Author's response to reviews

Title: Maximization of oscillatory frequencies during arteriovenous extracorporeal lung assist: a large-animal model of respiratory distress

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Author's response to reviews: see over
Dear Professor Norton,

I enclose the revised manuscript with the reviewers commentaries addressed. My co-authors and I thank the reviewers for their work. We hope that we have been able to address all concerns and implemented the valuable commentaries adequately into the manuscript.

Yours sincerely,

R.M. Muellenbach
We very much appreciated the reviewers’ comments. We hope that we can address the expressed concerns appropriately in order to improve the quality of the paper.

Reviewer: Klaus Markstaller

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

None.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

General:

“ARDS” by “baseline II” as it is confusing in the text to have one measurement named to a condition of the animals, which is present for all Measurements are named “baseline”, “ARDS”, PRM” etc. I would suggest to replace following measurements.

In order to be less confusing, we re-named the above mentioned measurement time points as “T_{Baseline}”, “T_{ARDS}” and “T_{RM}” throughout the manuscript.

Background:

The hypothesis of this study is clearly expressed. However, please point out more clearly in background and discussion, that this study won’t give any evidence that higher oscillatory frequencies are more protective to the lung (as you have correctly mentioned in the conclusion section).

The appropriate changes in the background and discussion have been made.

Original:

“Background”:

With these flow rates near total extracorporeal CO₂-removal could be achieved in animal models of respiratory failure, thereby allowing significant reduction of the respiratory minute volume and the peak inspiratory pressure (PIP) during CMV [16,17]. The current study was performed to evaluate the effects of different oscillatory frequencies on CO₂-elimination with and without av-ECLA in a large-animal model of ARDS. We hypothesized, that the application of high oscillatory frequencies, and thereby minimization of VT and alveolar stretch, requires the combination of HFOV with av-ECLA in order to maintain or reestablish normocapnia.

“Discussion”:
On the other hand, with near total CO$_2$-removal due to extracorporeal lung assist, the applied pressures and accordingly the applied VT could have been further reduced, thereby minimizing VT and alveolar shear stress. Finally, av-ECLA allows for decoupling of ventilation and oxygenation. Therefore, the application of more lung-protective ventilator settings is not only possible, but should also be examined.

**Revision:**

**“Background”:**

With these flow rates near total extracorporeal CO$_2$-removal could be achieved in animal models of respiratory failure, thereby allowing a more lung protective conventional ventilation strategy [17,18].

While significant reductions in minute ventilation and VTs can be obtained during the combination of av-ECLA and conventional mechanical ventilation, optimal oscillatory frequencies remain to be determined. The current study was performed to evaluate the effects of different oscillatory frequencies on CO$_2$-elimination with and without av-ECLA in a large-animal model of ARDS. We hypothesized, that the application of high oscillatory frequencies, and thereby minimization of VT and alveolar stretch, requires the combination of HFOV with av-ECLA in order to maintain or reestablish normocapnia.

**“Discussion”:**

On the other hand, with near total CO$_2$-removal due to extracorporeal lung assist, the applied pressures and accordingly the applied VT could have been further reduced, thereby minimizing VT and alveolar shear stress. Finally, av-ECLA allows for decoupling of ventilation and oxygenation. Therefore, the application of more lung-protective ventilator settings is not only possible, but should also be examined. However, it was not the purpose of this study to examine the effect of higher frequency HFOV on lung damage.

**Methods:**

Animal preparation, line 15: Please add to the sentence “The inspiratory oxygen fraction …” “… throughout the entire experiment”.

We changed the following sentence:

**Original:**

“Animal preparation”:
The inspiratory oxygen fraction (FiO$_2$) remained 1.0.

**Revision:**

“Animal preparation”:
The inspiratory oxygen fraction (FiO$_2$) remained 1.0 throughout the entire experiment.

**Statistical analysis:**

I do not see the rational why the frequencies have been modified in an incremental / decremental slope instead of a random order – please comment. Was the av-ECLA circuit opened and closed in random order at each oscillatory frequency? Please describe this part more in detail in the manuscript.
In our model we did randomize for the oscillatory frequencies (incremental (3 Hz) or decremental (15 Hz)) used at the beginning of the study. The rationale to modify the frequencies in an incremental/decremental fashion was to rule out a time effect in gas exchange and hemodynamic measurements due to recovery of the injured lung. We totally agree with the reviewers comment, that otherwise we could have modified the oscillatory frequencies in a random order. However, due to the little falling number and the numerous time points we did alter the frequencies in an incremental/decremental fashion.

In addition, we did open and close the av-ECLA in random order at each change of the oscillatory frequency.

The statistical analysis section has been revisited in order to be more precise:

Original:
“Statistical analysis”:

The data of the incremental and decremental oscillatory trial were pooled and analyzed using SigmaStat 2.03 (Systat Software Inc., Point Richmond, USA). The data were tested for normal distribution using the Kolmogorov-Smirnov test. Values are reported as mean ± SD. One and two-way analysis of variance (ANOVA) for repeated measurements were used for data analysis. Student-Newman-Keuls post hoc test was used for comparison of significant ANOVA results. Adjustment for multiple testing was performed. P values < 0.05 were considered significant.

Revision:
“Statistical analysis”:

In each animal both, the starting oscillatory frequency (3 Hz or 15 Hz in half of the animals each; variation of frequency in steps of 3 Hz every 30 min) and the order of opening and closing the av-ECLA at each frequency were randomized. The data of the incremental and decremental oscillatory trial were pooled and analyzed using SigmaStat 2.03 (Systat Software Inc., Point Richmond, USA). The data were tested for normal distribution using the Kolmogorov-Smirnov test. Values are reported as mean ± SD. One and two-way analysis of variance (ANOVA) for repeated measurements were used for data analysis. Student-Newman-Keuls post hoc test was used for comparison of significant ANOVA results. Adjustment for multiple testing was performed. P values < 0.05 were considered significant.

Results:
Please reduce the text to relevant results and present the data in a puristic and descriptive manner. E.g.: “Pulmonary shunt fraction was significantly ameliorated between 6 Hz and 12 Hz during HFOV compared with HFOV / av-ECLA …” – In this sentence “ameliorated” should be replaced by “higher”, as a valuation of this effect does not make any sense – this is part of the concept of the av – ECLA.

The results section has been revisited regarding the style, the length and the relevant findings.

Discussion:
Very well written. See comment above (background section).
We very much appreciated the reviewers’ comments. We hope that we can address the expressed concerns appropriately in order to improve the quality of the paper.

Reviewer: Gil Allen

The authors demonstrate that during HFOV in a porcine saline-lavage model of lung injury, the employment of av-ECLA is required to supplement CO2 removal during higher frequencies of ventilation in order to keep CO2 closer to normal physiologic range, particularly at frequencies of 9Hz and above. The authors conclude that because higher frequencies facilitate lower tidal volumes during HFOV, and hence limit the risk of overdistention injury, the concurrent use of av-ECLA with HFOV may help promote the true potential of HFOV in preventing. The authors appropriately point out that such markedly lower tidal volumes may be of less benefit when delivered with more conventional means of ventilation due to the potential for progressive derecruitment of lung, but they did not compare HFOV and CMV, nor did they directly measure the effects of frequency on tidal volume in their model. The authors also appropriately concede that the clinical relevance of this is called into question when one considers the potential therapeutic implications of permissive hypercapnea during HFOV without av-ECLA, but then also point out that clinical conditions still arise in which hypercapnea is contraindicated.

Overall, the question could be better defined in the introduction. The methods are sound and well described, as are the data. The discussion is well balanced but a bit brief at times, with limited references. The authors do clearly acknowledge previous work in the field. The title and abstract do not really convey the key points made by the paper. I suggest modifying both. The writing is acceptable, but punctuation is shaky, and sentence structure is weak at times. Suggest another proofread before resubmission.

The authors thank the reviewer for the recommendations. The title and the abstract have been modified. Another proofread has been made.

Major Points
1.) Although the hypothesis is clearly stated in the last sentence of the introduction, I had difficulty quickly discerning the purpose of the study until late in the discussion. The point that higher frequency HFOV facilitates lower tidal volumes at the expense of ventilation, and that av-ECLA facilitate higher frequency HFOV by “supplementing” CO2 removal needs to be more clearly stated in the introduction and reintroduced early in the discussion.

The appropriate changes have been made in the “Background” and “Discussion” section.

Original:
“Background”:

Line 10-14:
During HFOV, VT and thus carbon dioxide (CO\textsubscript{2})-elimination are directly related to the applied pressure amplitude (\(\Delta P\)) and the inspiratory/expiratory ratio, and are inversely related to the oscillatory frequency. In adult HFOV trials, the applied frequencies varied from 3-6 Hz and, in an attempt to maximize CO\textsubscript{2}-elimination, a \(\Delta P\) from 60-90 cmH\textsubscript{2}O was used [9,10].

Line 25-29:
With these flow rates near total extracorporeal CO\textsubscript{2}-removal could be achieved in animal models of respiratory failure, thereby allowing significant reduction of the respiratory minute volume and the peak inspiratory pressure (PIP) during CMV [16,17]. The current study was performed to evaluate the effects of different oscillatory frequencies on CO\textsubscript{2}-elimination with and without av-ECLA in a large-animal model of ARDS.

Original:
“Discussion”:

Line 14-21:
In most clinical studies using HFOV in adults, oscillatory frequencies between 3-6 Hz and pressure amplitudes between 60-90 cmH\textsubscript{2}O have been used [9,10]. If hypercapnia was evident during HFOV, oscillatory frequencies were decreased to 3 Hz and amplitudes were increased up to 90-100 cmH\textsubscript{2}O. This concept generates VTs resembling those during conventional lung-protective ventilation, thereby antagonizing the potential advantages of HFOV [11].

The goal of this large-animal study of ARDS was to evaluate the effects of HFOV during different oscillatory frequencies on CO\textsubscript{2}-elimination with and without the application of av-ECLA.

Revision:
“Background”:

Line 10-15:
During HFOV, VT and thus carbon dioxide (CO\textsubscript{2})-elimination are directly related to the applied pressure amplitude (\(\Delta P\)) and the inspiratory/expiratory ratio, and are inversely related to the oscillatory frequency – in other words: The lower the oscillatory frequency, the higher the resulting VT. In adult HFOV trials, the applied frequencies varied from 3-6 Hz and, in an attempt to maximize CO\textsubscript{2}-elimination, a \(\Delta P\) from 60-90 cmH\textsubscript{2}O was used [9,10].

2.) The abstract is obtuse, particularly in the results and conclusions. I would rephrase last sentence of the results to “Normocapnea during HFOV was only maintained with the addition of av-ECLA during frequencies of 9Hz and above.” Conclusions should really focus on what can be directly concluded from the study and if the authors wish to speculate on minimization of tidal volume, this should be clearly identified as speculation.

Line 26-33:
With these flow rates near total extracorporeal CO\textsubscript{2}-removal could be achieved in animal models of respiratory failure, thereby allowing a more lung protective conventional ventilation strategy [17,18].

While significant reductions in minute ventilation and VTs can be obtained during the combination of av-ECLA and conventional mechanical ventilation, optimal oscillatory frequencies remain to be determined. The current study was performed to evaluate the effects
of different oscillatory frequencies on CO₂-elimination with and without av-ECLA in a large-animal model of ARDS.

Revision:
“Discussion”:

Line 14-39:
In most clinical studies using HFOV in adults, oscillatory frequencies between 3-6 Hz and pressure amplitudes between 60-90 cmH₂O have been used [9,10]. If hypercapnia was evident during HFOV, oscillatory frequencies were decreased to 3 Hz and amplitudes were increased up to 90-100 cmH₂O. However, oscillatory frequencies below 4 Hz combined with maximum pressure amplitudes result in tidal volumes comparable to conventional lung-protective ventilation, thereby antagonizing the potential advantages of HFOV [12]. The idea of decoupling ventilation and oxygenation was introduced by Gattinoni et al [22-24]: Extracorporeal CO₂-elimination was provided by a veno-venous perfusion route with 20-30 % of the cardiac output, whereas oxygenation was maintained by mechanical ventilation. Several animal and human trials showed the feasibility of reducing ventilator requirements during pump-driven extracorporeal CO₂-removal [25-28]. However, despite technical advances of these systems, several side-effects and complications impede its benefits, e.g., pump-induced traumatisation of blood cells, plasma leakage of oxygenator membranes and activation of the coagulation and inflammatory system.

Pumpless extracorporeal lung assist became feasible with the introduction of low-resistance membrane lungs that could be interposed into a simple arteriovenous shunt between the femoral artery and vein [29]. With the patient’s heart as driving force, the transmembraneous blood flow is up to 25-30 % of the CO, thereby allowing comparable CO₂-elimination and reductions in ventilatory support achieved during pump-driven extracorporeal CO₂-removal [18]. In an adult sheep model of respiratory failure, av-ECLA allowed significant reductions in VTs (from 15 ± 1.6 to 3 ± 1.5 ml/kg), peak inspiratory pressures (40 ± 2.1 to 20 ± 7.5 cmH₂O) and minute ventilation (10 ± 1.4 to 0.5 ± 0 L/min) [18]. The goal of this large-animal study of ARDS was to evaluate the effects of HFOV during different oscillatory frequencies on CO₂-elimination with and without the application of av-ECLA.

3.) Since the main purpose of the study was to demonstrate that av-ECLA facilitates higher frequency HFOV and hence lower tidal volumes, I do not understand why tidal volumes weren’t measured. Surely a high sampling rate pneumotach could have been used to acquire what seems a critical measurement to the objective of the study. Without this, some initial measurement of impedance (post-RM) could have at least helped estimate the delivered tidal volumes from the oscillatory pressure amplitudes and inverse frequency. Why was this not done?

The authors very much agree with the reviewers that VTs measurements would have yielded very interesting additional information for this study. However, since two very high ranked publications (one experimental and one clinical each) did measure VTs during higher frequency HFOV (with almost the same frequencies and pressure amplitudes used in our study), the authors disclaimed to do so. But therefore the authors added this issue in the “Limitations” section of the discussion.

Original:
“Discussion”:
“Limitations”:
The study design implies the following limitations: First, we used an ARDS model based on surfactant-depletion. In adults, not surfactant deficiency, but alveolar flooding is the predominant mechanism in ARDS development with surfactant-depleted lungs responding better to lung recruitment. Second, the study protocol did not allow alterations of the pressure amplitude and I:E-ratio in order to improve CO$_2$-elimination. Both parameter settings refer to previous experimental large animal studies of HFOV and clinical practice [10,20,27].

Revision:
“Discussion”:
“Limitations”:

The study design implies the following limitations: First, we used an ARDS model based on surfactant-depletion. In adults, not surfactant deficiency, but alveolar flooding is the predominant mechanism in ARDS development with surfactant-depleted lungs responding better to lung recruitment [37]. Second, the Sensormedics 3100 B oscillator is a pressure cycling machine that can neither control nor directly measure VTs. Hence, in this study no direct statement regarding the applied VTs can be made. However, in a recent observational study VT was measured directly during HFOV in ARDS patients: It was shown, that the tidal volumes during HFOV with frequencies between 5 and 12 Hz are between 0.8 and 3.3 ml/kg predicted body weight [12].

4.) When comparing measurements before and after recruitment, the authors should refer to them as pre-RM and post-RM, as opposed to ARDS and post-RM. I understand that the reason for doing this is probably because of the differences in P:F ratios, but the RM does not reverse the lung injury. This makes the first sentence in 3rd para of results page (under heading “Hemodynamics and Oxygen Delivery”) and Table 1 confusing and difficult to follow.

In order to be less confusing, we re-named the above mentioned measurement time points as “$T_{Baseline}$”, “$T_{ARDS}$” and “$T_{RM}$” throughout the manuscript.

5.) On last page of discussion before conclusions, 1st through 3rd line, authors need to provide reference for statement, “In adults, not surfactant deficiency, but rather alveolar flooding……lungs responding better to lung recruitment.”

We added the following reference:

6.) Table 1 is cramped and difficult to follow. I recommend moving columns to the left to permit widening of columns for PRM and 3, 9, and 12 Hz so that standard errors don’t have to wrap under the mean values.

The appropriate changes have been made.

7.) The authors describe many findings in their results section and even outline them in figures, but then never really elaborate within the discussion. In fact, I believe the 3rd sentence of the last page of results is erroneous when I read the table. For instance, “CO
was significantly decreased and SVR and DO2 increased during HFOV/av-ECLA compared with HFOV...” Since PaO2 was relatively unchanged, the relative decrease in CO with HFOV/av-ECLA seems discordant with a relative increase in DO2. Could the authors please explain? Also, SvO2% is higher in the av-ECLA group at all frequencies. Was this predominantly due to the lower hemoglobin displacement imposed by the lower CO2 in the av-ECLA group? If anything, one would expect a lower CO to correspond with a lower SvO2%.

The authors have to apologize because the results as described in the 3rd sentence of the last page are vice versa. Since the av-ECLA is low-resistance membrane lung, that is interposed into a simple arteriovenous shunt between the femoral artery and vein, SVR is decreased and thus CO elevated. With the patient’s heart as driving force, the transmembranous blood flow is up to 25-30% of the CO. The av-ECLA increases mixed venous O2 content, since 25% of the CO is fully saturated with oxygen and drained back to the venous system.

The appropriate changes have been made in the results section.

Original:
“Results”:
“Hemodynamics and oxygen delivery”:

CO was significantly decreased and SVR and DO2 were significantly increased during HFOV/av-ECLA compared with HFOV from 3 Hz to 15 Hz (Table 1 and 2, p < 0.05).

Revision:
“Results”:
“Hemodynamics and oxygen delivery”:

SVR was significantly decreased and CO and DO2 were significantly increased during HFOV/av-ECLA compared with HFOV from 3 Hz to 15 Hz (Table 1 and 2, p < 0.05).

Minor Points
1.) Second page of discussion, line 9 of 2nd para: I believe the authors should rephrase as, “Nevertheless, mere VT-reduction during conventional ventilation leads to low peak pressure ventilation favoring further lung derecruitment.” I would also elaborate this in the following sentence describing the study by Dembinski and colleagues.

The appropriate changes have been made.

2.) Would rephrase sentence in lines 7-8 of last page of discussion (before conclusions), “Therefore, one might argue that without (eliminate “need for”) avECLA,

The appropriate changes have been made.

3.) Last line of first concluding paragraph, “Thereby” is misspelled, and next sentence ends with two periods.

The appropriate changes have been made.

4.) Need to update reference # 26.
Reference # 26 was updated.