (ΔCO_2) and O_2 (ΔO_2) extraction. CO_2 and O_2 measurements were corrected for sampling delays. Respiratory quotient was determined as $\Delta CO_2/\Delta O_2$. Pressure was measured at the patient tracheal tube connector.

Multiple Breath Washout

Multiple breath washout was performed using an adaptation of existing methodology (3). A sideport, positioned approximately 2 cm proximal to the patient tracheal tube connector, was used to blend a low flow sulfur-hexafluoride/oxygen mixture (79 % SF_6/21% O_2) with the bias flow, to achieve a concentration of 4% SF_6 at the airway opening during washin. The supplemental SF_6 flow was replaced by medical air during the washout to maintain constant flow and inspired oxygen concentrations throughout the study. Washout traces were analysed using commercial software (Wbreath v3.10.3.0, NddMedizintechnik Ag, Zurich, Ch). Functional residual capacity (FRC) was determined from the cumulative exhaled tracer gas (SF_6) divided by the difference in end tidal SF_6 concentration at the start and at completion of the washout. The number of lung volume turnovers at any given breath during the washout was calculated as the cumulative expired volume at that breath divided by the FRC determined at mid-sensor point. The cumulative expired volume was corrected for the external dead space between the sensor and the bias flow. The lung clearance index (LCI) was

calculated as the number of turnovers needed to lower the end tidal tracer gas concentration to $1/40^{th}$ of the starting concentration (4).

Forced Oscillatory Mechanics

The mechanical impedance of the lower respiratory system was measured with customized measuring equipment using the low-frequency forced oscillation technique (5, 6). Mechanical impedance of the lower respiratory system (Z_{lrs}) was obtained using the low-frequency forced oscillation technique (LFOT) as previously described (5, 6). Z_{lrs} spectra computed from four paired measurements of tracheal pressure and oscillatory flow (V') at each time point were averaged and a model comprising an airway compartment with a frequency-independent (Newtonian) resistance (R_{aw}), an inertance (I_{aw}) and a constant-phase tissue compartment characterized by coefficients of tissue damping (resistance) (G) and elastance (H) was fitted to the averaged spectra (see Figure 2) (7) according to Equation 1:

$$Z_{rs} = R_{aw} + jI_{aw} + \left[(G - jH)/\omega^{\alpha}\right] \qquad \text{Equation E1}$$

where R_{aw} is the airway resistance, I_{aw} is the airway inertance, $j = \sqrt{-1}$, G and H are the coefficients for tissue damping and tissue elastance respectively, ω is angular frequency, and α determines the frequency dependence of the real and imaginary parts of the impedance.

Lung Processing

Lambs were humanely killed with an overdose of pentobarbital at 3 h age and the tracheal tube was clamped for 3 min to facilitate oxygen absorption and lung collapse. The lamb thorax was opened and the pressure-volume relationship of the deflation limb was determined (8). Volumes were corrected for the system compliance.

Tissue from the right lower lobe was immediately frozen in liquid nitrogen for wet to dry ratio and Saturated Phosphatidylcholine (Sat PC) assay. An alveolar wash of the left lung was performed with 0.9 % NaCl at 4 °C. Three repeated saline bronchoalveolar lavages were pooled (1), and aliquots were saved for measurement of Sat PC (9, 10), and total protein (11) Sat PC was isolated from chloroform-methanol (2:1) extracts of alveolar washes by neutral

alumina column chromatography after exposure of lipid extracts to osmium tetroxide (9) and quantified by phosphorus assay (10).

Data Analysis and Statistics

Results are shown as mean (± SEM). Statistics were analysed using SPSS v14.0 (SPSS Inc, USA). Two tailed unpaired T-tests were used for comparing the effect of flow on variables within the bubble CPAP group. Two way ANOVA was used for all other statistical comparisons with CPAP level and applied bias flow as the independent factors. Significance was accepted as $p < 0.05$.

RESULTS

Supplemental results highlight the absence of any significant effect of bias flow on physiological outcome variables in the Bubble CPAP groups. Changes in gas exchange over time are shown in Figure E4, whilst Table E1 shows the comparison of physiological variables between lambs treated with 8 L/min or 12 L/min bias flow. Table E2 details the two-way ANOVA of the arterial blood gas time course data.

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Figure E1 – Representative Tidal Breathing Waveforms: Figure shows examples of tidal breathing waveforms recorded in lambs treated with constant pressure CPAP (left column) and bubble CPAP (right column): V_T - Tidal volume; V' - Flow; MM - Molar Mass; P_{ao} - pressure at the airway opening; O_2 % respired Oxygen; CO_2 - % respired Carbon Dioxide.

Figure E2 - Multiple Breath Washout in Lamb on Constant Pressure CPAP: Figure demonstrates changes in A) tidal volume (V_T); B) flow (V'); and C) molar mass (MM) over the course of a washout

Figure E3 -- Representative Impedance Spectra: Figure shows representative impedance spectra obtained from low-frequency forced oscillatory mechanics measurement of the lower respiratory system using a micropressure transducer positioned distal to the tip of the tracheal tube. The resistance is that portion of impedance in phase with flow and is the sum of two components: a frequency independent airway resistance and a frequency dependent tissue resistance. The reactance represents the portion of impedance out of phase with flow and is dominated at low frequencies by the tissue elastance (1/compliance) and at high frequencies by the airway inertance. The point at which reactance crosses the zero line (20 Hz in this example) represents the resonant frequency. The solid line represents the fit of an empirical model which describes the respiratory system as having a frequency independent airway resistance and inertance and a constant phase tissue compartment comprising an element of damping (resistance) and elastance.

Figure E 4 - Effect of Bubble CPAP bias flow on Arterial Gas Exchange Variables: There was no difference between a bias flow of 8 L/min (closed circle) or 12 L/min (open circle) in the arterial pH (panel A), $PaCO_2$ (panel B) or PaO_2 (panel C) at any timepoint over the course of the study.

Table E 1: Comparison of Physiological Variables between 8 L/min and 12 L/min Bias Flow in Bubble CPAP

Variable	Bubble 8 L/min (n=12)	Bubble 12 L/min (n=10)	p value
RR (breaths/min)	74.5	73.3	0.90
V_T (mL/kg)	5.8	6.5	0.20*
MV (mL/kg/min)	430	477	0.33
AFV (mL^2/kg/min)	578	621	0.34*
FRC (mL/kg)	25.6	21.0	0.14
LCI	20.4	25.1	0.08
Sat PC (μM/kg)	3.05	2.98	0.72*
Alveolar Protein (mg/kg)	4.88	6.41	0.16
PaO_2 (mmHg) at 2.5 hr	369	294	0.14
$PaCO_2$ (mmHg) at 2.5 hr	64.2	68.1	0.16
Delta O_2 (mL/kg/min)	7.31	6.98	0.33
Delta CO_2 (mL/kg/min)	6.45	6.52	0.88
RQ	0.93	0.98	0.60
Raw	3.33	1.49	0.07
G (L/cmH_2O)	131	119	0.25
Compliance (1/H) (L/cmH_2O)	1.70	1.74	0.81
V_{40} (mL/kg)	96.7	101.6	0.28

RR – respiratory rate; V_T – tidal volume; MV – minute volume; AFV – area under the flow volume curve; FRC – functional residual capacity; LCI – lung clearance index; Sat PC – saturated phosphatidylcholine; PaO_2 – partial pressure of oxygen in arterial blood; $PaCO_2$ – partial pressure of carbon dioxide in arterial blood; Delta O_2 – oxygen extraction; Delta CO_2 – CO_2 removal; RQ – respiratory quotient; R_{aw} – airway resistance; G – tissue damping; H – tissue elastance; V_{40} – static lung volume at 40 cmH_2O. P value for Unpaired Student's T-test

or Mann-Whitney Rank Sum Test. * indicates nonparametric data compared using Mann-Whitney Rank Sum Test.

Table E 2 – Results of Two-Way ANOVA of Time Course Arterial Blood Gas Measurements Comparing Effect of CPAP Type and Bias Flow

Variable	Time (min)	CP	B8	B12	P value CPAP vs Type	P value CPAP vs Flow
pH	0	7.41 (0.07)	7.41 (0.86)	7.31 (0.06)	0.97	0.87
	15	7.16 (0.10)	7.19 (0.10)	7.41 (0.03)	0.57	0.41
	30	7.21 (0.09)	7.25 (0.10)	7.22 (0.10)	0.36	0.94
	60	.27 (0.11)	7.32 (0.06)	7.24 (0.11)	0.15	0.94
	90	7.29 (0.08)	7.33 (0.02)	7.33 (0.07)	0.10	0.73
	120	7.26 (0.10)	7.35 (0.03)	7.27 (0.11)	0.007*	0.73
	150	7.27 (0.12)	7.35 (0.06)	7.29 (0.07)	0.033*	0.35
$PaCO_2$	0	64.4 (11.2)	62.3 (14.7)	56.5 (8.6)	0.66	0.27
(mmHg)	15	86.8 (12.6)	79.1 (14.7)	73.3 (6.2)	0.13	0.27
	30	79.8 (9.9)	70.4 (11.2)	72.2 (11.5)	0.040*	0.69
	60	76.6 (19.6)	66.7 (7.5)	64.5 (10.4)	0.088	0.71
	90	76.6 (18.2)	63.3 (7.5)	66.5 (8.2)	0.014*	0.55
	120	80.4 (21.3)	64.7 (4.9)	68.4 (6.63)	0.008*	0.53
	150	83.1 (30.9)	65.9 (7.5)	72.0 (8.1)	0.039*	0.47
PaO_2	0	9.4 (4.2)	9.7 (6.3)	8.6 3.7	0.89	0.63
(mmHg)	15	297 (164)	359 (147)	362 96.8	0.29	0.96
	30	243 (162)	315 (123)	275 96.2	0.19	0.49
	60	278 (158)	2659 (134)	296 87.2	0.87	0.63
	90	277 (127)	316 (96.5)	339 89.1	0.38	0.62
	120	270 (152)	358 (118)	312 96.7	0.099	0.40
	150	264 (163)	381 (92.8)	317 (77.9)	0.023*	0.22

Values shown as Mean (SD); CP – constant pressure; B8 - bubble CPAP 8 L/min bias flow; B12 - bubble CPAP 12 L/min bias flow; * $p < 0.05$

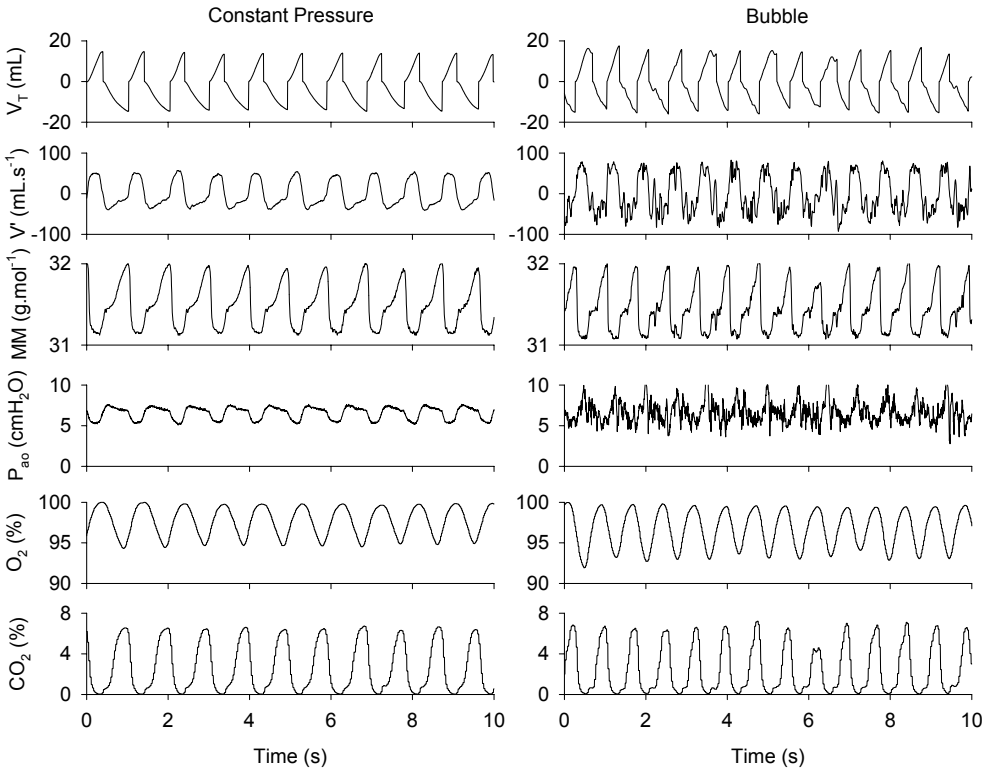
Constant Pressure
Bubble
V_T (mL)
V' (mL.s^{-1})
MM (g.mol^{-1})
P_{ao} (cmH_2O)
O_2 (%)
CO_2 (%)
Time (s)
Time (s)

Figure E1

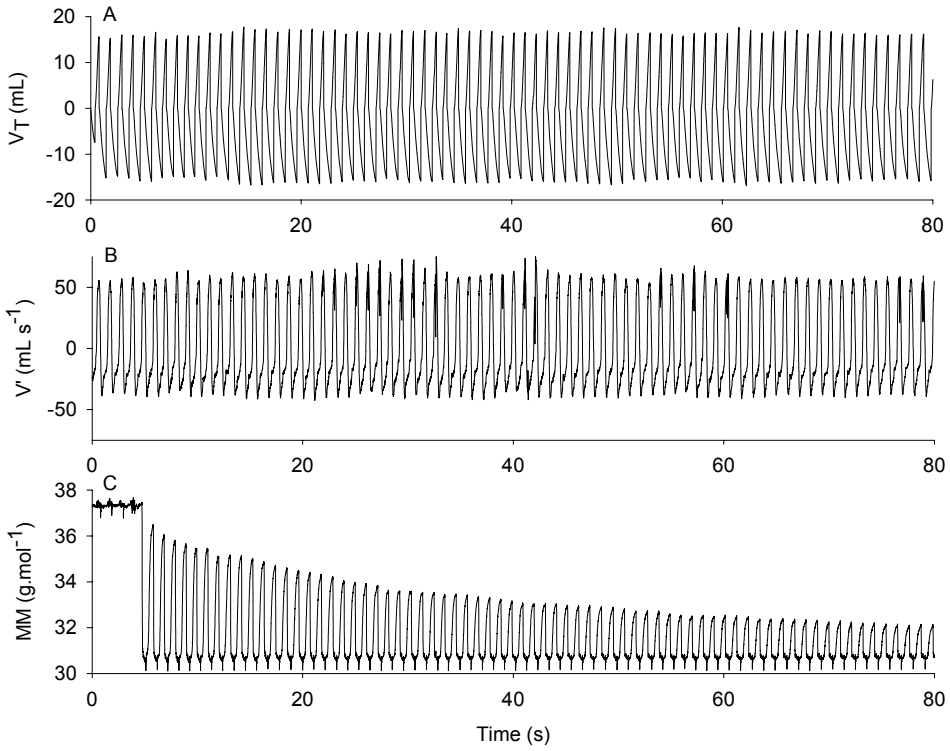
A
B
C
V_T (mL)
V' (mL s^{-1})
MM (g.mol^{-1})
Time (s)
20
10
0
-10
-20
50
-50
38
36
34
32
30
0
20
40
60
80

Figure E2

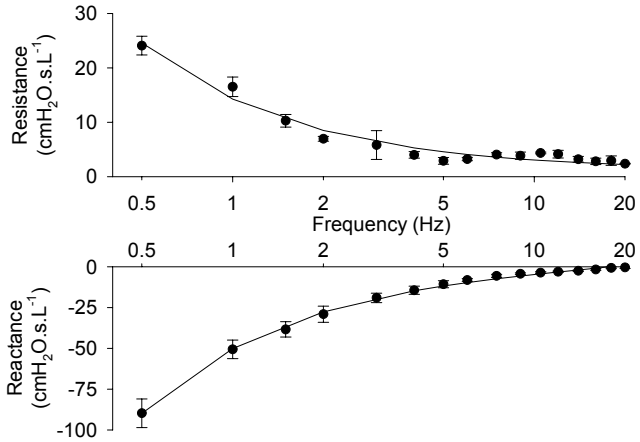
Resistance
(cmH2O.s.L-1)
30
20
10
0
0.5
1
2
5
10
20
Frequency (Hz)
0.5
1
2
5
10
20
Reactance
(cmH2O.s.L-1)
0
-25
-50
-75
-100

Figure E3

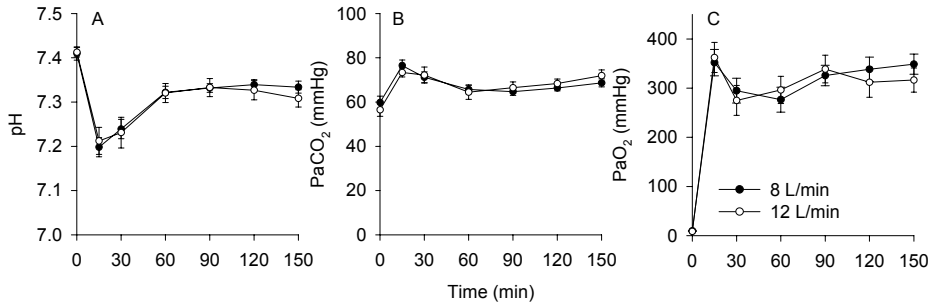

Figure E4