Author's response to reviews

Title: Pressure- versus volume-limited sustained inflations at resuscitation of premature newborn lambs

Authors:

Graeme R Polglase (graeme.polglase@monash.edu)
David G Tingay (David.Tingay@rch.org.au)
Risha Bhatia (Risha.Bhatia@thewomens.org.au)
Clare A Berry (clare.berry@uwa.edu.au)
Robert J Kopotic (rkopotic@CASMED.com)
Clinton P Kopotic (clinton@kopotic.com)
Yong Song (yong.song@uwa.edu.au)
Edguardo Szyld (eszyl@gmail.com)
Alan H Jobe (alan.jobe@cchmc.org)
J. Jane Pillow (jane.pillow@uwa.edu.au)

Version: 2
Date: 15 December 2013

Author's response to reviews: see over
Dear Sir/Madam,

RE: Pressure- versus volume-limited sustained inflations: recruiting the preterm lung.
MS ID: 1805975255104490
Authors: Graeme R Polglase, David G Tingay, Risha Bhatia, Clare A Berry, Robert J Kopotic, Clinton P Kopotic, Yong Song, Edguardo Szyld, Alan H Jobe and J. Jane Pillow
Journal: BMC Pediatrics

Thank you for the opportunity to revise our manuscript. We have taken time to respond to the reviewers’ comments, which are included below. We trust that the changes made to the manuscript will be acceptable to the Editor and Reviewers.

With kind regards,

Prof. Jane Pillow
BMedSci, MBBS, FRACP, PhD

Copyediting:
After reading through your manuscript, we feel that the quality of written English needs to be improved before the manuscript can be considered further.

We advise you to seek the assistance of a fluent English speaking colleague, or to have a professional editing service correct your language. Please ensure that particular attention is paid to the abstract.

We have carefully edited the manuscript for English. Additionally, we note that the manuscript has passed grammar and spelling checks within MS Word.

2. Authors' information: Please place the Authors’ Contributions section after Competing interests. Please check the instructions for authors on the journal website for the correct format to use for Authors’ Contributions.

Authors contributions have been added.

3. Competing interests:
Manuscripts should include a ?Competing interests? section.

A competing interests section has been included.
4. Acknowledgement: By way of a section? Acknowledgements?

An acknowledgement section has been added

5. Conclusions: This should state clearly the main conclusions of the research and give a clear explanation of their importance and relevance. Summary illustrations may be included.

A conclusions section has been added to the end of the discussion.

Reviewer 1.

SPECIFIC COMMENTS

Methods:

At page 7: Sustained inflation and ventilation strategies (first paragraph) The Authors could explain why the lambs were maintained in prone position. The human setting is different (preterm infants are supine in the "infant warmer" in the delivery room) and we wish to have some information to share in clinical practice. Can the Authors confirm that the results of the experiment could be the same changing the position (e.g. supine) of the lambs during respiratory assistance?

In general, four legged animals ventilate better in the prone position, as this is their natural position. Therefore we chose the prone position for this study. Physiologically, in a prone position the dependent region of the lung will be smaller than if the lamb was placed supine because the mass of the ventral surface is smaller. Given that the dependent lung is poorly aerated (Hooper et al. The FASEB Journal, Vol 21, 2007), placing the lamb in the prone position maximises the area of the lung to be aerated. Therefore it is likely that poorer oxygenation would result if a lamb were placed supine. The effect of body position on the initial oxygenation of preterm human infants at birth is not known, and for that reason, a current clinical trial is investigating the initiation of resuscitation of an infant on its left side; - in this situation the left lung (dependent) is smaller than the right lung.

At page 7: Sustained inflation and ventilation strategies (second paragraph) we know from the literature that the effect of SI is “maximum” when is immediately followed by a PEEP. Were the animals managed with a PEEP after the SI (both volume-limited and pressure-limited) before to receive a volume targeted ventilation (vt at 7 ml/Kg, plus PEEP)? Did the Authors evaluate the EEL with or without PEEP, after SI?

As per page 7, all lambs received a PEEP of 7 cmH2O immediately after the SI was complete. We did not evaluate EELV without PEEP as this was not the scientific question being asked in this study. Most devices used in high-income countries are able to deliver PEEP. Given the current ILCOR recommendations are to use PEEP during resuscitation, that a study without PEEP was not warranted.

At page 9: Post-mortem analysis (third paragraph): can the Authors present
some pictures showing the lung tissue after the two different SI approach? Even if the lung inflammation response analyzed by BAL was similar, may be interesting to evaluate the lung architecture.

We did not collect lung for histology as we did not expect to find significant differences in lung architecture for this brief 15 min study in which the only difference in management was the manner of sustained inflation delivery immediately after birth. Thus we are unable to provide lung images. Given the similarity between the inflammatory markers and early injury markers, it would be unlikely that lung histology would be differentially altered by these protocols. We have included this information in the discussion.

Results
At page 10: Arterial blood-gas and ventilation variables (second paragraph) in the volume-limited SI group the Authors have registered significantly higher FiO2 level at 15 min. The Authors in the discussion have suggested that was due to rapid delivery of the targeted initial inflating volume. Which was the flow used to deliver SI? In case of absence of PEEP immediately after SI, can we hypothesize that this event has amplified the negative effect on oxygenation by volume-limited SI?
The constant bias flow for sustained inflation and ventilation in all animals was 8 L/min.
As noted above, PEEP was given immediately after the SI, thus it is not possible that the negative effect of volume-limited SI on oxygenation was amplified by withdrawal of PEEP.

In the method (at page 7) the Authors have reported that the SI “was maintained for a further 15 s”. Which was the exact duration of the maneuver? In fact all the results (arterial blood-gas values and ventilator variables) are reported at 20 seconds of SI.
All SIs are of 20 s duration – The first 5 s of the SI was used to reach the target volume or pressure and subsequently held at this volume or pressure for a further 15 s. This detailed has been clarified in the methods section.

At page 10, hemodynamic measurements (first paragraph): The Authors are requested to explain the sentence: in both groups, heart rate and carotid arterial pressure decreased with time (p<0.001 for both). We know that SI acts not only on lung function but also on circulation (e.g. Klingerberg C, Arch Dis Child Fetal Neon ed 2013 “.....SI after birth improved speed of circulatory recovery...”). Can we speculate that the results reported by the Authors are considered as the recovery phase after SI? Is a positive or negative effect? Or they have other suggestions?
We thank the author for picking up this error in the manuscript, and also for the suggestion to study the effect of the SIs on the transition. We have not included data on Heart rate and arterial pressure during and after the SIs – we have added statements in the text and a figure (Figure 3) showing the effect of both SIs on the circulatory recovery. It is important to note that this study is focusing on circulatory transition not recovery, as per the Klingenberg paper (Arch Dis Child Fetal Neon ed 2013).
At page 11, regional aeration, (second paragraph): Why global end-expiratory lung volume significantly decreased after the sustained inflation in the VolSI group but not in the PressSI group? Can we hypothesize a better lung recruitment? Moreover, tissue resistance and tissue elastance were lower in SI pressure group: why? Can we hypothesize a different effect of volume-limited SI on lung recruitment?

The decrease in end-expiratory lung volume after the SI in the VOLSI group was due to the reduction in Peak-inspiratory pressure (by ~10 mmHg) used during the vol-limited SI to the subsequent maintenance of 7 ml/kg during volume guarantee ventilation. Pressure was not changed in the press-limited group. There was no difference between the groups in volume recruited, hence we cannot conclude that recruitment was better in either strategy. The differences in tissue resistance and elastance were not different between groups, with only time effects evident within each group. A comment has been included in the discussion.

Discussion

The Authors could underline better that the data need to be verified in human setting, where the possibility to set the Vt in course of SI is till now not available in delivery room;

A comment has been made to the conclusion highlighting the need for human equivalent studies.

Moreover, the worst oxygenation in volume-limited SI registered during transition (15 minutes after birth), could suggest a possible scenario of high oxidative stress with this modality used to deliver SI.

We do not know whether oxidative stress would be increased, given the lack of difference in cerebral oxygenation between groups at any time point. It more likely reflects worsening respiratory outcome due to the technique used to rapidly recruit the volume, as discussed on page 15. A volume-limited SI with more gradual delivery of the sustained inflation volume could have a substantially different effect.

Moreover, the Authors reported: “The 131 d lamb is approximately similar to 34-36 w GA in the human infant – a group that is still prone to respiratory distress due to surfactant deficiency”. Can we hypothesize that the two modalities to deliver SI could give the same results if the Authors will study lambs with lower GA and with higher surfactant deficiency?

The optimal SI strategy will be strongly influenced by the structural (maturational) integrity of the lung tissue as well as the lung mechanics. It is beyond the scope of this manuscript to speculate on the outcome of these approaches at other gestations without relevant data to support or refute this conjecture.

Can a different ventilator strategy amplify or reduce the inflammatory response after volume-limited and pressure-limited SI?

We did not investigate different ventilation strategies after the delivered SI, so the interaction between ventilation and the SI is not known and this question is
beyond the scope of the study undertaken. We, and others have shown that different tidal volumes and PEEP can alter the inflammatory response of the lung during the initial ventilation strategy (Polglase et al. 2008, Hillman et al. 2010, Wallace M et al. 2009) but these have not been tested after a SI. However, an initial ventilation strategy which included prophylactic surfactant, a SI and 7ml/kg volume guarantee reduced pulmonary and cerebral inflammation (Polglase et al. PLoS ONE, 2012) compared to a high tidal volume strategy.

The reason for the short ventilation time-frame in this study was to try and separate the inflammation and injury from the initial SI without the confounding pro-inflammatory and pro-injury influence of prolonged ventilation.

Reviewer 2
We thank the reviewer for their supportive comments.

2. Major compulsory revisions:
2.1. It is not clearly stated in the manuscript, but it seems that inspiratory volume was measured during the S.I. (primary outcome) The technique of this measurement should be given.
Inspiratory volume delivered during the sustained inflation was obtained from the FlexiVent ventilator. The value delivered to the trachea was used: tracheal volume was computed after accounting for the gas compression and impedance of the ventilator circuit

2.2. The animals were intubated with cuffed tubes. Using an upper pressure limit of 50 cmH2O in the volume-controlled arm may have resulted in an endotracheal tube leak which may be compensated in the pressure-controlled arm completely, but in the volume-controlled arm (controlling for inspiratory volume) only partially. The authors should delineate how the can be sure that different levels of leak may have caused a smaller FRC by the end of the 15s pressure/volume hold. Did they assure the absence of leak?
We did not observe any leak around the cuffed endotracheal tube. The delivered pressure and volume waveforms did not show evidence of either significant or differential leak between the two SI strategies.

2.3. Data on lung volume is the primary outcome measure. The legend of figure 6 does not even give the information if this is the mean or median and does not show variability. Please give the information and add standard deviations or 25/75th percentiles.
The figure showed a representative animal for simplicity. It has been redrawn to show group average data, however this required additional panels to show the variability.

2.4. The abstract states that "...both achieved similar end-expiratory lung volumes..." Figure 1 and 6 suggest that it is the end-inspiratory volume (at the end of the SI) which was measured. Please clarify!
We have adjusted this to read “inspiratory”. We thank the reviewer for picking this up.
2. Minor Essential Revisions:
2.1. Did the authors measure expired volume when animals were switched from the end of S.I. to volume-controlled ventilation? If yes, were these volumes consistent with the inspired volumes?
We were unable to measure this.

2.2. As stated in the methods section the animals were suctioned from the trachea after intubation. This is not the usual approach in the delivery room where only the upper airways are cleared from overt secretions. Furthermore, different suction volumes may have caused different filling conditions with amniotic fluids. This issue should be considered as a limitation in the discussion.

The lambs were suctioned because these are “normal” fetuses delivered by caesarean section with lungs full of liquid. In humans, all preterm deliveries are abnormal, and most fetuses will have decreased fetal lung liquid volume. As stated in the methods, we suctioned each lamb to the same degree, meaning the same depth and same pressure used. The suction protocol was conducted to standardise the level of lung liquid remaining in the lung after birth between all lambs, as this is known to alter the responsiveness to the SI. The suction protocol is also mentioned in the discussion (page 14).

There was no amniotic fluid remaining at delivery as the lambs were delivered (ie ex utero on the maternal abdomen at the time of suctioning. Does the reviewer mean lung liquid (not normally amniotic fluid in the lung). We do agree that the influence of SIs on different levels of lung liquid is not known, and will likely influence physiology and perhaps injury indices. We have referred to the unknown issue of lung liquid in the discussion within the Limitations of the study section.

2.3. Data on NIRS and on hemodynamics should be presented (i.e. in a table)
We have included these data based on recommendations from Reviewer 1 and 2 (see above comments).

2.4. Was the tube clamped to prevent spontaneous inspiration during instrumentation and delivery?
The reviewer is correct. The tube was clamped between intubation and commencement of the sustained inflation to prevent spontaneous inspiration during transfer to the bedside and initiation of sustained inflation. This detail has been added to the methods section.

3. Discretionary Revisions:
3.1. I am not sure if the US guidelines on newborn resuscitation advocate the use of S.I. (page 5, third paragraph). Please check and eventually revise.
The sentence is not discussing sustained inflations but the initial pressures that are used during positive pressure ventilation. The AHA do not advocate the use of the SI. We have clarified this sentence.
3.2. A more rapid filling with air in the presence of a fluid FRC may cause different distribution of fluid/air as compared to a slower filling procedure. It might be worthwhile to discuss this issue.

We totally agree with the reviewer, and we are currently submitting a paper that is looking precisely at this relationship in a different set of experimental animals. We feel it is outside the scope of this paper to discuss this in detail but have referred to the need to investigate this issue.

Reviewer 3

Minor Revisions –
Introduction –
Para 2. Line 1: A sustained inflation is used by some neonatologists ...... (5,6).
Wording change – Sustained lung inflation at birth is practiced at some centers for early establishment of infant's FRC at birth ().
The suggested change has been made.

Methods – Sustained inflation and Ventilation Strategies –
What was the FiO2 0.3 at birth in both the groups? Similarly what was the FiO2 at 20 secs at the end of sustained inflation? (again was it 0.3) – needs clarification.
FiO2 was 0.3 for the SI and for the initial ventilation. This has been clarified in the methods.

Results –
It would have been interesting to see the blood gases at the end of 20 secs in the two groups. Do we have a gas at the end of intervention?
We did not take a blood gas at the end of the 20 s SI – we agree this would have been an interesting observation. However, the practicality of achieving such is difficult with intermittent rather than continuous blood gas measurements, in the setting of an acute resuscitation.

PVR changes with FRC and pH. The pH was lower in the Pressure SI group before birth. However PVR is lowest at FRC. Both of them could influence FiO2 requirement depending on pulmonary hemodynamics.
We didn’t measure pulmonary hemodynamics in this study so are unable to comment on how PVR influenced our results.

Figure 5 can be deleted as it has not been discussed much in the discussion section.
We have deleted figure 5.

Discussion –
Para 3 – Physiologic changes seen in pressure / volume curves are not reflected in lung injury markers. The authors cannot make a conclusion of “slow rather than rapid delivery of the targeted initial inflating volume may identify a strategy that affords protection from both barotrauma and volutrauma”. The authors have to be careful about this.
We have tempered this comment and agree that more studies are required to investigate this relationship.

Page 15. Line 21/22 – Our failure to observe differences........similarly injurious. The reviewer feels that the authors have to say it straight that the initial sustained inflation with either pressure ventilation or volume ventilation does not make a difference as we did not find any differences at 15 minutes. We have added this comment to the discussion.

Limitations –
The reviewer is curious to know why the pressure was not adjusted based on end tidal volume to match that of volume SI. This would have enabled both groups to be somewhat similar.
The goal of the sustained inflation is to establish a functional residual capacity through slow delivery of a volume to the lung. Pressure-limited SI is what is normally practiced clinically and hence we opted to take the opposite approach to that suggested by Reviewer 3 – i.e. by matching as closely as possible the averaged sustained inflation volume delivered during pressure-limited SI.

Had we taken the approach suggested by Reviewer 3, matching the pressure used for pressure-limited SI to the average SI pressure used during the volume-limited SI, the average delivered SI volumes would have differed markedly between the two groups. We would have expected to see the same level of variability in the SI volume for different animals in the pressure-limited group. Although this question has some academic value, we considered it more important to try to match average delivered volume than average delivered pressure.

Abstract –
Methods - Delete “significance was determined using......ANOVA” (details of statistics in methods; also 1-way not mentioned in methods)
This has been removed from the abstract.

Major Revisions – Introduction –
P2, L2: recommended for the initial......in the recent European resuscitation guidelines (7). The reviewer thinks that the authors have to be careful with this statement. I do not think, the European RC guidelines do not make any recommendations regarding sustained inflation due to paucity of data on inflation time (may be wrong – please check this). If there are no concrete recommendations, then say it as it is and compare them to AAP resuscitation guidelines (2010). This will make the study more interesting.
The use of an initial sustained inflation, or a series of 5, 3 second inflations is indeed in the European resuscitation guidelines. The best way to deliver this SI is not known. We have made reference to this European RC statement in the manuscript.

Para 3, Line 1: Needs reworking – AAP does not recommend sustained inflation. We have reworded this sentence.
Also you have to discuss the three clinical studies somewhere (either in the introduction or in the discussion - Vyas et al 1981; Linder et al 2005; Harling et al 2005) and then go on to tell why sustained inflation delivered by pressure method is not favorable. The later part of the Para 3 in good.

We have included discussion of the trials in the first paragraph of the discussion.

Methods –
Postmortem analyses –
Why was the lungs ventilated with 100% oxygen for 2 minutes? 100% oxygen is toxic / inflammatory. This could have altered the results of gene expression analysis. Lambs were exposed to 2+3 = 5 minutes of 100% oxygen in an experimental duration of 20 minutes (15 + 5) = 25% of the time; for a sustained inflation of 20 secs (< 1% of the experiment). Why was oxygen reabsorption done? Lungs can be flash frozen without causing additional injury. Just curious? You just add additional variables that could influence the results. I am wondering whether you masked important results.

Was oxygen absorption done for Pressure volume studies?
Oxygen reabsorption is standard within our laboratory, and is conducted to make the lungs atelectatic as possible, in order to construct a pressure-volume curve without confounders of gas trapping. The lambs are heavily anaesthetized prior to oxygen reabsorption taking place and tend to become bradycardic immediately following clamping of the tracheal tube. We have published using this technique on >50 occasions. We acknowledge that 100% oxygen is toxic and pro-inflammatory however note that the exposure was at the end of the study with minimal time available to increase pro-inflammatory cytokines as a consequence of the brief 2 min oxygen exposure. Lambs became both bradycardic and hypoxic very rapidly during the 3 min oxygen resorption and hence it is unlikely that this 3 min period represented substantial further toxic oxygen exposure. A reference has been added to this technique and we have improved the description to include this information.

Discussion –
Para 1 – The authors have to simplify the wordings a little bit. They use FRC in one sentence (Line 5) and end expiratory volume in their data (Line 7). Do they mean they are the same? If they are the same then volume inflation was no more beneficial than pressure inflation to establish FRC. This has to be made clear. We don’t actually measure this do we, or does EELV give an indication of this?

The baseline pH were not similar between the groups and this has to be mentioned in the discussion.

While the fetal pH levels were different, there was no difference after delivery and ventilation. We have added comments in the discussion.

Page 14; Para 1 – Even though there was variability, it was not big enough for it to be statistically significant. Moreover, this was not accompanied by any differences in lung injury markers in their study. Even though the authors hypothesized that tidal volume inflation is better, their data does not suggest it. This has to be brought out and the factors discussed. We have clarified this result in our overall conclusion.
Thus the critical question.........................The authors have not answered this critical question as all the injury markers were similar in both the groups. So they have to modify this statement that in our experiments we did not find either pressure or volume ventilation sustained inflation make any difference in terms of lung injury at 15 minutes.......something like that. We had a statement in our discussion that the SI strategies were similarly injurious, but we have added text to this section to further clarify this position.

The authors need to explain why FiO2 went up in the volume ventilation group in the absence of inflammation? This happened despite the volumes being similar at 15 minutes (figure 6) in both the groups As discussed on page 16, the worse oxygenation may be due to barotrauma. We have expanded this section in the discussion. We do not stipulate that there was no inflammation, rather that the levels of inflammation were not different between the groups. Perhaps a longer duration of the study would have seen greater differences between the groups, and this is discussed later in the discussion.

Summary –
First sentence – In conclusion..........the efficacy of volume-limited sustained inflation for lung recruitment.......... The reviewer finds this statement surprising despite the volume group having high FiO2 requirement and no other differences between the groups. The authors have to rewrite that one is not better than the other based on this study. We have clarified the discussion to reinforce the lack of difference between groups.

Title –
It should reflect the results of the study. Also it does not tell whether this is in lambs or in infants. The title feels that it is a review article. Something like – Pressure versus Volume limited sustained inflation at Resuscitation in Premature newborn Lambs We thank the reviewer for the suggestion.

Abstract –
Conclusions – Also a sentence such as – “Pressure or volume ventilation sustained inflation at resuscitation did not make a significant difference in terms of physiologic variables or early markers of lung inflammation measured in preterm newborn lambs in our study. We have adjusted the conclusion within the abstract to clarify this.