

## BASIC SCIENCE ARTICLE OPEN



# Aeration strategy at birth does not impact carotid haemodynamics in preterm lambs

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**BACKGROUND:** The impact of different respiratory strategies at birth on the preterm lung is well understood; however, concerns have been raised that lung recruitment may impede cerebral haemodynamics. This study aims to examine the effect of three different ventilation strategies on carotid blood flow, carotid artery oxygen content and carotid oxygen delivery.

**METHODS:** 124–127-day gestation apnoeic intubated preterm lambs studied as part of a larger programme primarily assessing lung injury were randomised to positive pressure ventilation with positive end-expiratory pressure (PEEP) 8 cmH<sub>2</sub>O (No-RM;  $n = 12$ ), sustained inflation (SI;  $n = 15$ ) or dynamic PEEP strategy (DynPEEP; maximum PEEP 14 or 20 cmH<sub>2</sub>O,  $n = 41$ ) at birth, followed by 90 min of standardised ventilation. Haemodynamic data were continuously recorded, with intermittent arterial blood gas analysis.

**RESULTS:** Overall carotid blood flow measures were comparable between strategies. Except for mean carotid blood flow that was significantly lower for the SI group compared to the No-RM and DynPEEP groups over the first 3 min ( $p < 0.0001$ , mixed effects model). Carotid oxygen content and oxygen delivery were similar between strategies. Maximum PEEP level did not alter cerebral haemodynamic measures.

**CONCLUSIONS:** Although there were some short-term variations in cerebral haemodynamics between different PEEP strategies and SI, these were not sustained.

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## IMPACT:

- Different pressure strategies to facilitate lung aeration at birth in preterm infants have been proposed. There is minimal information on the effect of lung recruitment on cerebral haemodynamics.
- This is the first study that compares the effect of sustained lung inflation and dynamic and static positive end-expiratory pressure on cerebral haemodynamics.
- We found that the different ventilation strategies did not alter carotid blood flow, carotid oxygen content or carotid oxygen delivery.
- This preclinical study provides some reassurance that respiratory strategies designed to focus on lung aeration at birth may not impact cerebral haemodynamics in preterm neonates.

## INTRODUCTION

Lung aeration is a fundamental component of the transition to air-breathing at birth.<sup>1</sup> Achieving and maintaining lung aeration is challenging for many preterm infants. There has been considerable interest in the optimal methods of supporting lung aeration in preterm infants, including the use of adequate positive end-expiratory pressure (PEEP) or an initial sustained inflation (SI).<sup>2–6</sup> Preclinical studies have repeatedly demonstrated that the use of adequate PEEP at birth, and preferably dynamic PEEP manoeuvres, optimises lung mechanics, aeration and reduces the risks of lung injury compared to SI.<sup>2–6</sup> Recent clinical trials of fixed-duration SI in preterm infants have suggested potentially important adverse outcomes without lung protective benefits.<sup>7</sup> Studies assessing ventilation strategies have primarily focused on respiratory

outcomes. To date, there have been minimal reports detailing cerebrovascular responses to PEEP approaches at birth.

Cerebral blood flow (CeBF) decreases after birth in both healthy term lambs and infants as a proposed response to increasing oxygen saturation and cerebral oxygen delivery following spontaneous breathing.<sup>8,9</sup> However, the behaviour of CeBF and oxygen delivery in preterm infants aerated by PEEP recruitment or SI at birth remains largely unknown. Whilst the use of PEEP is ubiquitous, PEEP strategies at birth have not been subjected to systematic evaluation in preterm infants, resulting in the use of variable PEEP levels.<sup>10</sup> A SI at birth increased CeBF and carotid oxygen content compared to a low static PEEP with tidal inflations in preterm lambs.<sup>11</sup> Beyond the respiratory transition at birth, excessive PEEP levels impacts haemodynamics in preterm

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lambs.<sup>12,13</sup> The impact of higher PEEP levels at birth is less clear, with a recent pilot lamb study suggesting engorgement of microvessels and an increase in CeBF.<sup>14</sup>

We hypothesised that measures of cerebral haemodynamics will be impacted by different PEEP levels or a SI at birth. We aimed to determine the effect of static and dynamic PEEP level strategies or a SI to facilitate lung aeration at birth on carotid blood flow (CBF), carotid arterial blood oxygen (CAO) content and carotid oxygen delivery in intubated preterm lambs.

## METHODS

### Protocol

All techniques and procedures were approved by the Animal Ethics Committee of the Murdoch Children's Research Institute (MCRI), Melbourne, Australia in accordance with National Health and Medical Research Council (NHMRC) guidelines. This study was performed as part of a large inter-linked preterm lamb programme that aimed to determine the optimal methods of applying positive pressure ventilation (PPV) at birth. In the interest of animal reduction, and with the prior knowledge of our Animal Ethics Committee and funding body (NHMRC), some ventilation groups have been reported in different studies with distinctly separate aims.<sup>2–4</sup>

### Subjects

The detailed methodology and complete set of outcome measures for the broader study has been reported previously.<sup>2–5</sup> In summary, 124–127-day preterm lambs born via Caesarean section under general anaesthesia to betamethasone-treated (11.7 mg) ewes were instrumented before delivery. All ewes were from the same flock and studied over two breeding seasons of similar environmental factors. Instrumentation was performed on exteriorisation of the foetal chest and included endotracheal tube intubation (4.0 mm cuffed), passive drainage of foetal lung fluid, flow probe placement around a carotid artery (3 mm Transonic, AD Instruments, Sydney, Australia) and occlusive cannulation of the contralateral carotid artery and external jugular vein. At delivery, the umbilical cord was cut and the lamb moved to a resuscitator and weighed prior to commencing respiratory support (maximum delay between cord cut and first inflation 1 min). CBF, ventilation data, peripheral oxygen saturation (SpO<sub>2</sub>), heart rate and arterial blood pressure were recorded throughout (200 Hz, LabChart V7, AD Instruments, Sydney, Australia). Arterial blood gas (ABG) analysis was performed at foetal (Cord Intact; CI), 5, 15 min and then 15 min after birth. Ketamine and midazolam infusions were titrated to minimum doses that suppressed breathing and maintained analgesia.

### Measurements

As described in detail previously,<sup>2,4</sup> lambs were randomised to either (1) PPV at a PEEP 8 cmH<sub>2</sub>O with volume targeted ventilation (VTV) from birth for 90 min (No-RM), (2) an initial SI delivered at 35 cmH<sub>2</sub>O until aeration stability was achieved on real-time imaging of lung volume using electrical impedance tomography (mean (SD) 153.2 (24.3) s),<sup>2,4,6</sup> or (3) a dynamic PEEP manoeuvre delivered over 3 min using PEEP steps of 2 cmH<sub>2</sub>O to a maximum of 14 cmH<sub>2</sub>O (20 s increments/PEEP step) or 20 cmH<sub>2</sub>O (10 s increments), depending on group allocation, followed by the same stepwise decreases to a final PEEP of 8 cmH<sub>2</sub>O (DynPEEP). After the initial intervention, the SI and DynPEEP groups were managed as per No-RM. Fraction of inspired oxygen and tidal volume (V<sub>T</sub>) were started at 0.3 and 7 ml/kg respectively, and both titrated using a standardised strategy to maintain pre-ductal SpO<sub>2</sub> 88–94% and arterial carbon dioxide 45–60 mmHg following the 5 min ABG, with surfactant therapy at 10 min.<sup>2</sup>

A 2-min measurement of CBF and cardiorespiratory data were made immediately prior to cord clamping. CBF data were extracted at 10 s intervals during the foetal period, both intact cord (CI) and after cord clamping (CC) prior to commencing ventilation. CBF data were then extracted over 10 s at minutely intervals to 5 min after starting respiratory support, and over 20 s intervals at 10, 15, 30 and 90 min. In the DynPEEP groups, measures were made at maximum PEEP (80 s) and end of dynamic PEEP (8 cmH<sub>2</sub>O; 140 s) rather than 1 and 2 min. In all groups, measures from 3 to 90 min were at PEEP 8 cmH<sub>2</sub>O. From the CBF data, the mean waveform amplitude, maximum, minimum, and overall mean were extracted. Carotid blood flow is non-linear with dependency on heart rate and systemic blood pressure, complicated by the possibility of the presence of a ductus arteriosus in a foetus. Multiple waveform

measurements were, therefore, extracted to provide a comprehensive analysis of carotid blood flow. The maximum CBF corresponds to systolic blood pressure values, whilst the minimum corresponds to diastolic values. The amplitude measures the difference between the systolic and diastolic flow values (height of the waveform), while the mean incorporates all phases of the carotid blood flow. Values were then expressed by birth weight. In this study, the contralateral carotid artery was occluded for vascular access and, therefore, the absolute CBF refers to the flow measured in the remaining patent carotid artery. As occlusion of one carotid artery may influence the pattern of upper body flow distribution, we presented the data as both change from baseline for accuracy and in absolute values for ease of understanding. CAO content and carotid oxygen delivery were calculated from the ABG results using the following formulas:<sup>15,16</sup>

$$\text{CAO content (mlO}_2\text{/dLblood)} = (\text{Hb} \times 1.36 \times (\text{SaO}_2/100)) + (\text{PaO}_2 \times 0.0031)$$

Hb, haemoglobin (g/dl); SaO<sub>2</sub>, oxygen saturation of arterial blood (%); PaO<sub>2</sub>, arterial partial pressure of oxygen (mmHg).

$$\text{Carotid oxygen delivery (dL/min/kg)} = \text{CAO} \times \text{meanCBF.}$$

CBF, carotid blood flow (ml/min).

### Data analysis

Sample size was determined by the primary studies that were powered to lung injury outcomes and required larger group sizes (approximately 20 lambs/group) than studies powered to cardiorespiratory measures.<sup>2</sup> Previous preterm lamb studies reporting CBF and CAO content have shown important differences with 6/group.<sup>11,17</sup> Our broader study was designed to investigate different ventilation strategies at birth, specifically SI and DynPEEP. In the interest of reduction, the No-RM group acted as a control across multiple studies. This resulted in an unbalanced number of lambs in each group. As reported in the original studies, there was no difference in adverse event rates between groups. Data were analysed using a mixed-effect model (Tukey post-tests) using ventilation strategy and time as principle variables. Within the DynPEEP group, subgroup analysis comparing the maximum delivered PEEP (14 vs. 20 cmH<sub>2</sub>O) was performed using a two-way analysis of variance. Analysis was performed in PRISM V9 (GraphPad, San Diego, CA) and  $p < 0.05$  considered significant.

## RESULTS

A total of 89 lambs from our data set were available for analysis. Twenty-one lambs were excluded because of unreliable CBF measures related to probe-vessel contact. No lambs were excluded based on clinical stability. Table 1 and Online Supplement Table 1 describe the clinical characteristics of the 68 lambs included for analysis. Incomplete ABG data in 6 lambs resulted in 62 lambs available for calculation of CAO content and carotid oxygen delivery. The lambs were well matched. There were statistical but not clinically significant differences in the amount of foetal lung fluid drained before birth (No-RM higher), foetal PaO<sub>2</sub> (No-RM lower) and minimum CBF and PaCO<sub>2</sub> (both SI higher), with no significant differences in the absolute foetal CI mean and maximum CBF values.

Figure 1 shows the absolute CBF values over time from foetal CI (baseline). Strategy alone did not impact any CBF parameter ( $p = 0.62$ – $0.81$  mixed effects models) while time did (all  $p < 0.0001$ ). In all groups, an overall increase in mean and minimum and a decrease in maximum and amplitude occurred following cord clamping. Mean, maximum and minimum waveform values were higher in the No-RM group compared with DynPEEP at the time of first inflations. All other timepoints and comparison of different ventilation strategies were comparable with no significant differences found in later timepoints. There was no difference in the CBF measures for the 14 and 20 cmH<sub>2</sub>O DynPEEP levels (Online Supplementary Fig. 1).

There was high inter-subject variability in all groups, including the initial CI value. To account for this, Fig. 2 shows the change in CBF from foetal CI. Overall, time had the greatest impact on all CBF

**Table 1.** Lamb Characteristics.

Group	n	GA (days)	Weight (kg)	Gender (F:M)	Parity (S:T)	Foetal fluid (ml/kg)	Static $C_{Hb}$ (ml/kg/cmH <sub>2</sub> O)	Foetal arterial blood gas			Cerebral blood flow (ml/kg/min)				
								pH	PaCO <sub>2</sub> (mmHg)	PaO <sub>2</sub> (mmHg)	BIC (mmol)	Height	Min	Mean	Max
No-RM	12	126 (1.0)	3.37 (0.57)	6:6	1:11	19.0 (6.9)	1.35 (0.20)	7.34 (0.05)	46.0 (5.7)	23.3 (3.5)	24.0 (2.2)	50.3 (14.5)	0.9 (3.1)	15.6 (4.4)	51.3 (14.2)
SI	15	125.4 (1.0)	3.24 (0.43)	5:10	2:13	17.6 (5.8)	1.29 (0.26)	7.35 (0.07)	52.0 (7.6)	25.9 (6.1)	24.8 (2.8)	53.7 (31.1)	3.3 (5.9)	19.5 (10.8)	57.1 (30.5)
DynPEEP	41	125.3 (1.0)	3.39 (0.33)	26:15	3:38	17.4 (5.1)	1.21 (0.20)	7.34 (0.06)	47.3 (7.0)	27.5 (5.4)	23.5 (2.7)	51.0 (16.7)	-0.3 (4.2)	14.4 (5.9)	50.4 (15.8)
p value		0.13	0.43	0.13	0.78	<0.0001	0.11	0.95	0.049	0.009	0.28	0.87	0.04	0.64	0.06

All data are mean (SD) or ratio. All *p* values are derived from one-way ANOVA or chi-test as appropriate.

GA gestational age, F female, M male, S singleton, T multiparity,  $C_{Hb}$  compliance, PaCO<sub>2</sub> partial arterial pressure of carbon dioxide, PaO<sub>2</sub> partial arterial pressure of oxygen, BIC bicarbonate.

waveform measures (all  $p < 0.0001$  mixed effects models), with mean CBF increasing after cord clamping, before decreasing to pre-birth levels by 10 min. Strategy alone only impacted mean CBF ( $p = 0.049$ ), while the combined impact of time and strategy was significant for mean ( $p < 0.0001$ ), minimum ( $p = 0.0007$ ) and maximum ( $p = 0.0012$ ) CBF data, but not amplitude ( $p = 0.15$ ). The change in mean and minimum CBF from foetal values was less during the first 3 min for the SI group. Thereafter, the different ventilation strategies were comparable with no difference in later timepoints. In all groups, clamping of the umbilical cord caused the largest change in CBF (increase mean and minimum, decrease maximum and amplitude). Four lambs (3 DynPEEP; 7%, 1 SI; 7%) had a CBF that persisted at zero or negative values throughout the study (included in analysis).

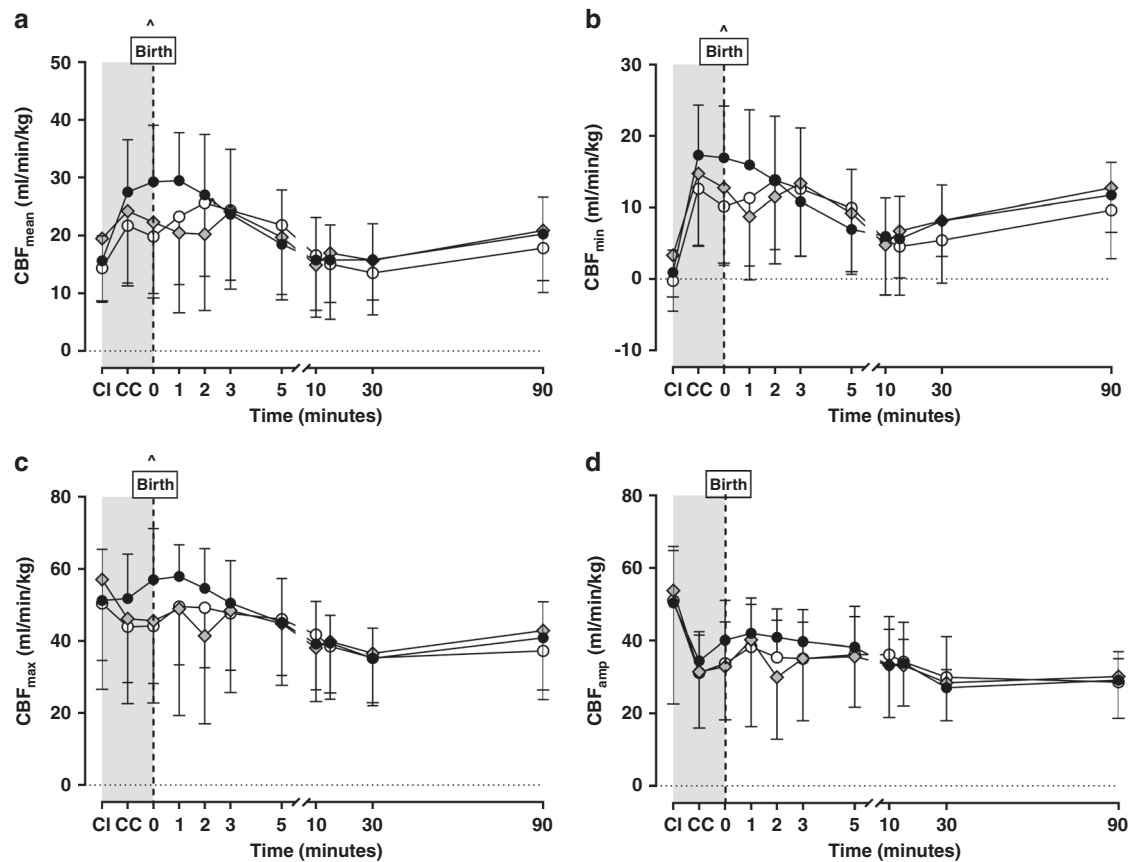
Figure 3 shows the absolute CAO content and carotid oxygen delivery over time. Irrespective of group, ventilation caused an increase in CAO content from foetal values ( $p < 0.0001$  for time). The combined impact of time and strategy was not statistically different ( $p = 0.19$ ). High variability in CAO content occurred in all groups at 5 min, with DynPEEP being lower; however, this was not significant. Carotid oxygen delivery increased similarly after birth for all groups ( $p < 0.0001$  for time and  $p = 0.50$  combined time and strategy). No difference was found in the CAO content and carotid oxygen delivery measures for the 14 and 20 cmH<sub>2</sub>O maximum PEEP levels (Online Supplementary Fig. 2).

## DISCUSSION

Supporting lung aeration at birth in preterm infants improves short-term respiratory outcomes.<sup>1,2,18</sup> Despite the interest in different approaches in supporting aeration of the preterm lung at birth, systematic evaluation of cerebral haemodynamics has been limited.<sup>11,14</sup> To our knowledge, our study is the first to assess the effect on cerebral haemodynamics of different PEEP strategies at birth. In general, we found that the aeration strategy did not cause short-term changes to CBF, CAO content or carotid oxygen delivery. Importantly, there was no significant difference in the use of transient PEEP levels between 8 and 20 cmH<sub>2</sub>O in the first 3 min of respiratory support. Foetal stability and cord clamping practices may play a bigger role in preterm cerebral haemodynamics at birth than the respiratory strategy.

In our study, the biggest change in CBF occurred after the umbilical cord was cut and before ventilation commenced, irrespective of group allocation. Cord clamping results in a wide range of vascular changes in preterm lambs, including an initial reduction in right ventricular output, initial increases in blood pressure over the initial 30 s and stability of heart rate.<sup>19</sup> This suggests that the effect of cord clamping itself is a prominent process that may influence CBF. CBF changes may have been different if the duration between cord clamping and ventilation was shorter or ventilation was commenced before cord clamping.<sup>20–22</sup> Although PEEP levels during ventilation prior to cord clamping have not been compared, our data suggest that the respiratory strategy is less likely to impact cerebral haemodynamics in the preterm infant than other factors such as cord care.

CBF is a commonly reported measure of cerebral haemodynamic physiology.<sup>23,24</sup> We found that the transient increase in mean and minimum CBF at birth was less during the SI compared to No-RM and DynPEEP (initial 3 min). The high intra-thoracic pressure and, therefore, higher pulmonary blood flow and left ventricular output created by SI ventilation may explain this difference compared to the other strategies which use tidal (cyclic) pressure changes. There is little agreement on the effects of SI on cerebral haemodynamics. A possible harmful effect of SI on cerebral oxygenation (measured with near-infrared spectroscopy) was found in a small study of preterm infants.<sup>25</sup> Both an increase in CBF and carotid oxygen delivery (with increased risk of cerebrovascular injury),<sup>17</sup> and a reduction in CBF compared to

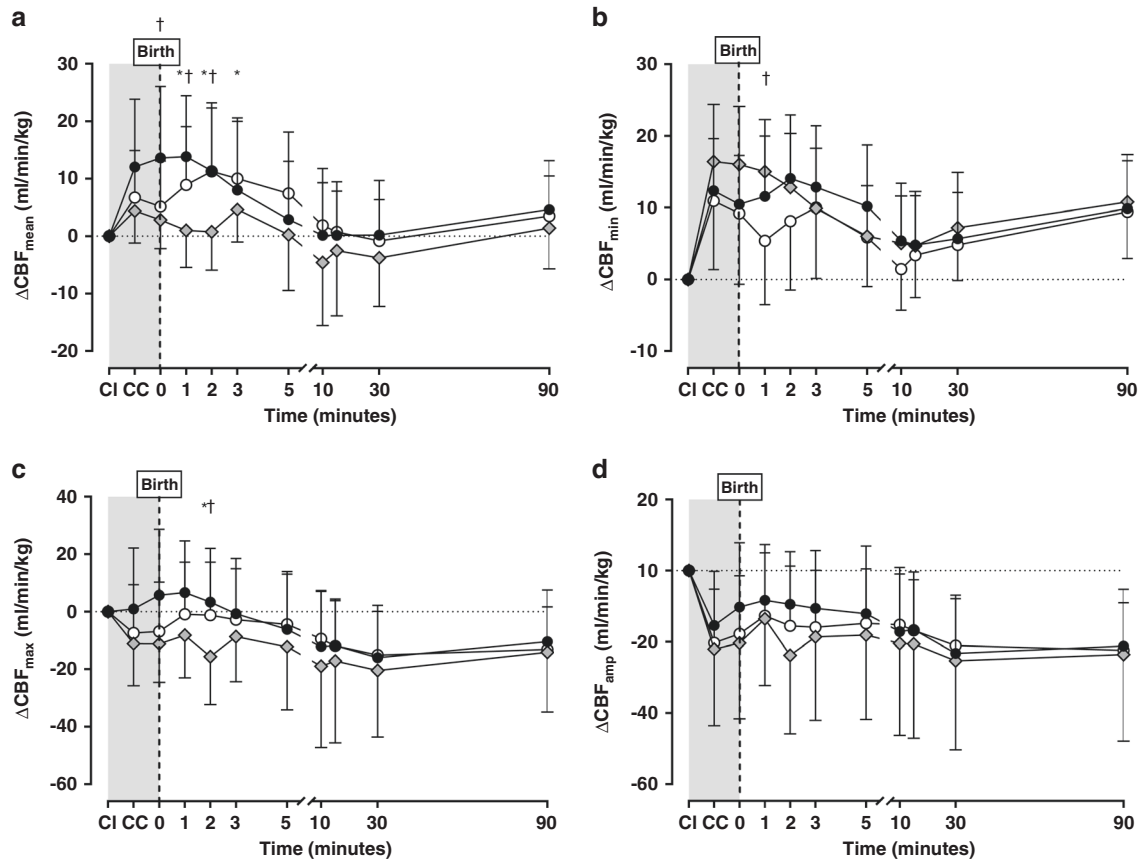


**Fig. 1** Impact of ventilation strategy at birth on carotid blood flow. Absolute CBF waveform measures including the mean (**a**;  $p < 0.0001$ ), minimum (**b**;  $p = 0.0007$ ), maximum (**c**;  $p < 0.0012$ ) and amplitude (**d**;  $p = 0.15$ ) during foetal measures with the cord intact (CI), following cord clamping (CC) before ventilation and 90 min of positive pressure ventilation in the No-RM (black circles), DynPEEP (open circles) and SI (grey diamond) recruitment groups. DynPEEP data represents pooled data for both the 14 and 20 cmH<sub>2</sub>O maximum PEEP. Foetal period without any ventilation shown in grey background. Birth; first 10 s after commencing allocated recruitment strategy. All  $p$  values represent the overall  $p$  value using a mixed effects model (time and strategy combined).  $\wedge$ No-RM vs DynPEEP  $p < 0.05$  Tukey post-tests. All data are mean and standard deviation.

non-SI interventions have been reported in preterm lambs.<sup>11</sup> These conflicting results may reflect the small sample sizes in each study (approximately 6/group). This is particularly relevant given the high level of inter-subject variability that we observed in our larger SI sample of 15, highlighting the importance of appropriately powered preclinical studies. We have also previously reported a high variability in the time and volume response needed to appropriately aerate the lung using a SI.<sup>6,26,27</sup> There is no accepted definition of an 'optimal' SI approach, with predefined, and fixed, time being the most common.<sup>7,28,29</sup> The use of a fixed duration SI is based on the false assumption that lung mechanics are the same in all recipients.<sup>6,26,27</sup> We have previously shown that titrating the SI duration to the aerated response in real-time results in better respiratory outcomes than the 30 s SI most commonly used in lamb studies.<sup>6</sup> In our earlier studies, a 30 s SI could be too short or unnecessarily excessive in terms of lung aeration.<sup>6</sup> This may explain the mixed results in preclinical and clinical preterm SI studies, especially the unexplained higher early mortality in the SI group in the Sustained Aeration of Infant Lungs (SAIL) trial.<sup>30</sup> While titrating the SI duration to response is not used clinically, in a preclinical study it is arguably preferable as it standardises the interventions' impact across all lambs irrespective of lung mechanics. Even with a titrated strategy to aeration, a SI provides no benefit in lung aeration and increases early injury markers compared to DynPEEP and No-RM.<sup>2</sup> The clinical implications of the different CBF patterns found in this study are

unknown and cannot be used to recommend one respiratory support strategy over another.

CAO content provides a measure to assess the availability of oxygen in the blood. CAO content increased from foetal values for all ventilation strategies, indicating that each group supported cerebral oxygenation similarly and successfully. Interestingly, the DynPEEP group did not show an initial increase in CAO content from the foetal baseline until 15 min, compared to 5 min in the SI and No-RM groups. The exact cause for this is unclear, and we can only speculate on the reasons. We have previously reported a lower dynamic respiratory system compliance prior to ventilation in this group of DynPEEP lambs at birth,<sup>2</sup> suggesting a possible foetal difference in the groups. The high variability indicates that the likely reason is a degree of transient hypoxia or asphyxia whilst moving to the resuscitator following cord clamping, which is known to occur with as little as 45 s delay in ventilation.<sup>15</sup> Importantly, the CAO content was similar in all groups by 10 min suggesting that any effect is transient. Irrespectively, these findings indicate that the higher PEEP at birth may impact cerebral oxygen delivery. Future well-powered studies with more arterial sampling intervals in the first 10 min are required. As we cannot conclude that there is not a potential impact on CAO content, clinical trials of DynPEEP, such as Positive End-Expiratory Pressure Levels during Resuscitation of Preterm Infants at Birth (the POLAR trial),<sup>31</sup> should include cerebral complications in their analysis.



**Fig. 2** Change in carotid blood flow (CBF) from foetal state. Change in mean (**a**;  $p < 0.0001$ ), minimum (**b**;  $p = 0.0007$ ), maximum (**c**;  $p = 0.0012$ ) and amplitude (**d**;  $p = 0.15$ ) of the CBF waveform from foetal measures with the cord intact (CI), following cord clamping (CC) before ventilation and then 90 min of positive pressure ventilation in the No-RM (black circles), DynPEEP (open circles) and SI (grey diamond) recruitment groups. DynPEEP data represents pooled data for both the 14 and 20 cmH<sub>2</sub>O maximum PEEP. Foetal period without any ventilation shown in grey background. Birth; first 10 s after commencing allocated recruitment strategy. All  $p$  values represent the overall  $p$  value using a mixed effects model (time and strategy combined). <sup>†</sup>SI vs No-RM, <sup>\*</sup>SI vs DynPEEP  $p < 0.05$  Tukey post-tests. All data are mean and standard deviation.

Carotid oxygen delivery, as a function of blood flow and oxygen content, is a thorough and accurate assessment of carotid haemodynamics. We found that the carotid oxygen delivery similarly increased from foetal levels in all groups, consistent with other comparisons between SI and No-RM in preterm lambs.<sup>11,17,32</sup> Some preterm lamb studies have indicated a significant increase in oxygen delivery in the No-RM group compared to the SI groups, when ventilated with 100% oxygen.<sup>11</sup> While this may indicate that the level of oxygenation influences cerebral haemodynamics, the lack of difference in our groups at 5 min (oxygenated in a standardised 30% inspired oxygen) supports our assertion that achieving lung aeration rather than the means of achieving it is the critical determinant of cerebral haemodynamics.<sup>2</sup>

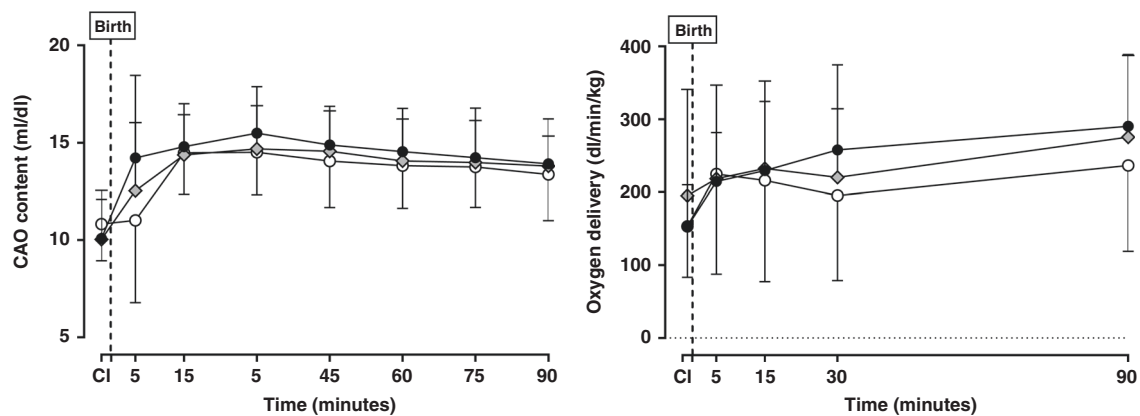
Despite the lung-protective benefits of DynPEEP at birth in preterm lambs,<sup>2,4</sup> this strategy may result in detrimental cardiovascular impacts. Impairment of pulmonary blood flow has been reported in a previous study of lambs managed with a DynPEEP approach between 4 and 10 cmH<sub>2</sub>O after respiratory transition.<sup>12,13</sup> It should be noted that lung aeration was not measured in these studies, limiting any interpretation of the role of volume state (overdistension or recruitment). Our study has been the first to assess the use of DynPEEP on cerebral haemodynamics during the respiratory transition, and we have previously reported that overdistension was not a feature in our lambs.<sup>2</sup> Thus, it is interesting that PEEP levels (between 8 and 20 cmH<sub>2</sub>O) did not significantly alter CBF or cerebral oxygen delivery. An important aspect of these PEEP strategies is the

transient nature of high PEEP exposure while the lung is clearing foetal lung fluid. Current clinical approaches to DynPEEP also constrain PEEP increases to clinical need, based on oxygen and heart rate response<sup>31,33</sup> rather than pre-fixed PEEP values that should further limit any potential risk of inadvertent high intrathoracic pressure on haemodynamics.

A redirection of CBF from the brain was noted in four lambs, most likely during diastole. It is possible that a redirection of blood flow to the ductal and pulmonary arteries caused this.<sup>15</sup> This cannot be confirmed as pulmonary or ductal blood flow was not measured in our study. As DynPEEP studies in infants are now being conducted, understanding the physiological impact of PEEP levels on pulmonary and ductal blood flow is needed.

This study has limitations. Data were sourced from a large biobank of inter-linked preterm lamb studies primarily assessing lung injury resulting in unbalanced group sizes. Cerebral haemodynamics were not the primary outcome of these studies, leading to some lambs being excluded due to unreliable CBF data (but not clinical outcomes). In addition, we found little difference between the three ventilation strategies. Our study was not designed as a non-inferiority or equivalence trial and conclusions regarding cardiovascular and cerebrovascular safety should be made with caution. We studied anaesthetised apnoeic and intubated lambs, a common preclinical model<sup>2,11,13,17,26</sup> but not representative of clinical practice. In our study, cerebral flow dynamics were assessed from a single carotid artery, with occlusion of the other. While this is common practice in lamb





**Fig. 3** Indicators of cerebral oxygen delivery. Absolute carotid arterial oxygen (CAO) content (**a**;  $p = 0.21$ ) and carotid oxygen delivery (**b**;  $p = 0.50$ ) in the foetal period (apnoeic cord intact (CI) and then at select timepoints during 90 min of positive pressure ventilation following the allocated 3 min aeration strategy at birth for the No-RM (black circles), DynPEEP (open circles) and SI groups (grey diamonds). Birth; first 10 s after commencing allocated recruitment strategy. All  $p$  values are derived from mixed effects model (time and strategy combined). All data are mean and standard deviation.

studies,<sup>22,34</sup> it is not possible in humans. Ultrasound measures or near-infrared spectroscopy have been used in humans, including preterm infants, to assess CBF and cerebral oxygenation,<sup>35–37</sup> and may have aided translatability. Extrapolating unilateral carotid blood flow measures to global cerebral haemodynamics requires caution, as unilateral occlusion may result in changes to the flow dynamics of the contralateral side.<sup>24</sup> Further, our measures should not be assumed to represent the cerebral microvascular bed, with a recent preterm lamb pilot study suggesting that large and small cerebral vessel behaviour is not always uniform.<sup>14</sup> Finally, we did not measure cerebral injury as an outcome.

## CONCLUSION

There is a paucity of information on the cerebral haemodynamic effects of respiratory strategy at birth in preterm infants, despite the clear risk of injury in this population. Reassuringly, respiratory strategy did not impact cerebral haemodynamics, suggesting that clinicians can focus on strategies that optimise cardiorespiratory outcomes during the transition to air breathing.

## DATA AVAILABILITY

All data, including raw data used for all figures and analysis, are available upon request to the corresponding author from 3 months following article publication to researchers who provide a methodologically sound proposal, with approval by an independent review committee ('learned intermediary'). Proposals should be directed to david.tingay@mcri.edu.au to gain access. Data requestors will need to sign a data access or material transfer agreement approved by MCRI. The study ARRIVE statement is also available at <https://doi.org/10.26188/19767463.v4>.

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## AUTHOR CONTRIBUTIONS

D.G.T. and P.M.P.-F. developed the concept and designed the experiment. D.G.T., P.M.P.-F., E.J.P., K.E.McC. and M.S. were involved in lamb experimental work. S.I.D., K.R.K. and D.S. performed the carotid and cerebral data analysis. All authors participated in data interpretation. D.G.T. supervised all aspects of the study and subsequent data analysis. S.I.D. wrote the first draft and all authors contributed to redrafting the manuscript.

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## COMPETING INTERESTS

The authors declare no competing interests.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Animal Ethics Committee of the Murdoch Children's Research Institute, Melbourne (MCRI), Australia (A780, A790, A822) in accordance with National Health and Medical Research Council guidelines and is reported as per the ARRIVE guidelines.

## ADDITIONAL INFORMATION

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