Effects of sustained inflation pressure during neonatal cardiopulmonary resuscitation of asphyxiated piglets

Gyu-Hong Shim, Seung Yeun Kim, Po-Yin Cheung, Tze-Fun Lee, Megan O’Reilly, Georg M. Schmöller

1 Centre for the Studies of Asphyxia and Resuscitation, Neonatal Research Unit, Royal Alexandra Hospital, Edmonton, Alberta, Canada, 2 Department of Pediatrics, Inje University Sanggye Paik Hospital, Seoul, Korea, 3 Department of Pediatrics, Eulji University Hospital, Daejeon, Korea, 4 Department of Pediatrics, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada

☯ These authors contributed equally to this work.

Abstract

Background
Sustained inflation (SI) during chest compression (CC = CC+SI) has been recently shown as an alternative method during cardiopulmonary resuscitation in neonates. However, the optimal peak inflation pressure (PIP) of SI during CC+SI to improve ROSC and hemodynamic recovery is unknown.

Objective
To examine if different PIPs of SI during CC+SI will improve ROSC and hemodynamic recovery in severely asphyxiated piglets.

Methods
Twenty-nine newborn piglets (1–3 days old) were anesthetized, intubated, instrumented and exposed to 30-min normocapnic hypoxia followed by asphyxia. Piglets were randomized into four groups: CC+SI with a PIP of 10 cmH₂O (CC+SI_PIP_10, n = 8), a PIP of 20 cmH₂O (CC+SI_PIP_20, n = 8), a PIP of 30 cmH₂O (CC+SI_PIP_30, n = 8), and a sham-operated control group (n = 5). Heart rate, arterial blood pressure, carotid blood flow, cerebral oxygenation, and respiratory parameters were continuously recorded throughout the experiment.

Results
Baseline parameters were similar between all groups. There was no difference in asphyxia-tion (duration and degree) between intervention groups. PIP correlated positively with tidal volume (VT) and inversely with exhaled CO₂ during cardiopulmonary resuscitation. Time to ROSC and rate of ROSC were similar between piglets resuscitated with CC+SI_PIP_10, CC+SI_PIP_20, and CC+SI_PIP_30 cmH₂O: median (IQR) 75 (63–193) sec, 94 (78–210)
sec, and 85 (70–90) sec; 5/8 (63%), 7/8 (88%), and 3/8 (38%) (p = 0.56 and p = 0.12, respectively). All piglets that achieved ROSC survived to four hours post-resuscitation. Piglets resuscitated with CC+SI_PIP_30 cmH\(_2\)O exhibited increased concentrations of pro-inflammatory cytokines interleukin-1\(\beta\) and tumour necrosis factor-\(\alpha\) in the frontoparietal cerebral cortex (both \(p<0.05\) vs. sham-operated controls).

**Conclusion**

In asphyxiated term newborn piglets resuscitated by CC+SI, the use of different PIPs resulted in similar time to ROSC, but PIP at 30 cmH\(_2\)O showed a larger \(V_T\) delivery, lower exhaled CO\(_2\) and increased tissue inflammatory markers in the brain.

**Introduction**

An estimated 10% of newborns need assistance to establish effective ventilation at birth, which remains the most critical step of neonatal resuscitation [1]. The need for chest compressions (CC) in the delivery room is rare (approximately 0.1% of term infants and up to 15% of preterm infants) [2–8]. A systemic review of newborns born between 1991 and 2004 who underwent prolonged chest compressions without signs of life at 10 minutes following birth noted 83% mortality, with 94% of survivors suffering death or severe disability [9]. Since the prognosis associated with receiving CC alone or epinephrine in the delivery room is poor, there is question whether the outcome can be improved with cardiopulmonary resuscitation methods specifically tailored to newborns [10, 11].

Current neonatal resuscitation guidelines recommend using an initial peak inspiratory pressure (PIP) of 20 to 25 cmH\(_2\)O if positive pressure ventilation (PPV) is required during neonatal resuscitation; this can potentially be increased to a PIP of 30 to 40 cmH\(_2\)O in some term infants [12–14]. There is evidence that a PIP of 20 to 25 cmH\(_2\)O causes high tidal volume \(V_T\) delivery in preterm infants [15, 16], and excessive \(V_T\) delivery induced by high PIP may cause hypocarbia [17], which is associated with brain injury [18]. Recently, Mian et al observed that preterm infants <29 weeks’ gestation who received mask PPV with \(V_T\)>6 mL/kg have significantly higher rates of intraventricular hemorrhage [19]. However, to date no study has measured the optimal PIP and the adequate \(V_T\) delivery during CC in the delivery room.

Despite extensive research over the past decade, the most effective way to deliver CC remains controversial. We have previously shown that providing a sustained inflation (SI) during CC (CC+SI) can significantly improve return of spontaneous circulation (ROSC) and survival in newborn piglets [20–23]. Our primary aim was to determine whether different PIP during CC+SI would affect time to ROSC and survival in newborn piglets with asphyxia-induced asystole. We hypothesized that using CC+SI with a PIP of 10 or 20 cmH\(_2\)O would reduce the time needed to achieve ROSC compare to using a PIP of 30 cmH\(_2\)O. The secondary aim was to examine the effect of PIP on hemodynamics and respiratory parameters and brain inflammation after resuscitation.

**Materials and methods**

Twenty-nine newborn mixed breed piglets were obtained on the day of experimentation from the University Swine Research Technology Centre. All experiments were conducted in accordance with the guidelines and approval of the Animal Care and Use Committee (Health
Sciences), University of Alberta AUP00002151, according to the ARRIVE guidelines [24], and registered at preclinicaltrials.eu (PCTE0000149). The study protocol is presented in Fig 1.

**Randomization**

Piglets were randomly allocated to control (sham-operated) or intervention (CC+SI with PIP of 10, 20, or 30 cmH₂O) groups. Allocation was block randomized with variable sized blocks using a computer-generated randomization program (http://www.randomizer.org). Sequentially numbered, sealed, brown envelopes containing the allocation were opened during the experiment (Fig 1).

**Sample size and power estimates**

Our primary outcome measure was time to achieve ROSC. Our previous studies showed a mean ± SD ROSC of 120±25sec during resuscitation using CC+SI with a PIP of 30 cmH₂O. To test the primary hypothesis that CC+SI with a PIP of 10 cmH₂O or 20 cmH₂O would reduce...
time to achieve ROSC, a sample size of 24 piglets (8 per group) would be was sufficient to
detect a clinically important (30%) reduction in time to achieve ROSC (i.e. 84sec vs. 120sec),
with 80% power and a 2-tailed alpha error of 0.05.

Animal preparation
Piglets were instrumented as previously described with modifications [20, 25, 26]. Following
the induction of anaesthesia using isoflurane, piglets were intubated via a tracheostomy, and
pressure-controlled ventilation (Acutronic Fabian HFO; Hirzel, Switzerland) was commenced
at a respiratory rate of 16–20 breaths/min and pressure of 20/5 cmH₂O. Oxygen saturation
was kept within 90–100%, glucose level and hydration was maintained with an intravenous
infusion of 5% dextrose at 10 mL/kg/hr. Throughout the entire experiment period, anaesthesia
was maintained with intravenous propofol 5–10 mg/kg/hr and morphine 0.1 mg/kg/hr. Addi-
tional doses of propofol (1–2 mg/kg) and morphine (0.05–0.1 mg/kg) were also given as
needed. The piglet’s body temperature was maintained at 38.5–39.5˚C using an overhead
warmer and a heating pad.

Hemodynamic parameters
A 5-French Argyle® (Klein-Baker Medical Inc. San Antonio, TX) double-lumen catheter was
inserted via the right femoral vein for administration of fluids and medications. A 5-French
Argyle® single-lumen catheter was inserted above the right renal artery via the femoral artery
for continuous arterial blood pressure monitoring in addition to arterial blood gas measure-
ments. The right common carotid artery was also exposed and encircled with a real-time ultrasonic
flow probe (2mm; Transonic Systems Inc., Ithaca, NY) to measure cerebral blood flow.

Piglets were placed in supine position and allowed to recover from surgical instrumentation
until baseline hemodynamic measures were stable (minimum of one hour). Ventilator rate
was adjusted to keep the partial arterial CO₂ between 35–45 mmHg as determined by periodic
arterial blood gas analysis. Mean systemic arterial pressure, systemic systolic arterial pressure,
heart rate, and percutaneous oxygen saturation were continuously measured and recorded
throughout the experiment with a Hewlett Packard 78833B monitor (Hewlett Packard Co.,
Palo Alto, CA).

Respiratory parameters
A respiratory function monitor (NM3, Respironics, Philips, Andover, MA) was used to contin-
uously measure V₇₅, airway pressures, gas flow, and exhaled CO₂ (ETCO₂). The combined gas
flow and ETCO₂ sensor was placed between the endotracheal tube and the ventilation device.
V₇₅ was calculated by integrating the flow signal [27]. ETCO₂ was measured using non-disper-
sive infrared absorption technique. The accuracy for gas flow is ±0.125 L/min, ETCO₂ ±2
mmHg [28].

Cerebral perfusion
Cerebral oxygenation (CrSO₂) was measured using the Invos⁴⁰⁰⁰ Cerebral/Somatic Oximeter
Monitor (Invos 5100, Somanetics Corp., Troy, MI). The sensors were placed on the right fore-
head of the piglet and secured with wrap and tape. Light shielding was achieved with a slim
cap. The Invos⁴⁰⁰⁰ Cerebral/Somatic Oximeter Monitor calculates crSO₂, which is expressed as
the percentage of oxygenated haemoglobin (oxygenated haemoglobin/total haemoglobin).
Values of regional oxygen saturation were stored every second with a sample rate of 0.13 Hz
[29].
Experimental protocol

Piglets were randomized into four groups: CC+SI with a PIP of 10 cmH\textsubscript{2}O (CC+SI\textsubscript{PIP}\_10, \( n = 8 \)), CC+SI with a PIP of 20 cmH\textsubscript{2}O (CC+SI\textsubscript{PIP}\_20, \( n = 8 \)), CC+SI with a PIP of 30 cmH\textsubscript{2}O (CC+SI\textsubscript{PIP}\_30, \( n = 8 \)), or sham-operated controls (\( n = 5 \)). To reduce selection bias, a two-step randomization process was used. Following surgical instrumentation and stabilization procedure, a subsequently numbered, sealed brown envelope containing the assignment “control” or “intervention” was opened (step one) (Fig 1). Piglets randomized to “intervention” underwent both hypoxia and asphyxia, whereas, the piglets randomized to “control” did not. The sham-operated control group received the same surgical protocol, stabilization, and equivalent experimental periods without hypoxia and asphyxia. The piglets that were randomized to “intervention” were exposed to 30 minutes of normocapnic hypoxia, which was followed by asphyxia. Asphyxia was achieved by disconnecting the ventilator and clamping the endotracheal tube until asystole. Asystole was defined as no heart rate audible during auscultation with standard stethoscope by a single investigator (GMS), who was blinded to HR displayed by ECG and carotid blood flow. After asystole was diagnosed a second subsequently numbered, sealed brown envelope containing the assignment “CC+SI\textsubscript{PIP}\_10 cmH\textsubscript{2}O”, “CC+SI\textsubscript{PIP}\_20 cmH\textsubscript{2}O”, or “CC+SI\textsubscript{PIP}\_30 cmH\textsubscript{2}O” was opened (step two) (Fig 1). Fifteen seconds after asystole was diagnosed PPV was performed for 30 seconds with a Neopuff T-Piece (Fisher & Paykel, Auckland, New Zealand). The default settings of the experiment were a PIP according to group allocation (CC+SI\textsubscript{PIP}\_10, 20, or 30 cmH\textsubscript{2}O), a positive end expiratory pressure of 5 cmH\textsubscript{2}O, and a gas flow of 8 L/min using a fraction of inspired oxygen of 0.21 (Fig 1). Using the two-thumb hand-encircling technique [12–14], CC was performed at a rate of 90/min using a metronome by a single operator in all piglets. After 30 seconds of PPV, CC was started and SI was given for 20 seconds, and then paused for 1 second before resuming another SI of 20 seconds. This was repeated until the piglet achieved ROSC. Epinephrine (0.02 mg/kg per dose) was administered intravenously 2 minutes after the start of PPV, and administered every 3 minutes as needed if no ROSC was observed, to a maximum of four doses. ROSC was defined as an unassisted heart rate \( \geq 100 \) bpm for 15 seconds. After ROSC, piglets recovered for four hours before being euthanized with an intravenous overdose of sodium pentobarbital (120 mg/kg).

Data collection and analysis

Demographics of study piglets were recorded. Transonic flow probes, heart rate and pressure transducer outputs were digitized and recorded with LabChart\textsuperscript{\textregistered} programming software (ADInstruments, Houston, TX). Airway pressures, gas flow, \( V\textsubscript{T} \), and ETCO\textsubscript{2} were measured and analyzed using Flow Tool Physiologic Waveform Viewer (Philips Healthcare, Wallingford, CT, USA). Following euthanization, the brain was removed from the skull and placed in ice-cold 2-methylbutane for 10 minutes before being stored at -80°C. Tissue samples were only collected from piglets that survived four hours after the resuscitation. The frontoparietal cortex was isolated from the whole brain and was homogenized in phosphate buffer (50mM containing 1mM EDTA, pH 7.0). Homogenates were centrifuged (3,000xg for 10 min at 4°C), the supernatants were collected, and protein concentration was quantified using the Bradford method. The concentrations of pro-inflammatory cytokines interleukin (IL)-1\( \beta \), IL-6, and tumour necrosis factor (TNF)-\( \alpha \) in brain tissue homogenates were determined using commercially available ELISA kits (PLB00B, P6000B, PTA00; R&D Systems, Minneapolis, USA). Cytokine concentrations were expressed relative to protein concentrations. The data are presented as mean (SD) for normally distributed continuous variables and median (IQR) when the distribution was skewed. For all respiratory parameters, continuous values during resuscitation
were analyzed. The data was tested for normality and compared using 2-way ANOVA for repeated measures using Bonferroni post-test. *P*-values are 2-sided and *p* < 0.05 was considered statistically significant. Statistical analyses were performed with SigmaPlot (Systat Software Inc., San Jose, USA).

**Results**

Twenty-nine newborn mixed breed piglets 1–3 days old, weighing 2.0 (0.13)kg, were obtained on the day of the experiment and were randomly assigned to either CC+SI_PIP_10, CC+SI_PIP_20, CC+SI_PIP_30, or sham-operated group. There were no significant differences in the baseline parameters between groups (Table 1).

**Resuscitation**

Although there were no significant differences in asphyxia time between groups, there was a trend for a shorter asphyxia time in the CC+SI_PIP_10 group (Table 2). However, this did not result in a difference in the degree of asphyxiation (as indicated by pH, PaCO₂, base excess, and lactate) between the intervention groups (Table 2). During resuscitation, the proportion of piglets that received epinephrine was similar between groups: 5/8 (63%) piglets in the CC+SI_PIP_10 group, 4/8 (50%) piglets in the CC+SI_PIP_20 group, and 5/8 (63%) piglets in the CC+SI_PIP_30 group (*p* = 0.84). Overall, 7/8 (88%) piglets in the CC+SI_PIP_20 group survived, compared to 5/8 (63%) piglets in the CC+SI_PIP_10 group and 3/8 (38%) piglets in the CC+SI_PIP_30 group (*p* = 0.12). Resuscitation time to achieve ROSC was similar between groups (Table 2). All piglets that achieved ROSC survived for four hours after resuscitation (Table 2).

**Respiratory parameters**

Respiratory parameters are presented in Table 3. As expected, there were significant differences in the respiratory parameters between groups, due to the use of different PIP to deliver the SI. The *Vₜ* increased as the PIP used to deliver SI increased: CC+SI_PIP_10: 7.3 (3.3) mL/kg; CC+SI_PIP_20: 10.3 (3.1) mL/kg; CC+SI_PIP_30: 14.0 (3.3) mL/kg; (p = 0.0018). Similarly, 30 cmH₂O PIP resulted in lower exhaled CO₂ compared to 10 and 20 cmH₂O (CC+SI_PIP_30:

Table 1. Baseline characteristics.

|                      | Sham-operated (n = 5) | CC+SI_PIP_10 (n = 8) | CC+SI_PIP_20 (n = 8) | CC+SI_PIP_30 (n = 8) |
|----------------------|-----------------------|----------------------|----------------------|----------------------|
| Age (days)           | 2.0 (1–2.5)           | 2.5 (1–3)            | 2.0 (1–2)            | 2.0 (1–3)            |
| Weight (kg)          | 2.0 (1.8–2.2)         | 2.0 (1.8–2.0)        | 2.1 (2.0–2.1)        | 2.1 (1.9–2.2)        |
| Heart rate (bpm)     | 198 (165–220)         | 196 (188–249)        | 209 (176–221)        | 176 (171–212)        |
| Mean Arterial blood pressure (mmHg) | 67 (60–72)         | 60 (52–69)           | 54 (49–59)           | 56 (53–58)           |
| Carotid flow (mL/min/kg) | 49 (30–57)       | 46 (31–49)           | 41 (30–54)           | 42 (38–51)           |
| Cerebral oxygenation (%) | 59 (54–64)       | 56 (50–63)           | 55 (48–62)           | 53 (46–60)           |
| pH                   | 7.48 (7.46–7.55)      | 7.44 (7.37–7.52)     | 7.52 (7.47–7.55)     | 7.50 (7.45–7.52)     |
| PaCO₂ (torr)         | 31.4 (28.1–34.9)      | 35.3 (25.1–38.3)     | 31.3 (28.0–36.6)     | 33.7 (31.1–34.4)     |
| SpO₂ (%)             | 98 (98–99)           | 98 (97–99)           | 98 (96–99)           | 99 (97–99)           |
| Base excess (mmol/L) | 2.0 (1–5)            | 0.5 (4.8–1.8)        | 4.0 (1–5)            | 2.5 (1.2–5.5)        |
| Lactate (mmol/L)     | 3.1 (3.0–3.9)         | 4.2 (3.1–4.9)        | 4.0 (2.9–5.3)        | 3.7 (3.0–4.6)        |

Data are presented as median (IQR)

https://doi.org/10.1371/journal.pone.0228693.t001
Changes in hemodynamic parameters

Hemodynamic changes of all groups are presented in Fig 2. At baseline, there was no significant difference in heart rate, mean arterial blood pressure, carotid blood flow, and cerebral oxygen saturation between all groups. At the end of asphyxia, all hemodynamic parameters were significantly reduced in the intervention groups compared to sham-operated controls. Following resuscitation and reoxygenation, heart rate returned to similar values as controls. Although mean arterial blood pressure increased towards baseline after resuscitation, values in the CC+SI_PIP_20 group remained lower than the baseline value at the end of the experiment. At the end of reoxygenation, the carotid blood flow was markedly reduced in CC+SI_PIP_20 and CC+SI_PIP_30 groups, resulting in lower cerebral oxygenation as compared with their own baseline values. However, there were no statistical differences between groups at the end of the reoxygenation period.

Brain injury markers

The concentrations of IL-1β and TNF-α in frontoparietal cortex tissue were significantly greater in the CC+SI_PIP_30 group compared to sham-operated controls (Fig 3). There was no significant difference in IL-6 concentrations between all groups (Fig 3).

Table 3. Respiratory parameters before ROSC.

|                      | CC+SI_PIP_10 (n = 8) | CC+SI_PIP_20 (n = 8) | CC+SI_PIP_30 (n = 8) | p value |
|----------------------|----------------------|----------------------|----------------------|---------|
| Peak Inflation Flow  | 4.5 (1.2)            | 6.7 (1.9)            | 8.4 (1.9)            | 0.0006  |
| Peak Expiration Flow | -7.1 (2.0)           | -9.2 (2.8)           | -12.2 (3.1)          | 0.0039  |
| Peak Inflation Pressure (cm H₂O) | 15.7 (4.7) | 25.6 (1.2) | 34.3 (3.8) | <0.0001 |
| Positive End Expiratory Pressure (cm H₂O) | 17.4 (5.6) | 26.5 (3.2) | 34.2 (5.4) | <0.0001 |
| Exhaled CO₂ (mmHg)   | 26.8 (8.5)           | 16.7 (10.6)          | 10.8 (4.5)           | 0.0032  |
| Tidal Volume (mL/kg) | 7.3 (3.3)            | 10.3 (3.1)           | 14.0 (3.3)           | 0.0018  |
| Rate (/min)*         | 90 (1)               | 90 (1)               | 90 (1)               | 1.000   |
| Minute Ventilation (mL/kg/min) | 657 (297) | 927 (279) | 1,260 (297) | 0.0018  |

Data are presented as mean (SD)

*Rate = Ventilation Rate = number of CC corresponds with number of ventilations per min

https://doi.org/10.1371/journal.pone.0228693.t003
Discussion

To optimize the effectiveness of CC+SI in newborn infants, the current study was performed to examine whether different PIP of the delivered SI would have an impact on ROSC, survival, and hemodynamic and respiratory outcomes in a newborn piglet model. To our knowledge, this is the first study investigating different PIP during CC+SI in resuscitation of asphyxiated piglets. The results of the study can be summarized as follows: i) resuscitation using CC+SI with pressures of 10, 20, and 30 cmH₂O resulted in a similar time to ROSC; ii) CC+SI_PIP_20 had a trend to more survival compared to CC+SI_PIP_10 or CC+SI_PIP_30, iii) resuscitation using CC+SI with a pressure of 30 cmH₂O delivered a larger VT and significantly lower exhaled CO₂; and iv) resuscitation using CC+SI with a pressure of 30 cmH₂O resulted in significantly higher pro-inflammatory cytokine concentrations in the brain.

We have previously shown that using CC+SI with a PIP of 30 cmH₂O can significantly improve ROSC and survival in asphyxiated newborn piglets, compared to using the...
coordinated 3:1 compression-to-ventilation ratio (C:V) [20, 21, 23]. However, the optimal PIP for delivering SI during CC remains unknown. Current neonatal resuscitation guidelines recommend using a PIP of 20 to 25 cmH\textsubscript{2}O for preterm infants and up to 30 to 40 cmH\textsubscript{2}O in full-term infants as initial inflating pressure [12–14]. Although there is sufficient evidence that an inflation pressure of 20 to 25 cmH\textsubscript{2}O delivers a high V\textsubscript{T} in preterm infants [15, 16], no study has ever measured the optimal PIP and the delivery of adequate V\textsubscript{T} during CC in the delivery room. Sølevåg et al reported the need for a PIP of approximately 25 cmH\textsubscript{2}O to achieve adequate V\textsubscript{T} in piglet cadavers and determined distending pressure during CC+SI [30]. This study suggests that chest recoil produces an inflation pressure-dependent V\textsubscript{T} allowing passive ventilation during CCs [30]. A similar result was reported while observing chest recoil after the application of a downward force on the chest of infants undergoing surgery requiring general anesthesia [31]. Additionally, the first randomized controlled trial comparing CC+SI and 3:1 C:V in preterm infants <33 weeks’ gestation during neonatal resuscitation in the delivery room used a distending pressure of 24 cmH\textsubscript{2}O reported adequate V\textsubscript{T} delivery [32]. All of the aforementioned studies suggest the optimal PIP for CC+SI is approximately 20–25 cmH\textsubscript{2}O.

Fig 3. Concentrations of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 in frontoparietal cortex tissue homogenates in sham-operated controls (n = 5), and piglets resuscitated using CC+SI with a PIP of 10 cmH\textsubscript{2}O (CC+SI_PIP_10, n = 5), a PIP of 20 cmH\textsubscript{2}O (CC+SI_PIP_20, n = 7), and a PIP of 30 cmH\textsubscript{2}O (CC+SI_PIP_30, n = 3). Results represent the median (solid bar), IQR (box margin), and 95% confidence interval. Asterisk (*) indicates a significant difference compared to sham-operated controls (p<0.05).

https://doi.org/10.1371/journal.pone.0228693.g003
Notably, appropriate distending pressure is significant for proper ventilation of the neonate. While it is known that delivery of an adequate V\textsubscript{T} during CC is vital, it can be difficult to measure V\textsubscript{T} in the delivery room during 3:1 C:V using mask or endotracheal intubation [20, 27, 33]. Moreover, although several studies have demonstrated CC+SI can provide adequate V\textsubscript{T}, relatively high distending pressure may cause higher V\textsubscript{T} [20, 33, 34]. Therefore, clinicians should be aware of the V\textsubscript{T} used during PPV and deliver \(<6–8\) mL/kg, particularly in very preterm infants [19, 27, 33]. In this study, the delivered V\textsubscript{T} was significantly greater in piglets resuscitated with CC+SI\textsubscript{PIP_30}, 14.0 (3.3) mL/kg, compared to the V\textsubscript{T} delivered when using CC+SI\textsubscript{PIP_10} and CC+SI\textsubscript{PIP_20}: 7.3 (3.3) and 10.3 (3.1) mL/kg, respectively. High V\textsubscript{T} delivery during PPV (>8 mL/kg) has been shown to cause brain inflammation and injury in several animal studies [35–37]. Recently, Sobotka et al also reported that using a single SI for 30 seconds, followed by PPV in an asphyxiated near-term lamb model could result in the disruption of the blood brain barrier and cerebral vascular leakage [38]. These injuries may be caused either by a direct result of the initial SI or a higher V\textsubscript{T} delivered during subsequent ventilations [38].

Furthermore, a recent observational study in preterm infants <29 weeks’ gestation observed a four-fold increase in intraventricular hemorrhage in infants ventilated even with a V\textsubscript{T} >6 mL/kg [19]. Overall, these studies showed the link between high V\textsubscript{T} delivery and the progression of brain injury. It is understood that high V\textsubscript{T} may cause brain inflammation and pathology through two mechanisms: hemodynamic instability, and a localized cerebral inflammatory response arising from ventilator-induced lung injury [39]. Interestingly, in our study, piglets resuscitated with CC+SI\textsubscript{PIP_20} showed lower mean arterial pressure, carotid blood flow, and crSO\textsubscript{2} while CC+SI\textsubscript{PIP_30} showed a lower carotid blood flow and crSO\textsubscript{2}, when compared to baseline levels at the end of reoxygenation. This decrease in carotid blood flow for CC+SI\textsubscript{PIP_20} and CC+SI\textsubscript{PIP_30} may be due to alterations to pulmonary blood flow resulting from a high V\textsubscript{T}, which have previously been shown in preterm lamb studies [37, 40]. This decrease in carotid blood flow may also cause hemodynamic instability, which can lead to brain injuries.

In addition, our study showed that piglets resuscitated with CC+SI\textsubscript{PIP_30} also exhibited higher levels of the pro-inflammatory cytokines IL-1\textbeta and TNF-\alpha in frontoparietal cortex tissue. In a review by Polglase et al, it was suggested that excessive V\textsubscript{T} may initiate a pulmonary pro-inflammatory response and a systemic inflammatory cascade leading to brain injury [37]. This is also consistent with our findings, which suggested the large V\textsubscript{T} delivered by using a PIP of 30 cmH\textsubscript{2}O during CC+SI leads to the initiation of an inflammatory cascade, which in-turn increased pro-inflammatory cytokine levels in the brain. Brain injury may therefore be increased in the CC+SI\textsubscript{PIP_30} group by either of these two mechanisms. Although our use of 20 cmH\textsubscript{2}O PIP generated a V\textsubscript{T} >8 mL/kg, the lack of an inflammatory response in the brain tissue suggests it may be less injurious than a PIP of 30 cmH\textsubscript{2}O. In terms of brain protection, a PIP of 10 cmH\textsubscript{2}O may appear to be the optimal pressure. Even though there were no statistically significant differences between groups, the hemodynamic recovery of the CC+SI\textsubscript{PIP_10} group tended to be better than both CC+SI\textsubscript{PIP_20} and CC+SI\textsubscript{PIP_30} groups during the four-hour observation period. However, in a clinical setting where the newborn is undergoing the fetal-to-neonatal transition, using a PIP of 10 cmH\textsubscript{2}O may not provide enough pressure to drive against any lung liquid retained within the airways. Further studies are needed to verify its effectiveness in the clinical setting.

Current neonatal resuscitation guidelines recommend 100% oxygen during neonatal CC [12–14], however the most effective oxygen concentration during CC remains controversial. To date, there are no available clinical studies regarding oxygen use during neonatal CC. Sole-våg et al reported that asphyxiated piglets resuscitated with 21% vs. 100% oxygen have similar
time to ROSC (ranging from 75 to 592 s) with very high mortality rates (50–75%) in both groups during CC [41]. In addition, a recent meta-analysis of eight animal trials (n = 323 animals) comparing various oxygen concentrations during CC showed no difference in mortality rates and time to ROSC [42]. These results suggest that 21% oxygen has similar time to ROSC and mortality as 100% oxygen during CC. Therefore, we used 21% oxygen during CC in our experiment.

In the current study, the time to achieve ROSC for all groups was similar. Although there were more piglets that achieved ROSC following resuscitation with CC+SI_PIP_20, this was not significantly different compared to using CC+SI_PIP_10 and CC+SI_PIP_30. During CC +SI, improved carotid blood flow, mean arterial pressure, % change in ejection fraction, cardiac output, alveolar oxygen delivery and lung aeration may all result in faster ROSC by increasing intrathoracic pressure and improving minute ventilation [20–23, 32]. In our study there were significant differences in minute ventilation between groups, but there were no differences in carotid blood flow at 1 hour after ROSC. As there were no differences in carotid blood flow and the time to ROSC, carotid blood flow may be a major indicator in determining the time to ROSC.

In our study, the use of 30 cmH₂O PIP resulted in lower exhaled CO₂ compared to 10 and 20 cmH₂O (CC+SI_PIP_30: 10.8 (4.5) mmHg vs. CC+SI_PIP_10: 26.8 (8.5) mmHg vs. CC+SI_PIP_20: 16.7 (10.6) mmHg; p = 0.0032). Li et al reported that exhaled CO₂, partial pressure of exhaled CO₂, and volume of expired CO₂, were significantly higher in surviving piglets compared to non-surviving piglets during resuscitation [33]. Chalak et al additionally reported a cut-off of 14 mmHg for exhaled CO₂ to be the most reliable indicator for ROSC with 92% sensitivity and 81% specificity [43]. The CC+SI_PIP_30 group had the lowest exhaled CO₂, which was below the 14 mmHg cut-off. Our results suggest that high V₇ and low exhaled CO₂ with a PIP 30 cmH₂O may be associated with hemodynamic instability and increased pro-inflammatory cytokines, which may increase brain injury and decrease survival.

Our use of a piglet asphyxia model is a great strength of this translational study, as this model closely simulates delivery room events, with the gradual onset of severe asphyxia leading to bradycardia [23, 44]. However, several limitations should be considered before implementing CC during SI in the delivery room. Our asphyxia model uses piglets that have already undergone the fetal-to-neonatal transition, and piglets were sedated/anesthetized [21, 44]. Furthermore, our model requires piglets to be intubated with a tightly sealed endotracheal tube to prevent any endotracheal tube leak [23, 44]. Although endotracheal intubation may not occur in the delivery room as mask ventilation is frequently used, it is recommended when CC is administered [12–14]. The use of SI as an initial respiratory support technique might also be harmful, as indicated by a recent multicenter trial comparing SI versus PPV (SAIL [Sustained Aeration of Infant Lungs] trial) [45]. However, in our model SI was used during CC, rather than initial respiratory support as in the SAIL trial. Nevertheless, our findings are still clinically relevant as the distribution of cardiac output in the fetus and post-transitional neonate during asphyxia episodes are qualitatively similar.

**Conclusion**

In asphyxiated term newborn piglets resuscitated by CC+SI, the use of different PIPs resulted in similar time to ROSC, but the use of a PIP 30 cmH₂O showed a larger V₇ delivery, lower exhaled CO₂ and increased brain inflammation compared to using of a PIP 10 or 20 cmH₂O. Future studies in animal models and/or during neonatal resuscitation are needed to examine the optimal SI delivery parameters during CC.
Author Contributions

Conceptualization: Po-Yin Cheung, Megan O’Reilly, Georg M. Schmölder.

Data curation: Gyu-Hong Shim, Seung Yeun Kim, Po-Yin Cheung, Tze-Fun Lee, Megan O’Reilly, Georg M. Schmölder.

Formal analysis: Tze-Fun Lee, Megan O’Reilly.

Funding acquisition: Georg M. Schmölder.

Investigation: Gyu-Hong Shim, Seung Yeun Kim, Po-Yin Cheung, Georg M. Schmölder.

Methodology: Gyu-Hong Shim, Seung Yeun Kim, Po-Yin Cheung, Tze-Fun Lee, Megan O’Reilly, Georg M. Schmölder.

Project administration: Tze-Fun Lee, Georg M. Schmölder.

Resources: Georg M. Schmölder.

Validation: Georg M. Schmölder.

Writing – original draft: Gyu-Hong Shim.

Writing – review & editing: Gyu-Hong Shim, Seung Yeun Kim, Po-Yin Cheung, Tze-Fun Lee, Megan O’Reilly, Georg M. Schmölder.

References

1. Aziz K, Chadwick M, Baker M, Andrews W. Ante- and intra-partum factors that predict increased need for neonatal resuscitation. Resuscitation. 2008; 79(3):444–52. https://doi.org/10.1016/j.resuscitation.2008.04.008 PMID: 18952348

2. Wyckoff MH, Perlman JM. Cardiopulmonary resuscitation in very low birth weight infants. Pediatrics. 2000; 106(3):616–20. https://doi.org/10.1542/peds.106.3.616 PMID: 11012336

3. Shah PS, Tai KFY. Chest compression and/or epinephrine at birth for preterm infants <32 weeks gestational age: matched cohort study of neonatal outcomes. J Perinatol. 2009; 29(10):693–7. https://doi.org/10.1038/jp.2009.70 PMID: 19554013

4. Shah PS. Extensive cardiopulmonary resuscitation for VLBW and ELBW infants: a systematic review and meta-analyses. J Perinatol. 2009; 29(10):655–61. https://doi.org/10.1038/jp.2009.71 PMID: 19554016

5. Wyckoff MH, Salhab WA, Heyne RJ, Kendrick DE, Stoll B, Lapook AR, et al. Outcome of extremely low birth weight infants who received delivery room cardiopulmonary resuscitation. J Pediatr. 2012; 160(2):239–44.e2. https://doi.org/10.1016/j.jpeds.2011.07.041 PMID: 21930284

6. Kapadia V, Wyckoff MH. Chest compressions for bradycardia or asystole in neonates. Clin Perinatol. 2012; 39(4):833–42. https://doi.org/10.1016/j.clp.2012.09.011 PMID: 23164181

7. Finer N, Tarin T, Vaucher YE, Barrington KJ, Bejar R. Intact survival in extremely low birth weight infants after delivery room resuscitation. Pediatrics. 1999; 104(4):e40. https://doi.org/10.1542/peds.104.4.e40 PMID: 10506265

8. DeMauro SB, Roberts RS, Davis PG, Alvaro R, Bairam A, Schmidt B, et al. Impact of delivery room resuscitation on outcomes up to 18 months in very low birth weight infants. J Pediatr. 2011; 159(4):546–50.e1. https://doi.org/10.1016/j.jpeds.2011.03.026 PMID: 21592510

9. Harrington DJ, Redman CW, Moulden M, Greenwood CE. The long-term outcome in surviving infants with Apgar zero at 10 minutes: a systematic review of the literature and hospital-based cohort. Am J Obstet Gynecol. 2007; 196(5):463.e1-e5. https://doi.org/10.1016/j.ajog.2006.10.077 PMID: 17466703

10. Solevåg AL, Cheung PY, O’Reilly M, Schmölder GM. A review of approaches to optimise chest compressions in the resuscitation of asphyxiated newborns. Arch Dis Child Fetal Neonatal Ed. 2016; 101(3):F272–6. https://doi.org/10.1136/archdischild-2015-309761 PMID: 26627554

11. Solevåg AL, Schmölder GM. Optimal Chest Compression Rate and Compression to Ventilation Ratio in Delivery Room Resuscitation: Evidence from Newborn Piglets and Neonatal Manikins. Front Pediatr. 2017; 5:3. https://doi.org/10.3389/fped.2017.00003 PMID: 28168185

12. Wyckoff MH, Aziz K, Escobedo MB, Kapadia VS, Kattwinkel J, Perlman JM, et al. Part 13: Neonatal Resuscitation: 2015 American Heart Association Guidelines Update for Cardiopulmonary Resuscitation
Sustained inflation pressure during neonatal cardiopulmonary resuscitation

13. Perlman JM, Wryllie J, Kattwinkel J, Wyckoff MH, Aziz K, Guinsburg R, et al. Part 7: Neonatal Resuscitation: 2015 International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science With Treatment Recommendations. Circulation. 2015; 132(16 Suppl 1):S204–41. https://doi.org/10.1161/CIR.0000000000000276 PMID: 26472855

14. Wryllie J, Perlman JM, Kattwinkel J, Wyckoff MH, Aziz K, Guinsburg R, et al. Part 7: Neonatal resuscitation: 2015 International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science with Treatment Recommendations. Resuscitation. 2015; 95:e169–201. https://doi.org/10.1016/j.resuscitation.2015.07.045 PMID: 26477424

15. Schmolzer GM, te Pas AB, Davis PG, Morley CJ. Reducing lung injury during neonatal resuscitation of preterm infants. Arch Dis Child Fetal Neonatal Ed. 2004; 89(1):F84–7. https://doi.org/10.1136/adc.89.1.f84 PMID: 14711865

16. Damann O, Allred EN, Kuban KC, van Marter LJ, Stewart JE, Pagano M, et al. Hypocarbia during the first 24 postnatal hours and white matter echolucencies in newborns or < or = 28 weeks gestation. Pediatr Res 2001; 49(3):388–93. https://doi.org/10.1203/00006450-200103000-00013 PMID: 11228265

17. Mian Q, Cheung PY, O’Reilly M, Barton SK, Polglase GR, Schmolzer GM. Volume targeted versus pressure-limited ventilation in neonates. Cochrane Database Syst Rev. 2017; 10:CD003666. https://doi.org/10.1002/14651858.CD003666.pub4 PMID: 29039883

18. Kilkenny C, Altman DG, Browne WJ, Cuthill IC, Emerson M. Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PLoS Biol 2010; 8(6):e1000412. https://doi.org/10.1371/journal.pbio.1000412 PMID: 20613859

19.樱桃树M, Gorens I, Cheung PY, Lee TF, Lu M, O’Reilly M, et al. Chest Compressions during Sustained Inflation Improve Recovery When Compared to a 3:1 Compression: Ventilation Ratio during Cardiopulmonary Resuscitation in a Neonatal Porcine Model of Asphyxia. Neonatology. 2017; 112(4):337–46. https://doi.org/10.1159/000477998 PMID: 28768280

20. Schmolzer GM, O’Reilly M, LaBossiere J, Lee TF, Cowan S, Qin S, et al. Cardiopulmonary resuscitation with chest compressions during sustained inflations: a new technique of neonatal resuscitation that improves recovery and survival in a neonatal porcine model. Circulation. 2013; 128(23):2495–503. https://doi.org/10.1161/CIRCULATIONAHA.113.002289 PMID: 24086527

21. Li ES, Gorens I, Cheung PY, Lee TF, Lu M, O’Reilly M, et al. Chest Compressions during Sustained Inflations Improve Recovery When Compared to a 3:1 Compression: Ventilation Ratio during Cardiopulmonary Resuscitation in a Neonatal Porcine Model of Asphyxia. Neonatology. 2017; 112(4):337–46. https://doi.org/10.1159/000477998 PMID: 28768280

22. Li ES, Cheung PY, Lee TF, Lu M, O’Reilly M, Schmolzer GM. Return of spontaneous Circulation Is Not Affected by Different Chest Compression Rates Superimposed with Sustained Inflations during Cardiopulmonary Resuscitation in Newborn Piglets. PLoS One. 2016; 11(6):e0157249–14. https://doi.org/10.1371/journal.pone.0157249 PMID: 27304210

23. Mustofa J, Cheung PY, Patel S, Lee TF, Lu M, Pasquin MP, et al. Effects of different durations of sustained inflation during cardiopulmonary resuscitation on return of spontaneous circulation and hemodynamic recovery in severely asphyxiated piglets. Resuscitation. 2018; 129:82–89. https://doi.org/10.1016/j.resuscitation.2018.06.013 PMID: 29928955

24. Lilly C, Altman DG, Browne WJ, Cuthill IC, Emerson M. Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PLoS Biol 2010; 8(6):e1000412. https://doi.org/10.1371/journal.pbio.1000412 PMID: 20613859

25. Schmolzer GM, O’Reilly M, LaBossiere J, Lee TF, Cowan S, Nicoll J, et al. 3:1 compression to ventilation ratio versus continuous chest compression with asynchrony ventilation in a porcine model of neonatal resuscitation. Resuscitation. 2014; 85(2):270–5. https://doi.org/10.1016/j.resuscitation.2013.10.011 PMID: 24161768

26. Cheung PY, Gill RS, Bigam DL. A swine model of neonatal asphyxia. J Vis Exp. 2011; 56:e3166. https://doi.org/10.3791/3166 PMID: 22006174

27. Schmolzer GM, Kamlin COF, Dawson JA, Pas te A, Morley CJ, Davis PG. Respiratory monitoring of neonatal resuscitation. Arch Dis Child Fetal Neonatal Ed. 2010; 95(4):F295–303. https://doi.org/10.1161/adc.2009.165878 PMID: 19776023

28. van Os S, Cheung PY, Pichler G, Aziz K, O’Reilly M, Schmolzer GM. Exhaled carbon dioxide can be used to guide respiratory support in the delivery room. Acta Paediatr. 2014; 103(8):796–806. https://doi.org/10.1111/apa.12650 PMID: 24698203

29. Pichler G, Binder-Heschl C, Avian A, Beckenbach E, Schmolzer GM. Reference ranges for regional cerebral tissue oxygen saturation and fractional oxygen extraction in neonates during immediate
30. Solevåg Lee TF, Lu M Schmölzer GM, Cheung PY. Tidal volume delivery during continuous chest compressions and sustained inflation. Arch Dis Child Fetal Neonatal. 2017; 102(1):F85–7. https://doi.org/10.1136/archdischild-2016-311043 PMID: 27566670

31. Tsui BCH, Horne S, Tsui J, Corry GN. Generation of tidal volume via gentle chest pressure in children over one year old. Resuscitation. 2015; 92:148–53. https://doi.org/10.1016/j.resuscitation.2015.02.021 PMID: 25749553

32. Schmölzer GM, O'Reilly M, Fray C, van Os S, Cheung PY. Chest compression during sustained inflation versus 3:1 chest compression:ventilation ratio during neonatal cardiopulmonary resuscitation: a randomised feasibility trial. Arch Dis Child Fetal Neonatal Ed. 2018; 103(5):F455–60. https://doi.org/10.1136/archdischild-2017-313037 PMID: 28988159

33. Schmölzer GM. Chest compressions during sustained inflation during cardiopulmonary resuscitation in newborn infants translating evidence from animal studies to the bedside. JACC Basic Transl Sci. 2019; 4(1):116–21. https://doi.org/10.1016/j.jacbts.2018.12.004 PMID: 30847426

34. Li ES, Cheung PY, O’Reilly M, Schmölzer GM. Change in tidal volume during cardiopulmonary resuscitation in newborn piglets. Arch Dis Child Fetal Neonatal Ed. 2015; 100(6):F530–3. https://doi.org/10.1136/archdischild-2015-308363 PMID: 26139543

35. Polglase GR, Miller SL, Barton SK, Baburamani AA, Wong FY, Aridas JD, et al. Initiation of resuscitation with high tidal volumes causes cerebral hemodynamic disturbance, brain inflammation and injury in preterm lambs. PLoS One. 2012; 7(6):e39535. https://doi.org/10.1371/journal.pone.0039535 PMID: 22761816

36. Polglase GR, Nitssos I, Baburamani AA, Crossley KJ, Slater MK, Gill AW, et al. Inflammation in utero exacerbates ventilation-induced brain injury in preterm lambs. J Appl Physiol. 2012; 112(3):481–9. https://doi.org/10.1152/japplphysiol.00995.2011 PMID: 22052871

37. Polglase GR, Miller SL, Barton SK, Kluckow M, Gill AW, Hooper SB, et al. Respiratory support for premature neonates in the delivery room: effects on cardiovascular function and the development of brain injury. Pediatr Res. 2014; 75(6):682–8. https://doi.org/10.1038/pr.2013.40 PMID: 24614803

38. Sobotka K, Hooper SB, Crossley KJ, Ong T, Schmölzer GM, Barton SK, et al. Single sustained inflation followed by ventilation leads to rapid cardiorespiratory recovery but causes cerebral vascular leakage in asphyxiated near-term lambs. PLoS One. 2016; 11(1):e0146574. https://doi.org/10.1371/journal.pone.0146574 PMID: 26765258

39. Barton SK, Tolcos M, Miller SL, Roehr CC, Schmölzer GM, Davis PG, et al. Unraveling the links between the initiation of ventilation and brain injury in preterm infants. Front Pediatr. 2015; 3:97. https://doi.org/10.3389/fped.2015.00397 PMID: 26618148

40. Anderson CC, pillow Jj, Gill AW, et al. The cerebral critical oxygen threshold of ventilated preterm lambs and the influence of antenatal inflammation. J Appl Physiol. 2011; 111(3):775–81. https://doi.org/10.1152/japplphysiol.00214.2011 PMID: 21719729

41. Solevåg A.; Schmölzer G.M.; O’Reilly M.; Lu M.; Lee T.-F.; Hornberger L.K.; et al. Myocardial perfusion and oxidative stress after 21% vs. 100% oxygen ventilation and uninterrupted chest compressions in severely asphyxiated piglets. Resuscitation. 2016; 106:7–13. https://doi.org/10.1016/j.resuscitation.2016.06.014 PMID: 27344929

42. Garcia-Hidalgo C, Cheung PY, Solevåg AL, Vento M, O’Reilly M, Saugstad O, et al. A review of oxygen use during chest compressions in newborns. Front Pediatr. 2018; 6:400. https://doi.org/10.3389/fped.2018.00400 PMID: 30619794

43. Chalak LF, Barber CA, Hynan L, Garcia D, Christie L, Wyckoff MH. End-tidal CO₂ detection of an audible heart rate during neonatal cardiopulmonary resuscitation using asystole in asphyxiated piglets. Pediatr Res. 2011; 69(3 Pt 1):401–5. https://doi.org/10.1203/PDR.0b013e31821257f1 PMID: 21283051

44. Pasquin MP, Cheung PY, Patel S, Lu M, Lee TF, Wagner M, et al. Comparison of Different Ventilation Ratios (2:1, 3:1, and 4:1) during Cardiopulmonary Resuscitation in a Porcine Model of Neonatal Asphyxia. Neonatology. 2018; 114(1):37–45. https://doi.org/10.1159/000487988 PMID: 29649792

45. Kirpalani H, Ratcliffe SJ, Keszer M, Davis PG, Foglia EE, Te Pas A, et al. Effect of sustained inflations vs intermittent positive pressure ventilation on bronchopulmonary dysplasia or death among extremely preterm infants: The SAIL randomized clinical trial. JAMA. 2019; 321(12):1165–75. https://doi.org/10.1001/jama.2019.1660 PMID: 30912836