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Title: The effect of high frequency oscillatory ventilation combined with tracheal gas insufflation on extravascular lung water in patients with acute respiratory distress syndrome: a randomized, crossover, physiological study.

Article Type: Research Paper

Keywords: Acute Respiratory Distress Syndrome; Extravascular Lung Water; High Frequency Ventilation

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Abstract: Purpose: High frequency oscillation combined with tracheal gas insufflation (HFO-TGI) improves oxygenation in patients with Acute Respiratory Distress Syndrome (ARDS). There is limited physiologic data regarding the effects of HFO-TGI on hemodynamics and pulmonary edema during ARDS. The aim of this study was to investigate the effect of HFO-TGI on extravascular lung water (EVLW).
Materials and Methods: We conducted a prospective, randomized, crossover study. Consecutive eligible patients with ARDS received sessions of conventional mechanical ventilation (CMV) with recruitment maneuvers (RMs), followed by HFO-TGI with RMs, or vice versa. Each ventilatory technique was administered for 8 hours. The order of administration was randomly assigned. Arterial/central venous blood gas analysis and measurement of hemodynamic parameters and EVLW were performed at baseline and after each 8-hour period using the single-indicator thermodilution technique.
Results: Twelve patients received 32 sessions. PaO2/FiO2 and respiratory system compliance were higher ($p<0.001$ for both), while EVLW indexed to predicted body weight (EVLWI) and oxygenation index were lower ($p=0.021$ and 0.029, respectively) in HFO-TGI compared with CMV. There was a significant correlation between PaO2/FiO2 improvement and EVLWI drop during HFO-TGI ($Rs=-0.452$, $p= 0.009$).
Conclusions: HFO-TGI improves gas exchange and lung mechanics in ARDS, and potentially attenuates EVLW accumulation.

Response to Reviewers: Dear Professor Lumb,

Thank you for considering our manuscript for publication in the Journal of Critical Care. We would also like to thank the reviewers for carefully reading the manuscript and providing insightful comments.

Please, find attached the revised version of the manuscript and the Electronic Supplementary Material. Please, also find a separate manuscript file (mmarkedchanges.doc) where changes are highlighted in red.

We had to add 5 items to the reference list in order to address the reviewers' comments appropriately. The word count is now 3217.

Our detailed responses to the reviewer's comments follow below.

Abbreviations: ESM=electronic supplementary material.; HFO= high-frequency oscillation; TGI=tracheal gas insufflation; CMV=conventional mechanical ventilation. Revision-related text changes are highlighted in red script.

Reviewers' comments:

Reviewer 1:
Specific comments
#1. Throughout the manuscript : the proper timing of the measurements should be better defined: "following" is used at many places, but the methods section refers to the end of the treatment period. The figures seem to indicate they were performed shortly (?) after the end of the treatment period.
Response: Besides baseline, there were two time points of physiologic measurements in each ventilation session (Figure E1). One after 8 hours of CMV ventilation and one after 8 hours of HFO-TGI ventilation. These measurements were taken while the patient was still on CMV or HFO-TGI ventilation respectively, and lasted 10-15 minutes. Since we could not assess respiratory mechanics on HFO-TGI ventilation, this assessment was performed within 5 minutes after return to CMV. The timing of each measurement is now better defined as suggested by the reviewer:
- The last sentence of the Materials and Methods paragraph in the Abstract now reads: "Arterial/central venous blood gas analysis and measurement of hemodynamic parameters and EVLW were performed at baseline and after each 8-hour period using the single-indicator thermodilution technique."
- In the main manuscript, the first sentence of the Measurements paragraph in the Patients and Methods section now reads:"Patients underwent three assessments in every session: at baseline, after 8 hours of CMV and after 8 hours of HFO-TGI (Fig. 1a, Fig. E1, ESM). Each assessment lasted 10 to 15 minutes, while the patient was still on CMV or HFO-TGI ventilation respectively"
-The phrasing in the results section has also been changed to "after using HFO-TGI" and "after 8 hours of HFO-TGI" instead of "following HFO-TGI".
-The legend to Figure 2b now reads: "Individual differences in EVLWI between the two ventilation strategies. Each symbol corresponds to one session and represents the difference in EVLWI after 8 hours of CMV minus EVLWI after 8 hours of HFO-TGI…." and in figure 3 "Scatter plot for values of the change in extravascular lung water index (ΔEVLWI) and in PaO2/FiO2 (ΔPaO2/FiO2) over the 8-hour HFO-TGI period…."
-In the ESM the duration of each assessment is now denoted with symbols:
Physiological measurements were performed after the end of the 8-hour treatment period and required 10-15 minutes during which the patient was still on CMV ventilation.
¶ Physiological measurements were performed after the end of the 8-hour treatment period and required 10-15 minutes during which the patient was still on HFO-TGI ventilation.
§ Respiratory mechanics were assessed 1-5 minutes after return to CMV.

#2. The summary should indicate the method of measuring EVLW (PiCCO).
Response: The requested information has been added to the last sentence of the Materials and Methods paragraph of the Abstract which now reads: "Arterial/central venous blood gas analysis and

measurement of hemodynamic parameters and EVLW were performed at baseline and after each 8-hour period using the single-indicator thermodilution technique".

#3. Patients and methods : ejection fraction : I suppose it is left ventricular (it should be mentioned).
Response : This is correct, thank you. "Left ventricular ejection fraction" is now mentioned in the Patients and Methods section.

#4. Results : "SAPS scores were enrolled": some words are missing
Response: The beginning of the first paragraph of the Results section now reads: "Of 20 consecutive patients (15 males) assessed for eligibility, 12 patients (10 males) were enrolled in the study; [age 45.0 (31.3-47.5) years; body mass index 25.0 (23.0-26.9) kg/m2; predicted body weight 72.7 (66.8-80.2) kg; Simplified Acute Physiology Score II 35.0 (28.0-40.9)]."

#5. Table : how was the fluid balance recorded ?
Response: Fluid balance (i.e. fluid intake minus fluid output comprising urine and any drain output if present) is documented hourly by the nursing staff in the ICU. The reported values refer to the cumulative 8-hour fluid balance at each assessment point. We have added this information in the Patients and Methods section, at the end of the first paragraph of Measurements which now reads as follows:
"EVLW, hemodynamic parameters, respiratory system mechanics, arterial and central venous blood gases and the cumulative fluid balance over the preceding 8 hours (8-hour fluid intake minus 8-hour fluid output) were documented for each assessment."
The legend to Table 2 now includes the statement: " * Fluid balance refers to the 8-hour period of HFO-TGI or CMV ventilation. Baseline fluid balance refers to the 8-hour period preceding the baseline measurements."

Minor comments
1. The P/F ratio does not need a decimal
2. GVEDVI and ITBVI : the "V" are missing
3. FiO2 could be abbreviated
Response:
1. We omitted the decimal from the P/F ratio values in Table1.
2. Global End Diastolic Volume and Intra Thoracic Blood Volume Index are now abbreviated GEDVI and ITBVI respectively in the main manuscript and Table 2.
3. We abbreviated FiO2 throughout the main manuscript, Tables and Figures.

Reviewer 2:
#1: The length of time each patient was exposed to HFO is rather short and thus differences between the two groups although interesting is not necessarily indicative of any lasting affect. This should probably be discussed in the "limitations" section.
Response: We now address this point in the limitations section as follows: "Longer HFO-TGI sessions could have resulted in a larger and more uniform reduction in EVLWI. Notably, in the prone position the time required to observe a significant reduction in EVLWI compared to CMV was 18 hours [35]. Although our physiological data do not describe a potentially lasting effect of HFO-TGI, we have previously shown a progressively sustained improvement in oxygenation and lung mechanics with repetitive (for up to 10 days) HFO-TGI sessions [3]."

#2: Statistical analysis of the data: I don't know how well the Wilcoxon-matched paired test, although recommended given the lack of washout-period, truly compensates for this absence. More concerning, however, is that some of the patients had multiperiod crossover trials. It is my understanding that for patients who receive multiperiod treatments (ABAB, for example-where A is the first treatment and B is the second), their data should be analyzed differently than those who undergo the standard AB

treatment (1. Jones B, Kenward MG 2nd edition, Boca Raton: Chapman & Hall/CRC; 2003. Design and analysis of cross-over trials).
Response:
Regarding the choice of test: Given the absence of a wash-out period, the analysis proposed by Tudor and Koch (Review of nonparametric methods for the analysis of cross-over trials, Statistical Methods in Medical Research (1994), 3:345-381) using the Wilcoxon matched paired test is recommended, and does not suffer from the disadvantages of preliminary testing for carry-over (Jones-Kenward, Design and analysis of cross-over trials (2003), CRC, §2.12.6). However, we do not claim that the method of analysis itself compensates for the lack of wash-out period. The absence of a carry-over effect is an assumption based on the fact that measurements were taken 8h after the cross-over and, to some extent, corroborated by the negative testing for carry-over. A different design with four groups (AA/BB/AB/BA) would have allowed a better estimation of the difference in treatment effect, if a carry-over effect were present. On the other hand, the analysis based on the design we have followed is more robust to departures from normality, as well as assumptions on the covariance structure of the variables (Jones & Kenward §5.7.2).
We have added the following comment to the limitations section: “The crossover 2x2 design without a washout period introduces some limitations in the interpretation of the data due to the possibility of a carry-over effect even if statistical tests to detect it are negative. This limitation applies, to some extent, to any crossover trial [34]." The book by Jones & Kenward suggested by the referee has also been added to the references.
Regarding the analysis of patients with multiperiod treatments: What we actually did was randomizing the patients again to a treatment group if they were eligible to receive more than two sessions, and treating the subsequent sessions as new cases. This is described in the first paragraph of the Protocol section, as well as in the Legend to Figure E1 (supplementary material). Naturally, this may raise some concerns regarding the interpretation of the results, as we may no longer assume that different cases come from independent observations. However, it is not hard to check that, under mild assumptions, cross-over differences over different sessions of the same patient are uncorrelated (e.g. a uniform covariance structure would suffice).
In principle, we could also assume that all patients underwent multiperiod sessions, but for some of them data from the last periods are missing. We could then proceed with a Bayesian analysis (as in Jones & Kenward §2.9) that can handle missing data effectively. However, this would require a different set of assumptions, and it would make the analysis much more technical.
Figure 2b and Figure 3 essentially depict our raw data on EVLWI. Each symbol corresponds to one session and represents the difference in EVLWI at the end of CMV minus EVLWI at the end of HFO-TGI. Ventilation sessions of the same patient are represented by the same kind of symbol. One reason for providing this detailed information is to show that patients with four sessions (□, ▽, ■ and ●) had variable responses to HFO-TGI, and they do not seem to introduce a bias in the interpretation of the results.

#3. More information regarding the study subjects, specifically: A. Had all the patients diagnosed with ARDS within the preceding 96H of study entry been mechanically ventilated during those 96H (your separating these two criteria imply they may not have been). B. What does "volume loading" mean? How much volume did each patient actually receive prior to the initiation of vasopressors? C. How did you define "chronic therapy"? Was this just regular use of albuterol or did this also mean patients who are on mono-therapy with one agent in addition to albuterol or patients who are on LABA/ICS and albuterol or patents who are on LABA/ICS, LAMA with albuterol?
Response: A. Patients included in the study were diagnosed with ARDS within the preceding 96 hours of study entry but they might have been ventilated for a longer period of time. Pre-enrollment ventilation duration was variable, and we added this information to the first paragraph of the Results section: “The time of mechanical ventilation prior to study enrollment was 108 (66-120) hours.” B. Patients with low blood pressure received 20-30 ml/Kg bolus crystalloid targeting a CVP of 12 prior to the initiation of noradrenaline. In the Patients and Methods section, the first paragraph of Study

Subjects regarding hemodynamic instability as exclusion criterion now reads: “2) severe hemodynamic instability (systolic arterial pressure <90 mmHg despite volume loading with up to 30 mL/kg crystalloid targeting a central venous pressure (CVP) of 12 mmHg and norepinephrine infusion ≥0.5 μg/kg/min)”. C. We defined chronic therapy for COPD/asthma patients as chronic corticosteroid therapy of any kind (inhaled or oral). These eligibility criteria were also applied in Mentzelopoulos SD, Malachias S, Zintzaras E, et al: Intermittent recruitment with high-frequency oscillation/tracheal gas insufflation in acute respiratory distress syndrome. Eur Respir J 2012; 39:635–647.Exclusion of COPD patients is now defined as: “significant chronic obstructive pulmonary disease (COPD) or asthma (previous hospital admissions for COPD/asthma, chronic corticosteroid therapy, oral or inhaled, for COPD/asthma, and/or documented chronic CO2 retention with baseline PaCO2 >45 mmHg);”

#4. There are a few grammatical errors (which I will leave to the editors) and a couple word-choice errors: A) I think you would prefer to write "bronchoscopy" instead of "endoscopy'" on page 6. B) I think you mean barotrauma instead of biotrauma on page 12.
Response: We did our best to eliminate grammatical errors. A) we changed “endoscopy” to “bronchoscopy” as suggested by the referee in the last paragraph of page 6 which now reads: “correct positioning of tracheal tube tip (~4 cm above the carina) was verified by chest radiography, and tracheal tube patency was confirmed by a ≤10 sec lasting bronchoscopy.” B) At this point we were actually referring to biotrauma, so the phrasing has now been changed to better convey the message that: “avoidance of repeated opening and collapsing of affected alveoli with HFO, prevents ventilator induced inflammatory response, promotes effective tissue repair, and ameliorates alveolar and interstitial edema.”

Reviewer 3:
#1: Unfortunately, in the rationale of the study and in the discussion, the investigators fail to mention the recent study of Ferguson et al (N Engl J Med. 2013 Feb 28;368(9):795-805) where it was reported that high-frequency oscillatory ventilation may increase in-hospital mortality of patients with ARDS. In other words, the investigators must strongly defend the role - if any - of HFO-TGI in clinical practice. By not providing a robust defence of potential uses of HFO-TGI then the current study could be interpreted by a potential reader as an academic exercise.
Response: We would like to thank the reviewer for giving us the opportunity to elaborate. The protocol we use for HFO-TGI ventilation is totally different than the protocol used in the study by Ferguson et al. In the recent large randomized control trial by Ferguson et al. showing worse outcomes with HFO in early ARDS, high mPaws were used. This could potentially contribute to hemodynamic compromise by directly affecting right ventricular afterload and end organ failure, thus leading to an increased need for vasopressors in the HFO group [Guervilly et al. Crit Care Med 2012; 40(5):1539-45, Ferguson et al. N Engl J Med 2013; 368:795-805]. The present study’s combination of HFO-TGI with short lasting RMs and cuff leak was almost identical to the previously employed HFO-TGI/RMs protocol that resulted in substantial physiological benefit without hemodynamic compromise and improved survival when it was applied intermittently during lung protective CMV and targeting improved oxygenation [Mentzelopoulos et al. Eur Respir J 2012; 39:635–647]. This protocol was also successfully applied as rescue ventilation in patients with ARDS and traumatic brain injury and succeeded to control PaCO2 [Vrettou et al. Crit Care 2013; 17(4):R136]. Possible mechanisms contributing to hemodynamic stability during HFO-TGI include a) recruitment of the dependent lung units with the addition of TGI to HFO, which might decrease pulmonary vascular resistance and thus reduce the risk of right ventricular dysfunction [Mentzelopoulos et al. Intensive Care Med 2011; 37:990-999, Guervilly et al. Crit Care Med 2012; 40(5):1539-45], and b) enhanced CO2 elimination with the use of TGI and cuff leak, hence further protecting right ventricular function [Mekontso et al. Intensive Care Med 2009; 35(11): 1850-8]. Moreover the intermittent use of HFO-TGI may have prevented long term HFO-related adverse effects.
The last paragraph of the Discussion section now reads as follows:

“The protocol we use for HFO-TGI ventilation is totally different from the protocol used in the study by Ferguson et al. [30]. In this recent large randomized controlled trial showing worse outcomes with HFO in early ARDS, high mPaws were used [30]. This could potentially contribute to hemodynamic compromise by directly affecting right ventricular afterload, thus leading to an increased need for vasopressors in the HFO group and end organ failure [30,31]. The present study’s combination of HFO-TGI with short lasting RMs and cuff leak was almost identical to the previously employed HFO-TGI/RMs protocol that resulted in substantial physiological benefit without hemodynamic compromise and improved survival when it was applied intermittently during lung-protective CMV, with a target of improved oxygenation [3]. This protocol was also successfully applied as rescue ventilation in patients with ARDS and traumatic brain injury [32]. Possible mechanisms contributing to hemodynamic stability during HFO-TGI include a) recruitment of the dependent lung units with the addition of TGI to HFO, which may decrease pulmonary vascular resistance and reduce the risk of right ventricular dysfunction [4,31], and b) enhanced CO2 elimination with the use of TGI and cuff leak, hence further protecting right ventricular function [33]. Moreover the intermittent use of HFO-TGI may have prevented long term HFO-related adverse effects. Table 2 shows that parameters of right ventricular function such as the CVP were similar in the two ventilation strategies without a need for excess fluid volume administration or vasopressor dose escalation.”

Specific Comments
#1: Which criteria were used to define/identify the presence of ARDS?
Response: We have followed the American-European Conference definition of ARDS (1994) that was the broadly used definition at the time of study design. We have added the appropriate reference in the main manuscript [16].

#2: The investigators must better stress/discuss that in one third of the HFO-TGI sessions extravascular lung water were not lower with HFO-TGI as compared with conventional mechanical ventilation.
Response: We now stress this point in the legend to Figure 2 “ … Those below the zero line correspond to sessions where EVLWI was lower following CMV (9/32)” and in the end of the first paragraph of the Discussion section: “Figure 2b shows that although EVLWI was significantly lower following HFO-TGI/RM ventilation, this response was not uniform neither among patients nor within sessions of the same patient.” The addition we made to the limitations section, requested by Reviewer 2 is also relevant: “Longer HFO-TGI sessions could have resulted in a larger and more uniform reduction in EVLWI. Notably, in the prone position the time required to observe a significant reduction in EVLWI compared to CMV was 18 hours [35]. ”

Reviewer 4:
#1: The authors have used the so-called 40/40 rule as their recruitment manoeuvre for HFOV. However, from a physiological perspective this is not the most optimal method. Although time-consuming, a stepwise incremental-decremental titration of the CDP allows for a better lung volume, better oxygenation and oscillation on the deflation limb of the pressure volume loop. Do the authors think their results would have been different if they would have used such an approach?
Response: To the best of our knowledge, the optimal method for lung recruitment has not yet been established. However, the method you suggest is obviously physiologically sound. Nevertheless, we cannot speculate on the effect of such a method on our results. Therefore, we added in the 4th paragraph of the Discussion section the following:
“We applied RMs by using the 40/40 rule during CMV and HFO-TGI. Another physiologically sound approach could be to apply a stepwise incremental-decremental titration of the continuous distending pressure [29]. However, we cannot speculate on how this approach would have affected our results.”

#2: After their recruitment on HFOV, the authors turned down the CDP to approximately 6 - 8 cmH2O above the mean airway pressure during conventional mechanical ventilation. Such an approach does not make sense to me. Can the authors elaborate on why they did this? One can speculate that such an approach would lead to serious derecruitment.
Response: We adopted this approach based on physiological measurements obtained in previously published studies published by our group in patients with ARDS of similar severity. Indeed, following a recruitment meneuver, we performed inspiratory and expiratory pressure-volume curves and found that the point of maximal curvature (PMC) of the expiratory limb had mean value 25-26 cmH2O [Mentzelopoulos SD et al. Crit Care Med 2007; 35:1500–1508]. Therefore, in the current study by setting the mPaw during HFO-TGI at approximately 6-8 cmH2O above the mPaw of the preceding CMV, we expected to have a mPaw at the level or slightly above the PMC where lung recruitment is maintained. Indeed, mPaw during HFO-TGI in the current study was 27-29 cmH2O, a pressure rather unlikely to lead to serious derecruitment.

#3a: Regarding Figure 2, can the authors explain why there was no effect on extravascular lung water at 16 hours in patients who crossed over from CMV to HFOV?
Response: Statistical analysis of a 2X2 cross-over trial involves the cross-over differences (EVLWI in HFO-TGI - EVLWI in CMV) in both groups. Restricting the analysis in group HF-1 does not necessarily yield statistically significant results, possibly due to the small sample size.
#3b: Am I right by interpreting that the effect of HFOV+TI is only short-lived?
Response: Indeed, the effect of HFO-TGI ventilation on EVLW may be short-lived. In fact, we have implicitly assumed the short lived effect of a single, 8-hour HFO-TGI period in our study design, when we assumed that 8-hour intervals would suffice to minimize the carry-over effect of the previous treatment. However, repetitive periods of HFO-TGI have previously resulted in a progressively sustained respiratory physiological benefit [3]. We have added a relevant comment to the first paragraph of the Limitations section: “Although our physiological data do not indicate a potentially lasting effect of HFO-TGI, we have shown a progressively sustained improvement in oxygenation and lung mechanics with repetitive (for up to 10 days) HFO-TGI sessions [3].”

*Cover Letter

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Professor Philip D. Lumb
Editor-in-Chief
Journal of Critical Care

January 31st 2014

Dear Professor Lumb,

Re: Revision of manuscript JCRC-D-13-00655R1

Thank you for considering our manuscript titled "The effect of high frequency oscillatory ventilation combined with tracheal gas insufflation on extravascular lung water in patients with acute respiratory distress syndrome: a randomized, crossover, physiological study" for publication in the Journal of Critical Care. We would also like to thank the reviewers for carefully reading the manuscript and for providing insightful comments that, we believe, helped considerably to improve the quality and clarity of the article.

Please, find attached the revised version of the manuscript (revisedmanuscript.doc) and the Electronic Supplementary Material (revisedESM.doc). Please, also find a separate manuscript file (mmarkedchanges.doc) where changes are highlighted in red. Our response to the reviewers' comments is included in the file responsetoreviewers.doc.

We had to add 5 items to the reference list in order to address the reviewers' comments appropriately. The word count is now 3217.

Sincerely,

Dr Charikleia S. Vrettou
MD, MRCP,
Research Fellow in Intensive Care Medicine

The effect of high frequency oscillatory ventilation combined with tracheal gas insufflation on extravascular lung water in patients with acute respiratory distress syndrome: a randomized, crossover, physiological study.

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Keywords Acute Respiratory Distress Syndrome; Extravascular Lung Water; High Frequency Ventilation.

Abstract

*Purpose***:** High frequency oscillation combined with tracheal gas insufflation (HFO-TGI) improves oxygenation in patients with Acute Respiratory Distress Syndrome (ARDS). There is limited physiologic data regarding the effects of HFO-TGI on hemodynamics and pulmonary edema during ARDS. The aim of this study was to investigate the effect of HFO-TGI on extravascular lung water (EVLW).

*Materials and Methods***:** We conducted a prospective, randomized, crossover study. Consecutive eligible patients with ARDS received sessions of conventional mechanical ventilation (CMV) with recruitment maneuvers (RMs), followed by HFO-TGI with RMs, or vice versa. Each ventilatory technique was administered for 8 hours. The order of administration was randomly assigned. Arterial/central venous blood gas analysis and measurement of hemodynamic parameters and EVLW were performed at baseline and after each 8-hour period using the single-indicator thermodilution technique.

*Results***:** Twelve patients received 32 sessions. PaO_2/FiO_2 and respiratory system compliance were higher ($p<0.001$ for both), while EVLW indexed to predicted body weight (EVLWI) and oxygenation index were lower ($p=0.021$ and 0.029, respectively) in HFO-TGI compared with CMV. There was a significant correlation between PaO_2/FiO_2 improvement and EVLWI drop during HFO-TGI ($Rs=-0.452$, $p= 0.009$).

*Conclusions***:** HFO-TGI improves gas exchange and lung mechanics in ARDS, and potentially attenuates EVLW accumulation.

Introduction

Intermittent high-frequency oscillation combined with tracheal gas insufflation (HFO-TGI), tracheal tube cuff leak, and recruitment meneuvres (RMs) improves gas exchange and lung mechanics in patients with acute respiratory distress syndrome (ARDS) [1-4]. Underlying mechanisms may include: 1) HFO-RMs lung recruitment, likely augmented by TGI's positive end expiratory pressure (PEEP) effect [1-3] 2) preferential recruitment of previously non-aerated, dependent lung regions [4] 3) enhancement of HFO-related gas transport mechanisms [5] by the TGI jet stream, and 4) improved washout of the anatomic dead space and CO_2 elimination [6].

An unstudied-to-date, but plausible beneficial mechanism of HFO-TGI could comprise a reduction in pulmonary edema. The role of extravascular lung water (EVLW) measurement has been recently proposed to be central in the diagnosis, monitoring and decision making in ARDS and it is feasible by the single thermodilution technique [7-10]. In the present study, we tested the hypothesis that HFO-TGI with RMs reduces EVLW compared with CMV with RMs, without adversely affecting other hemodynamic parameters.

Patients and Methods

The study was conducted from June to December 2011 in the intensive care unit (ICU) of Evaggelismos Hospital, which is a 30 bed multidisciplinary unit, admitting medical and surgical patients, including trauma. The study protocol was approved by the Evaggelismos Hospital Scientific and Ethics committee. Written next-of-kin consent was obtained for all patients.

Study subjects

Patients who met the following criteria were considered eligible for enrollment: 1) age 18-75 years; 2) body weight >40 kg; 3) ARDS diagnosis established within preceding 96 hours [11]; 4) endotracheal intubation and mechanical ventilation; 5) oxygenation disturbances with PaO_2/FiO_2 ratio<200 mmHg at PEEP ≥ 5 cmH_2O. Exclusion criteria were in accordance with previously published exclusion criteria for HFO-TGI use [3]: 1) active air leak or recent persistent (for >72 hours) air leak; 2) severe hemodynamic instability (systolic arterial pressure <90 mmHg despite volume loading with up to 30 mL/kg crystalloid to target a central venous pressure (CVP) of 12 mmHg and norepinephrine infusion ≥0.5 μg/kg/min); 3) significant heart disease (left ventricular ejection fraction <40%, and/or history of pulmonary edema, active coronary ischemia or myocardial infarction); 4) significant chronic obstructive pulmonary disease (COPD) or asthma (previous hospital admissions for COPD/asthma, chronic corticosteroid therapy for COPD/asthma, and/or documented chronic CO_2 retention with baseline $PaCO_2$ >45 mmHg); 5) chronic interstitial lung disease; 6) lung biopsy or resection at current admission; 7) known or suspected thromboembolic disease; 8) intracranial hypertension (intracranial pressure ≥20 mmHg despite deep sedation, analgesia, hyperosmolar therapy and minute ventilation titrated to $PaCO_2$ ≤35mmHg); 9) pregnancy; 10) morbid obesity with body mass index (BMI) >40 kg/m^2; and 11) enrollment in another interventional study.

Protocol

Following study enrollment, baseline measurements were obtained while patients were ventilated with lung protective volume assist CMV with constant

inspiratory flow as prescribed by the attending physicians (Table 1). Subsequently, patients received either a session comprising 8 hours of CMV followed by 8 hours of HFO-TGI (HF-1), or vice versa (HF-2) (Fig.1a). A schematic presentation of the study protocol is detailed in Fig. E1 in the electronic supplementary material (ESM). We used constrained randomization [12] to ensure equal number of HF-1 and HF-2 sessions and equal representation of each patient in the two groups. Each patient could receive at least two sessions, the first session being randomly assigned to one of the two groups, HF-1 or HF-2, and the second session to the opposite group. If oxygenation criteria were met for ≥6 hours after the second session and the 96-hour criterion was also met, a patient could additionally receive two more sessions.

The 8-hour duration of each ventilatory technique was chosen because we know from previous studies that application of HFO-TGI for >6 hours is associated with significant improvement in oxygenation and lung mechanics [3]. In-between consecutive sessions, patients were ventilated with CMV, while ventilation settings, sedation, and analgesia were adjusted by the attending physicians. Any episodes of hypotension related to RMs or HFO-TGI application were to be treated with norepinephrine and a 300-500 ml bolus of crystalloid [3]. In the event of pneumothorax, severe hemodynamic instability or intracranial hypertension at any point during the study period, the patient was withdrawn from the study.

CMV application

During every CMV period, patients were ventilated with the square-wave inspiratory flow, volume assist-control mode. Ventilatory settings were as follows: Tidal volume 6-8 mL/kg predicted body weight (PBW), combinations of PEEP (cmH_2O) and FiO_2 according to the ARDSnet PEEP/FiO_2 protocol (10/0.6, 10-14/0.7,

14/0.8, 14-18/0.9, 18-24/1.0) [13], inspiratory-to-expiratory time ratio 1:2, target pH 7.20-7.45, and target end-inspiratory plateau airway pressure <30 cmH_2O. Target PaO_2 was 60-80 mmHg, except in patients with traumatic brain injury, where we aimed at PaO_2 >90 mmHg. RMs were administered during CMV by applying continuous positive airway pressure (CPAP) at 40 cmH_2O for 40 sec (see also Fig. E1 in the ESM).

Patients were sedated with midazolam and/or propofol to a Ramsay score of 4-6. If patient-ventilator dyssynchrony [14] was observed despite a Ramsay score of 6, continuous infusion of cis-atracurium was initiated at 0.1-0.2 mg/kg/h. A bolus dose of cis-atracurium was administered 30 mins before each RM. Continuous infusion of fentanyl at 1-3 μg/kg/h was used for analgesia in the presence of clinically obvious factors mandating pain control, e.g., cases of trauma or surgery within the preceding 48-72 h.

HFO-TGI application

Before HFO-TGI initiation, orotracheal tubes (inner diameter 8.0-9.0 mm) were cut-down to 26 cm, correct positioning of tracheal tube tip (~4 cm above the carina) was verified by chest radiography, and tracheal tube patency was confirmed by a ≤10 sec lasting bronchoscopy [1]. A 4.8 cm long circuit adapter with angled side arms (Smiths Medical International, Watford, UK) was introduced in-between the tracheal tube connector and the Y-piece of the ventilator breathing circuit. A rigid-wall catheter (Vygon, Ecouen, France; inner diameter 1.0 mm, outer diameter 2.0 mm) was passed through the side arm of the adapter and was used for the administration of TGI. The TGI catheter length was tailored to the placement of its tip at 0.5-1.0 cm beyond the tip of the tracheal tube.

Patients were sedated with midazolam and/or propofol to a Ramsay score of 6 and paralyzed with cis-atracurium. HFO was provided using a 3100B high frequency ventilator (Sensormedics, Yorba Linda, CA, USA). Patients were connected to the high frequency ventilator and a 40 sec RM was performed by pressurizing the HFO breathing circuit at 40 cmH_2O with the oscillator piston off. We then resumed HFO and placed a 3-5 cmH_2O tracheal tube cuff leak. We returned mPaw to its pre-leak level by adjusting the mPaw valve, and mPaw was set 6-8 cmH_2O above its value during the preceding CMV [2]. Subsequently, we connected the TGI catheter to a variable-orifice O_2 flowmeter providing humidified O_2 at room temperature, and started TGI at a flow equal to 50% of the preceding CMV minute ventilation. TGI initiation caused a 1-2 cmH_2O rise in mPaw, which we reversed by adjusting the mPaw valve. FiO_2 was set at 1, oscillatory pressure amplitude (ΔP) was set at 65-90 cmH_2O (30 cmH_2O above the preceding CMV $PaCO_2$ value), and oscillation frequency (f) at 3.5-5.5 Hz. ΔP and f were further adjusted to achieve a target arterial pH of 7.20-7.45. The catheter used for TGI administration was removed when switching to the conventional ventilator.

Measurements

Patients underwent three assessments in every session: at baseline, after 8 hours of CMV and after 8 hours of HFO-TGI (Fig. 1a, Fig. E1, ESM). Each assessment lasted 10 to 15 minutes while the patient remained on CMV or HFO-TGI, respectively. EVLW, hemodynamic parameters, respiratory system mechanics, arterial and central venous blood gases and the cumulative fluid balance over the preceding 8 hours (8-hour fluid intake minus 8-hour fluid output) were documented for each assessment.

The single indicator transpulmonary thermodilution technique (PiCCOplus®, Pulsion Medical Systems, Munich, Germany) was used for EVLW measurement and hemodynamic monitoring. This technique correlates well with the gold standard gravimetric method in animals, as well as with the double indicator dilution technique in humans for the estimation of EVLW [7]. Patients had a femoral arterial 5-F thermistor catheter and a central venous catheter in situ. For each set of hemodynamic measurements, 15 mL of 0.9% saline (<8 ^{o}C) were administered by central venous injection with an adequate thermodilution curve displayed on the monitor screen in duplicate and the mean values were included in the analysis [15]. Respiratory mechanics were assessed by the end-inspiratory and end-expiratory technique [1], always at CMV with square-wave inspiratory flow. Following HFO-TGI, respiratory mechanics were measured immediately after return to CMV (see also Fig. E1 in the ESM).

Statistical analysis

Statistical procedures were based on recommendations for analysis of crossover trials [16]. Non-parametric statistics were applied. Differences between group characteristics at baseline in quantitative and qualitative data were evaluated by the Mann-Whitney U-test and Fisher's exact test, respesctively. Within-group changes of variables were analyzed with Wilcoxon-matched paired test, when indicated. Correlations were determined by using the non-parametric Spearman's (Rs) test. The treatment effect of HFO-TGI (individual crossover difference between HFO-TGI and CMV) was analyzed by comparing the values at the end of each period with Wilcoxon-matched paired test with "HFO-TGI dependent" p-value (p_{hd}) indicating significance. The possibility of a carryover period, or other treatment effect was

assessed by comparing the values of the differences at the end of each period between HF-1 and HF-2 groups with Mann-Whitney U signed rank sum test with "HFO-TGI independent" p-value (p_{hi}) indicating significance (Fig. 1). The false discovery rate procedure [17] revealed a corrected p-value of less than 0.03 to be significant for multiple comparisons. Statistical analyses were performed using SPSS Statistics version 20 (SPSS Inc., Chicago, Illinois, USA) and "R" statistical software version 3.0 (R Foundation for Statistical Computing, Vienna, Austria). An *a priori* power analysis was performed with GPower 3.1 (Franz Faul, University of Kiel, Germany). The minimum sample size was calculated based on 90% power and a two-sided 0.05 significance level. The sample size capable of detecting a between-ventilatory techniques difference of 1 mL/kg was estimated for the decrease in EVLW indexed to PBW (EVLWI) using data from a previous human study [18]. The critical sample size was estimated to be 25 ventilation sessions. Data are presented as median (interquantile range) unless otherwise specified.

Results

Of 20 consecutive patients (15 males) assessed for eligibility, 12 patients (10 males) were enrolled in the study; [age 45.0 (31.3-47.5) years; body mass index 25.0 (23.0-26.9) kg/m^2; PBW 72.7 (66.8-80.2) kg; Simplified Acute Physiology Score II 35.0 (28.0-40.9)]. Of these patients eleven had primary (pulmonary) ARDS, and three patients had co-existing head trauma not associated with intracranial hypertension. The reasons for the exclusion of 8 patients were: hemodynamic instability requiring high-dose vasopressors (3 patients), traumatic brain injury with intracranial hypertension (1 patient), COPD diagnosis with documented chronic CO_2 retention (2 patients), suspected thromboembolic disease (1 patient), and echocardiographic

evidence of severe cardiac dysfunction (1 patient). The time of mechanical ventilation prior to study enrollment was 108 (66-120) hours.

A total number of 32 ventilation sessions were administered. Sixteen sessions were assigned to the HF-1 group and 16 to the HF-2 group. Four patients received 4 sessions, and 8 patients received 2 sessions. There were no protocol related complications and all patients completed the study uneventfully. No significant difference in baseline ventilation settings was evident between the two groups (Table 1). Ventilatory settings at each assessment time-point are shown in Fig. E1 in the ESM.

Gas exchange and lung mechanics

PaO_2/FiO_2 increased after using HFO-TGI compared with CMV (p_{hd} <0.001, Fig. 1b), while the oxygenation index decreased following HFO-TGI compared with CMV [15.1 (10.6-22.3) *versus* 19.1 (16.0-27.7), p_{hd} =0.029]. Respiratory system compliance was higher after HFO-TGI [36.8 (24.7-44.0) *versus* 31.7 (24.8-39.1) mL/cmH_2O, p_{hd} <0.001]. A lower $PaCO_2$ was maintained with HFO-TGI compared with CMV (42.5 (38.7-54.0) *versus* 46.3 (40.9-63.5) mmHg, p_{hd} =0.045], but this difference did not remain significant following correction for multiple comparisons and did not significantly affect the pH [7.38 (7.33-7.44) *versus* 7.36 (7.31-7.41), p_{hd} =0.166].

EVLWI and hemodynamic measurements

EVLWI was lower after 8 hours of HFO-TGI in comparison with CMV (p_{hd} =0.021, Fig. 2a). In 23/32 sessions EVLWI values were lower in HFO-TGI compared

with CMV, while in 9/32 sessions EVLWI was higher in HFO-TGI (Fig. 2b) (mean crossover difference 1.25 mL/kg, or 10%).

Central venous oxygen saturation was higher in HFO-TGI (p_{hd} =0.001), while the pulmonary vascular permeability index (PVPI) was lower (p_{hd} =0.047), but this difference did not remain significant after correcting for multiple comparisons (Table 2). No significant difference between the two ventilatory techniques was detected in the rest of the hemodynamic parameters (Table 2). There was a negative correlation between changes in EVLWI and changes in PaO_2/FiO_2 during HFO-TGI (Rs=-0.452, p=0.009) (Fig. 3).

Fluid balance and vasopressor support

There was no significant difference between the two groups regarding fluid balance or vasopressor support (Table 2).

Carryover effect

Carryover or treatment effect of the application of CMV on the result of HFO-TGI in HF-1 group and of the application of HFO-TGI on the result of CMV in HF-2 group was not significant for PaO_2/FiO_2 (Fig. 1b), EVLWI (Fig. 2a), respiratory system compliance, oxygenation index, $PaCO_2$, pulmonary vascular permeability index (PVPI) and hemodynamic parameters (Table 2). Therefore, it can be assumed that possible carryover period or treatment effect could not have affected the results.

Discussion

We have shown that HFO-TGI with interspersed RMs applied for 8 hours in patients with early ARDS resulted in improved gas exchange and lung mechanics and

in reduced EVLW accumulation compared with CMV with RMs. Hemodynamic parameters including cardiac index and intrathoracic lung volume index (ITBVI) were not significantly different between the two ventilation strategies. These results are consistent with amelioration of pulmonary edema formation related to enhanced lung recruitment with HFO-TGI [1,6]. Figure 2b shows that although EVLWI was significantly lower following HFO-TGI/RM ventilation, this response was not uniform neither among patients nor within sessions of the same patient.

Lung recruitment may affect the EVLWI in different and opposing ways [15]; it can lower the EVLWI by collapsing the pulmonary small vessels [19] or reducing the cardiac output [20,21], whereas it may increase the EVLWI by increasing lung volume [22], redistributing the pulmonary blood flow [23], or elevating the central venous pressure [23]. During HFO-TGI, the pattern of lung recruitment is different. The continuous forward TGI flow exerts a PEEP-effect by impeding the opposite directed expiratory flow [1,2,6]. Scanographic data with HFO-TGI show preferential recruitment of the dependent, subcarinal lung regions without overdistention of the already aerated lung [4].

In a porcine ARDS model [20] it has been shown that the addition of PEEP and the use of small tidal volumes during CMV can attenuate lung injury and EVLW accumulation. According to other animal ARDS studies, avoidance of repeated opening and collapsing of affected alveoli with HFO, prevents ventilator induced inflammatory response, promotes effective tissue repair, and ameliorates alveolar and interstitial edema [24-27]. With the addition of TGI to HFO, we combined these lung protective approaches by minimizing tidal volume and enhancing lung recruitment in the lower and dependent lung areas [4]. An explanation for our findings based on a lung protective recruitment effect that attenuates permeability edema formation is also

corroborated by the marginally lower value of the PVPI (Table 2), which is a marker of pulmonary vascular permeability in ARDS [10].

We applied RMs by using the 40/40 rule during CMV and HFO-TGI. Another physiologically sound approach could be to apply a stepwise incremental-decremental titration of the continuous distending pressure [28]. However, we cannot speculate on how this approach would have affected our results. Figure 3 shows the correlation between improvement in EVLWI and PaO_2/FiO_2 during HFO-TGI. This correlation has not been observed when RMs alone were administered in ARDS patients during CMV [29]. If a sustained and less traumatic lung recruitment is the cause of EVLWI decrease, then the amount of recruitable lung tissue with HFO-TGI can explain this correlation. However, we do not have the scanographic data to further support this argument.

The protocol we use for HFO-TGI ventilation is totally different from the protocol used in the study by Ferguson et al. [30]. In this recent large randomized controlled trial showing worse outcomes with HFO in early ARDS, high mPaws were used [30]. This could potentially contribute to hemodynamic compromise by directly affecting right ventricular afterload, thus leading to an increased need for vasopressors in the HFO group and end organ failure [30,31]. The present study's combination of HFO-TGI with short lasting RMs and cuff leak was almost identical to the previously employed HFO-TGI/RMs protocol that resulted in substantial physiological benefit without hemodynamic compromise and improved survival when it was applied intermittently during lung-protective CMV, with a target of improved oxygenation [3]. This protocol was also successfully applied as rescue ventilation in patients with ARDS and traumatic brain injury [32]. Possible mechanisms contributing to hemodynamic stability during HFO-TGI include a) recruitment of the

dependent lung units with the addition of TGI to HFO, which may decrease pulmonary vascular resistance and reduce the risk of right ventricular dysfunction [4,31] and b) enhanced CO_2 elimination with the use of TGI and cuff leak, hence further protecting right ventricular function [33]. Moreover the intermittent use of HFO-TGI may have prevented long term HFO-related adverse effects. Table 2 shows that parameters of right ventricular function such as the CVP were similar in the two ventilation strategies without a need for excess fluid volume administration or vasopressor dose escalation.

Study Limitations

The crossover 2x2 design without washout period introduces some limitations in the interpretation of data due to the possibility of a carry-over effect even if statistical tests to detect it are negative. This limitation applies, to some extent, to every crossover trial [34]. Longer HFO-TGI sessions could have resulted in a larger and more uniform reduction in EVLWI. Notably, in the prone position the time required to observe a significant reduction in EVLWI compared with supine CMV was 18 hours [35]. Although our physiological data do not indicate a potentially lasting effect of HFO-TGI, we have previously shown a progressively sustained improvement in oxygenation and lung mechanics with repetitive (for up to 10 days) HFO-TGI sessions [3].

Limitations of the single indicator thermodilution technique include the underestimation of EVLW in diseases that block the thermal indicator's passage through the lung, e.g., in massive pulmonary embolism, severe pulmonary edema, hypoxic pulmonary vasoconstriction, and lung resection [15]. In the present study,

patients with lung resection and diagnosed or suspected pulmonary embolism were excluded, while all patients were on prophylactic anticoagulation.

Limitations of the long-term use of TGI are described elsewhere [3]. Right heart catheterization would have allowed measurement of pulmonary arterial and capillary wedge pressures, but attending physicians preferred the less invasive single indicator transpulmonary thermodilution technique for the hemodynamic monitoring of their ARDS patients.

Conclusions

Intermittent recruitment with the use of HFO-TGI improves lung mechanics and may reduce EVLW in patients with ARDS compared with protective CMV. This effect is associated with improved lung oxygenation.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Acknowledgements

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Figure legends:

Figure 1

Study design and PaO_2/FiO_2 values. a) Crossover study design. After baseline assessment, patients of the first group (HF-1) initially underwent 8 hours of Conventional Mechanical Ventilation (CMV) followed by 8 hours of High Frequency Oscillatory (HFO) ventilation combined with Tracheal Gas Insufflation (TGI),

whereas patients of the second group (HF-2) initially underwent 8 hours of HFO-TGI ventilation followed by 8 hours of CMV. b) HFO-TGI significantly increased PaO_2/FiO_2 in both periods of administration, compared with CMV. Data are presented as mean ± standard error. #: $p<0.05$ *versus* previous assessment value. The possibility of a carryover period or other treatment effect was assessed by comparing the values of the differences at the end of each period between HF-1 and HF-2 groups with Mann-Whitney U signed rank sum test (d minus a *versus* c minus b) and "HFO-TGI independent" p-value (p_{hi}) below 0.03 indicating significance. The HFO-TGI treatment effect (individual crossover difference between HFO-TGI and CMV) was analyzed by comparing the values at the end of each period (a plus b *versus* c plus d) with Wilcoxon-matched paired test and "HFO-TGI dependent" p-value (p_{hd}) of less than 0.03 indicating significance.

Figure 2

Results on extravascular lung water index (EVLWI). a) HFO-TGI combined with RMs decreased EVLWI in HF-2 whereas CMV had no effect on EVLWI in either group. Data are presented as mean ± standard error. #: $p<0.05$ *versus* previous assessment value. For further explanation please, see legend to Fig. 1.

b) Individual differences in EVLWI between the two ventilation strategies. Each symbol corresponds to one session and represents the difference in EVLWI after 8 hours of CMV minus EVLWI after 8 hours of HFO-TGI. Ventilation sessions in the same patient are represented with the same kind of symbol. Symbols above the zero line correspond to sessions where EVLWI was lower following HFO-TGI (23/32). Those below the zero line correspond to sessions where EVLWI was lower following CMV (9/32).

Figure 3

Scatter plot for values of the change in extravascular lung water index (ΔEVLWI) and in PaO_2/FiO_2 ($\Delta PaO_2/FiO_2$) over the 8-hour HFO-TGI period. Regression equation (solid line) is shown (Rs=-0.452, p=0.009), i.e., there was a significant correlation between PaO_2/FiO_2 improvement and EVLWI drop during HFO-TGI. Each symbol corresponds to one session. Ventilation sessions in the same patient are represented with the same kind of symbol.

The effect of high frequency oscillatory ventilation combined with tracheal gas insufflation on extravascular lung water in patients with acute respiratory distress syndrome: a randomized, crossover, physiological study.

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Keywords Acute Respiratory Distress Syndrome; Extravascular Lung Water; High Frequency Ventilation.

Abstract

*Purpose***:** High frequency oscillation combined with tracheal gas insufflation (HFO-TGI) improves oxygenation in patients with Acute Respiratory Distress Syndrome (ARDS). There is limited physiologic data regarding the effects of HFO-TGI on hemodynamics and pulmonary edema during ARDS. The aim of this study was to investigate the effect of HFO-TGI on extravascular lung water (EVLW).

*Materials and Methods***:** We conducted a prospective, randomized, crossover study. Consecutive eligible patients with ARDS received sessions of conventional mechanical ventilation (CMV) with recruitment maneuvers (RMs), followed by HFO-TGI with RMs, or vice versa. Each ventilatory technique was administered for 8 hours. The order of administration was randomly assigned. Arterial/central venous blood gas analysis and measurement of hemodynamic parameters and EVLW were performed at baseline and after each 8-hour period using the single-indicator thermodilution technique.

*Results***:** Twelve patients received 32 sessions. PaO_2/FiO_2 and respiratory system compliance were higher ($p<0.001$ for both), while EVLW indexed to predicted body weight (EVLWI) and oxygenation index were lower ($p=0.021$ and 0.029, respectively) in HFO-TGI compared with CMV. There was a significant correlation between PaO_2/FiO_2 improvement and EVLWI drop during HFO-TGI ($Rs=-0.452$, $p=0.009$).

*Conclusions***:** HFO-TGI improves gas exchange and lung mechanics in ARDS, and potentially attenuates EVLW accumulation.

Introduction

Intermittent high-frequency oscillation combined with tracheal gas insufflation (HFO-TGI), tracheal tube cuff leak, and recruitment meneuvres (RMs) improves gas exchange and lung mechanics in patients with acute respiratory distress syndrome (ARDS) [1-4]. Underlying mechanisms may include: 1) HFO-RMs lung recruitment, likely augmented by TGI's positive end expiratory pressure (PEEP) effect [1-3] 2) preferential recruitment of previously non-aerated, dependent lung regions [4] 3) enhancement of HFO-related gas transport mechanisms [5] by the TGI jet stream, and 4) improved washout of the anatomic dead space and CO_2 elimination [6].

An unstudied-to-date, but plausible beneficial mechanism of HFO-TGI could comprise a reduction in pulmonary edema. The role of extravascular lung water (EVLW) measurement has been recently proposed to be central in the diagnosis, monitoring and decision making in ARDS and it is feasible by the single thermodilution technique [7-10]. In the present study, we tested the hypothesis that HFO-TGI with RMs reduces EVLW compared with CMV with RMs, without adversely affecting other hemodynamic parameters.

Patients and Methods

The study was conducted from June to December 2011 in the intensive care unit (ICU) of Evaggelismos Hospital, which is a 30 bed multidisciplinary unit, admitting medical and surgical patients, including trauma. The study protocol was approved by the Evaggelismos Hospital Scientific and Ethics committee. Written next-of-kin consent was obtained for all patients.

Study subjects

Patients who met the following criteria were considered eligible for enrollment: 1) age 18-75 years; 2) body weight >40 kg; 3) ARDS diagnosis established within preceding 96 hours [11]; 4) endotracheal intubation and mechanical ventilation; 5) oxygenation disturbances with PaO_2/FiO_2 ratio<200 mmHg at PEEP ≥ 5 cmH_2O. Exclusion criteria were in accordance with previously published exclusion criteria for HFO-TGI use [3]: 1) active air leak or recent persistent (for >72 hours) air leak; 2) severe hemodynamic instability (systolic arterial pressure <90 mmHg despite volume loading with up to 30 mL/kg crystalloid to target a central venous pressure (CVP) of 12 mmHg and norepinephrine infusion ≥0.5 μg/kg/min); 3) significant heart disease (left ventricular ejection fraction <40%, and/or history of pulmonary edema, active coronary ischemia or myocardial infarction); 4) significant chronic obstructive pulmonary disease (COPD) or asthma (previous hospital admissions for COPD/asthma, chronic corticosteroid therapy for COPD/asthma, and/or documented chronic CO_2 retention with baseline $PaCO_2$ >45 mmHg); 5) chronic interstitial lung disease; 6) lung biopsy or resection at current admission; 7) known or suspected thromboembolic disease; 8) intracranial hypertension (intracranial pressure ≥20 mmHg despite deep sedation, analgesia, hyperosmolar therapy and minute ventilation titrated to $PaCO_2$ ≤35mmHg); 9) pregnancy; 10) morbid obesity with body mass index (BMI) >40 kg/m^2; and 11) enrollment in another interventional study.

Protocol

Following study enrollment, baseline measurements were obtained while patients were ventilated with lung protective volume assist CMV with constant inspiratory flow as prescribed by the attending physicians (Table 1). Subsequently,

patients received either a session comprising 8 hours of CMV followed by 8 hours of HFO-TGI (HF-1), or vice versa (HF-2) (Fig.1a). A schematic presentation of the study protocol is detailed in Fig. E1 in the electronic supplementary material (ESM). We used constrained randomization [12] to ensure equal number of HF-1 and HF-2 sessions and equal representation of each patient in the two groups. Each patient could receive at least two sessions, the first session being randomly assigned to one of the two groups, HF-1 or HF-2, and the second session to the opposite group. If oxygenation criteria were met for ≥6 hours after the second session and the 96-hour criterion was also met, a patient could additionally receive two more sessions.

The 8-hour duration of each ventilatory technique was chosen because we know from previous studies that application of HFO-TGI for >6 hours is associated with significant improvement in oxygenation and lung mechanics [3]. In-between consecutive sessions, patients were ventilated with CMV, while ventilation settings, sedation, and analgesia were adjusted by the attending physicians. Any episodes of hypotension related to RMs or HFO-TGI application were to be treated with norepinephrine and a 300-500 ml bolus of crystalloid [3]. In the event of pneumothorax, severe hemodynamic instability or intracranial hypertension at any point during the study period, the patient was withdrawn from the study.

CMV application

During every CMV period, patients were ventilated with the square-wave inspiratory flow, volume assist-control mode. Ventilatory settings were as follows: Tidal volume 6-8 mL/kg predicted body weight (PBW), combinations of PEEP (cmH_2O) and FiO_2 according to the ARDSnet PEEP/FiO_2 protocol (10/0.6, 10-14/0.7, 14/0.8, 14-18/0.9, 18-24/1.0) [13], inspiratory-to-expiratory time ratio 1:2, target pH 7.20-7.45, and target end-inspiratory plateau airway pressure <30 cmH_2O. Target

PaO_2 was 60-80 mmHg, except in patients with traumatic brain injury, where we aimed at PaO_2 >90 mmHg. RMs were administered during CMV by applying continuous positive airway pressure (CPAP) at 40 cmH_2O for 40 sec (see also Fig. E1 in the ESM).

Patients were sedated with midazolam and/or propofol to a Ramsay score of 4-6. If patient-ventilator dyssynchrony [14] was observed despite a Ramsay score of 6, continuous infusion of cis-atracurium was initiated at 0.1-0.2 mg/kg/h. A bolus dose of cis-atracurium was administered 30 mins before each RM. Continuous infusion of fentanyl at 1-3 μg/kg/h was used for analgesia in the presence of clinically obvious factors mandating pain control, e.g., cases of trauma or surgery within the preceding 48-72 h.

HFO-TGI application

Before HFO-TGI initiation, orotracheal tubes (inner diameter 8.0-9.0 mm) were cut-down to 26 cm, correct positioning of tracheal tube tip (~4 cm above the carina) was verified by chest radiography, and tracheal tube patency was confirmed by a ≤10 sec lasting bronchoscopy [1]. A 4.8 cm long circuit adapter with angled side arms (Smiths Medical International, Watford, UK) was introduced in-between the tracheal tube connector and the Y-piece of the ventilator breathing circuit. A rigid-wall catheter (Vygon, Ecouen, France; inner diameter 1.0 mm, outer diameter 2.0 mm) was passed through the side arm of the adapter and was used for the administration of TGI. The TGI catheter length was tailored to the placement of its tip at 0.5-1.0 cm beyond the tip of the tracheal tube.

Patients were sedated with midazolam and/or propofol to a Ramsay score of 6 and paralyzed with cis-atracurium. HFO was provided using a 3100B high frequency ventilator (Sensormedics, Yorba Linda, CA, USA). Patients were connected to the

high frequency ventilator and a 40 sec RM was performed by pressurizing the HFO breathing circuit at 40 cmH_2O with the oscillator piston off. We then resumed HFO and placed a 3-5 cmH_2O tracheal tube cuff leak. We returned mPaw to its pre-leak level by adjusting the mPaw valve, and mPaw was set 6-8 cmH_2O above its value during the preceding CMV [2]. Subsequently, we connected the TGI catheter to a variable-orifice O_2 flowmeter providing humidified O_2 at room temperature, and started TGI at a flow equal to 50% of the preceding CMV minute ventilation. TGI initiation caused a 1-2 cmH_2O rise in mPaw, which we reversed by adjusting the mPaw valve. FiO_2 was set at 1, oscillatory pressure amplitude (ΔP) was set at 65-90 cmH_2O (30 cmH_2O above the preceding CMV $PaCO_2$ value), and oscillation frequency (f) at 3.5-5.5 Hz. ΔP and f were further adjusted to achieve a target arterial pH of 7.20-7.45. The catheter used for TGI administration was removed when switching to the conventional ventilator.

Measurements

Patients underwent three assessments in every session: at baseline, after 8 hours of CMV and after 8 hours of HFO-TGI (Fig. 1a, Fig. E1, ESM). Each assessment lasted 10 to 15 minutes while the patient remained on CMV or HFO-TGI, respectively. EVLW, hemodynamic parameters, respiratory system mechanics, arterial and central venous blood gases and the cumulative fluid balance over the preceding 8 hours (8-hour fluid intake minus 8-hour fluid output) were documented for each assessment.

The single indicator transpulmonary thermodilution technique (PiCCOplus®, Pulsion Medical Systems, Munich, Germany) was used for EVLW measurement and hemodynamic monitoring. This technique correlates well with the gold standard gravimetric method in animals, as well as with the double indicator dilution technique

in humans for the estimation of EVLW [7]. Patients had a femoral arterial 5-F thermistor catheter and a central venous catheter in situ. For each set of hemodynamic measurements, 15 mL of 0.9% saline (<8 ^{o}C) were administered by central venous injection with an adequate thermodilution curve displayed on the monitor screen in duplicate and the mean values were included in the analysis [15]. Respiratory mechanics were assessed by the end-inspiratory and end-expiratory technique [1], always at CMV with square-wave inspiratory flow. Following HFO-TGI, respiratory mechanics were measured immediately after return to CMV (see also Fig. E1 in the ESM).

Statistical analysis

Statistical procedures were based on recommendations for analysis of crossover trials [16]. Non-parametric statistics were applied. Differences between group characteristics at baseline in quantitative and qualitative data were evaluated by the Mann-Whitney U-test and Fisher's exact test, respesctively. Within-group changes of variables were analyzed with Wilcoxon-matched paired test, when indicated. Correlations were determined by using the non-parametric Spearman's (Rs) test. The treatment effect of HFO-TGI (individual crossover difference between HFO-TGI and CMV) was analyzed by comparing the values at the end of each period with Wilcoxon-matched paired test with "HFO-TGI dependent" p-value (p_{hd}) indicating significance. The possibility of a carryover period, or other treatment effect was assessed by comparing the values of the differences at the end of each period between HF-1 and HF-2 groups with Mann-Whitney U signed rank sum test with "HFO-TGI independent" p-value (p_{hi}) indicating significance (Fig. 1). The false discovery rate procedure [17] revealed a corrected p-value of less than 0.03 to be significant for multiple comparisons. Statistical analyses were performed using SPSS Statistics

version 20 (SPSS Inc., Chicago, Illinois, USA) and "R" statistical software version 3.0 (R Foundation for Statistical Computing, Vienna, Austria). An *a priori* power analysis was performed with GPower 3.1 (Franz Faul, University of Kiel, Germany). The minimum sample size was calculated based on 90% power and a two-sided 0.05 significance level. The sample size capable of detecting a between-ventilatory techniques difference of 1 mL/kg was estimated for the decrease in EVLW indexed to PBW (EVLWI) using data from a previous human study [18]. The critical sample size was estimated to be 25 ventilation sessions. Data are presented as median (interquantile range) unless otherwise specified.

Results

Of 20 consecutive patients (15 males) assessed for eligibility, 12 patients (10 males) were enrolled in the study; [age 45.0 (31.3-47.5) years; body mass index 25.0 (23.0-26.9) kg/m^2; PBW 72.7 (66.8-80.2) kg; Simplified Acute Physiology Score II 35.0 (28.0-40.9)]. Of these patients eleven had primary (pulmonary) ARDS, and three patients had co-existing head trauma not associated with intracranial hypertension. The reasons for the exclusion of 8 patients were: hemodynamic instability requiring high-dose vasopressors (3 patients), traumatic brain injury with intracranial hypertension (1 patient), COPD diagnosis with documented chronic CO_2 retention (2 patients), suspected thromboembolic disease (1 patient), and echocardiographic evidence of severe cardiac dysfunction (1 patient). The time of mechanical ventilation prior to study enrollment was 108 (66-120) hours.

A total number of 32 ventilation sessions were administered. Sixteen sessions were assigned to the HF-1 group and 16 to the HF-2 group. Four patients received 4 sessions, and 8 patients received 2 sessions. There were no protocol related complications and all patients completed the study uneventfully. No significant

difference in baseline ventilation settings was evident between the two groups (Table 1). Ventilatory settings at each assessment time-point are shown in Fig. E1 in the ESM.

Gas exchange and lung mechanics

PaO_2/FiO_2 increased after using HFO-TGI compared with CMV (p_{hd} <0.001, Fig. 1b), while the oxygenation index decreased following HFO-TGI compared with CMV [15.1 (10.6-22.3) *versus* 19.1 (16.0-27.7), p_{hd} =0.029]. Respiratory system compliance was higher after HFO-TGI [36.8 (24.7-44.0) *versus* 31.7 (24.8-39.1) mL/cmH_2O, p_{hd} <0.001]. A lower $PaCO_2$ was maintained with HFO-TGI compared with CMV (42.5 (38.7-54.0) *versus* 46.3 (40.9-63.5) mmHg, p_{hd} =0.045], but this difference did not remain significant following correction for multiple comparisons and did not significantly affect the pH [7.38 (7.33-7.44) *versus* 7.36 (7.31-7.41), p_{hd} =0.166].

EVLWI and hemodynamic measurements

EVLWI was lower after 8 hours of HFO-TGI in comparison with CMV (p_{hd} =0.021, Fig. 2a). In 23/32 sessions EVLWI values were lower in HFO-TGI compared with CMV, while in 9/32 sessions EVLWI was higher in HFO-TGI (Fig. 2b) (mean crossover difference 1.25 mL/kg, or 10%).

Central venous oxygen saturation was higher in HFO-TGI (p_{hd} =0.001), while the pulmonary vascular permeability index (PVPI) was lower (p_{hd} =0.047), but this difference did not remain significant after correcting for multiple comparisons (Table 2). No significant difference between the two ventilatory techniques was detected in the rest of the hemodynamic parameters (Table 2). There was a negative correlation

between changes in EVLWI and changes in PaO_2/FiO_2 during HFO-TGI (Rs=-0.452, p=0.009) (Fig. 3).

Fluid balance and vasopressor support

There was no significant difference between the two groups regarding fluid balance or vasopressor support (Table 2).

Carryover effect

Carryover or treatment effect of the application of CMV on the result of HFO-TGI in HF-1 group and of the application of HFO-TGI on the result of CMV in HF-2 group was not significant for PaO_2/FiO_2 (Fig. 1b), EVLWI (Fig. 2a), respiratory system compliance, oxygenation index, $PaCO_2$, pulmonary vascular permeability index (PVPI) and hemodynamic parameters (Table 2). Therefore, it can be assumed that possible carryover period or treatment effect could not have affected the results.

Discussion

We have shown that HFO-TGI with interspersed RMs applied for 8 hours in patients with early ARDS resulted in improved gas exchange and lung mechanics and in reduced EVLW accumulation compared with CMV with RMs. Hemodynamic parameters including cardiac index and intrathoracic lung volume index (ITBVI) were not significantly different between the two ventilation strategies. These results are consistent with amelioration of pulmonary edema formation related to enhanced lung recruitment with HFO-TGI [1,6]. Figure 2b shows that although EVLWI was significantly lower following HFO-TGI/RM ventilation, this response was not uniform neither among patients nor within sessions of the same patient.

Lung recruitment may affect the EVLWI in different and opposing ways [15]; it can lower the EVLWI by collapsing the pulmonary small vessels [19] or reducing the cardiac output [20,21], whereas it may increase the EVLWI by increasing lung volume [22], redistributing the pulmonary blood flow [23], or elevating the central venous pressure [23]. During HFO-TGI, the pattern of lung recruitment is different. The continuous forward TGI flow exerts a PEEP-effect by impeding the opposite directed expiratory flow [1,2,6]. Scanographic data with HFO-TGI show preferential recruitment of the dependent, subcarinal lung regions without overdistention of the already aerated lung [4].

In a porcine ARDS model [20] it has been shown that the addition of PEEP and the use of small tidal volumes during CMV can attenuate lung injury and EVLW accumulation. According to other animal ARDS studies, avoidance of repeated opening and collapsing of affected alveoli with HFO, prevents ventilator induced inflammatory response, promotes effective tissue repair, and ameliorates alveolar and interstitial edema [24-27]. With the addition of TGI to HFO, we combined these lung protective approaches by minimizing tidal volume and enhancing lung recruitment in the lower and dependent lung areas [4]. An explanation for our findings based on a lung protective recruitment effect that attenuates permeability edema formation is also corroborated by the marginally lower value of the PVPI (Table 2), which is a marker of pulmonary vascular permeability in ARDS [10].

We applied RMs by using the 40/40 rule during CMV and HFO-TGI. Another physiologically sound approach could be to apply a stepwise incremental-decremental titration of the continuous distending pressure [28]. However, we cannot speculate on how this approach would have affected our results. Figure 3 shows the correlation between improvement in EVLWI and PaO_2/FiO_2 during HFO-TGI. This correlation has not been observed when RMs alone were administered in ARDS patients during

CMV [29]. If a sustained and less traumatic lung recruitment is the cause of EVLWI decrease, then the amount of recruitable lung tissue with HFO-TGI can explain this correlation. However, we do not have the scanographic data to further support this argument.

The protocol we use for HFO-TGI ventilation is totally different from the protocol used in the study by Ferguson et al. [30]. In this recent large randomized controlled trial showing worse outcomes with HFO in early ARDS, high mPaws were used [30]. This could potentially contribute to hemodynamic compromise by directly affecting right ventricular afterload, thus leading to an increased need for vasopressors in the HFO group and end organ failure [30,31]. The present study's combination of HFO-TGI with short lasting RMs and cuff leak was almost identical to the previously employed HFO-TGI/RMs protocol that resulted in substantial physiological benefit without hemodynamic compromise and improved survival when it was applied intermittently during lung-protective CMV, with a target of improved oxygenation [3]. This protocol was also successfully applied as rescue ventilation in patients with ARDS and traumatic brain injury [32]. Possible mechanisms contributing to hemodynamic stability during HFO-TGI include a) recruitment of the dependent lung units with the addition of TGI to HFO, which may decrease pulmonary vascular resistance and reduce the risk of right ventricular dysfunction [4,31] and b) enhanced CO_2 elimination with the use of TGI and cuff leak, hence further protecting right ventricular function [33]. Moreover the intermittent use of HFO-TGI may have prevented long term HFO-related adverse effects. Table 2 shows that parameters of right ventricular function such as the CVP were similar in the two ventilation strategies without a need for excess fluid volume administration or vasopressor dose escalation.

Study Limitations

The crossover 2x2 design without washout period introduces some limitations in the interpretation of data due to the possibility of a carry-over effect even if statistical tests to detect it are negative. This limitation applies, to some extent, to every crossover trial [34]. Longer HFO-TGI sessions could have resulted in a larger and more uniform reduction in EVLWI. Notably, in the prone position the time required to observe a significant reduction in EVLWI compared with supine CMV was 18 hours [35]. Although our physiological data do not indicate a potentially lasting effect of HFO-TGI, we have previously shown a progressively sustained improvement in oxygenation and lung mechanics with repetitive (for up to 10 days) HFO-TGI sessions [3].

Limitations of the single indicator thermodilution technique include the underestimation of EVLW in diseases that block the thermal indicator's passage through the lung, e.g., in massive pulmonary embolism, severe pulmonary edema, hypoxic pulmonary vasoconstriction, and lung resection [15]. In the present study, patients with lung resection and diagnosed or suspected pulmonary embolism were excluded, while all patients were on prophylactic anticoagulation.

Limitations of the long-term use of TGI are described elsewhere [3]. Right heart catheterization would have allowed measurement of pulmonary arterial and capillary wedge pressures, but attending physicians preferred the less invasive single indicator transpulmonary thermodilution technique for the hemodynamic monitoring of their ARDS patients.

Conclusions

Intermittent recruitment with the use of HFO-TGI improves lung mechanics and may reduce EVLW in patients with ARDS compared with protective CMV. This effect is associated with improved lung oxygenation.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Figure legends:

Figure 1

Study design and PaO_2/FiO_2 values. a) Crossover study design. After baseline assessment, patients of the first group (HF-1) initially underwent 8 hours of Conventional Mechanical Ventilation (CMV) followed by 8 hours of High Frequency Oscillatory (HFO) ventilation combined with Tracheal Gas Insufflation (TGI), whereas patients of the second group (HF-2) initially underwent 8 hours of HFO-TGI ventilation followed by 8 hours of CMV. b) HFO-TGI significantly increased PaO_2/FiO_2 in both periods of administration, compared with CMV. Data are presented as mean ± standard error. #: $p<0.05$ *versus* previous assessment value. The possibility of a carryover period or other treatment effect was assessed by comparing the values of the differences at the end of each period between HF-1 and HF-2 groups with Mann-Whitney U signed rank sum test (d minus a *versus* c minus b) and "HFO-TGI independent" p-value (p_{hi}) below 0.03 indicating significance. The HFO-TGI treatment effect (individual crossover difference between HFO-TGI and CMV) was analyzed by comparing the values at the end of each period (a plus b *versus* c plus d) with Wilcoxon-matched paired test and "HFO-TGI dependent" p-value (p_{hd}) of less than 0.03 indicating significance.

Figure 2

Results on extravascular lung water index (EVLWI). a) HFO-TGI combined with RMs decreased EVLWI in HF-2 whereas CMV had no effect on EVLWI in either group. Data are presented as mean ± standard error. #: $p<0.05$ *versus* previous assessment value. For further explanation please, see legend to Fig. 1.

b) Individual differences in EVLWI between the two ventilation strategies. Each symbol corresponds to one session and represents the difference in EVLWI after 8 hours of CMV minus EVLWI after 8 hours of HFO-TGI. Ventilation sessions in the

same patient are represented with the same kind of symbol. Symbols above the zero line correspond to sessions where EVLWI was lower following HFO-TGI (23/32). Those below the zero line correspond to sessions where EVLWI was lower following CMV (9/32).

Figure 3

Scatter plot for values of the change in extravascular lung water index (ΔEVLWI) and in PaO_2/FiO_2 ($\Delta PaO_2/FiO_2$) over the 8-hour HFO-TGI period. Regression equation (solid line) is shown (Rs=-0.452, p=0.009), i.e., there was a significant correlation between PaO_2/FiO_2 improvement and EVLWI drop during HFO-TGI. Each symbol corresponds to one session. Ventilation sessions in the same patient are represented with the same kind of symbol.

Table 1. Baseline ventilation settings and physiological measurements

	HF-1	HF-2
Sessions (n)	16	16
Tidal volume (mL/kg PBW)[a,b,c]	6.73 (6.01-7.27)	7.17 (6.22-7.76)
Respiratory rate (breaths/min)[b,c]	24 (24-28)	27 (23-31)
Minute ventilation (L/min)[b,c]	12.3 (11.6-15.3)	14.4 (12.1-15.3)
Inspiratory-to-expiratory time ratio[b,c]	1:2	1:2
Positive end-expiratory pressure (cmH_2O)[b,c]	12 (11-12)	12 (11-16)
FiO_2[b,c]	0.7 (0.7-0.8)	0.7 (0.7-0.9)
PaO_2/FiO_2 (mmHg)[b,c]	130 (94-187)	131 (90-181)
$PaCO_2$ (mmHg)[b,c]	42.3 (39.5-53.2)	39 (35.6-45.3)
Oxygenation index[b,c,d]	18.2 (10.3-22.3)	16.8 (10.5-18.7)
End-inspiratory plateau airway pressure (cmH_2O)[b,c]	28.5 (24-31)	26.5 (25.7-30.2)
Mean airway pressure (cmH_2O)[b,c]	20.0 (17.0-22.0)	19.5 (19.5-22.3)
Quasistatic respiratory system compliance (mL/cmH_2O)[b,c,e]	35.2 (21.5-45.2)	35.9 (27.9-38.7)
Sequential Organ Failure Assessment (SOFA) score[b]	10.0 (8.25-11.0)	10.5 (9.0-12.0)
Lung injury score[b]	3.00 (2.75-3.50)	2.88 (2.65-3.44)

Values are median (interquantile range) unless otherwise specified. HF-1, patients who initially underwent 8 hours of Conventional Mechanical Ventilation (CMV) followed by 8 hours of High Frequency Oscillatory (HFO) ventilation combined with Tracheal Gas Insufflation (TGI); HF-2, patients who initially underwent 8 hours of HFO-TGI followed by 8 hours of CMV; PBW, Predicted Body Weight.

[a], For males PBW was calculated as 50 + [height (cm)-152.4] x 0.91; for females as 45.5 + [height (cm)-152.4] x 0.91.

[b], Determined/calculated prior to the initiation of each ventilation session.

[c], Determined on volume assist-control mode with square-wave inspiratory flow.

[d], Calculated as mean airway pressure divided by the PaO_2/FiO_2 x 100.

[e], Calculated as tidal volume divided by the difference between the end-inspiratory and end-expiratory plateau airway pressure.

Table 2: Hemodynamic parameters

Parameter		Baseline	8 hours	16 hours	p_{hd}	p_{hi}
MAP (mmHg)	HF-1	85.5 (75.8-98.0)	84.5 (78.5-92.5)	85.6 (81.8-93.8)	0.140	0.669
	HF-2	81.0 (72.5-93.3)	83.8 (78.2-94.5)	80.0 (72.5-93.2)		
HR (beats/min)	HF-1	100.5 (81.0-113.7)	101.5 (84.8-112.8)	109.0 (81.0-119.5)	0.266	0.361
	HF-2	100.0 (81.0-113.0)	98 (75.0-112.5)	101.5 (81.8-112.5)		
CI ($L/min/m^2$)	HF-1	4.17 (2.72-6.64)	4.22 (3.51-5.48)	3.96 (3.22-6.17)	0.144	0.239
	HF-2	4.44 (2.70-5.69)	4.42 (3.44-5.23)	4.82 (3.47-5.92)		
SVI (mL/m^2)	HF-1	46.9 (33.5-55.5)	47.3 (32.3-50.5)	43.0 (28.5-62.6)	0.164	0.445
	HF-2	49.9 (30.3-60.4)	45.3 (33.6-58.0)	48.3 (32.3-67.7)		
$ScvO_2$ (%)	HF-1	78.3 (64.9-81.6)	73.0 (58.5-82.4)	82.4 (76.8-88.3)	0.001	0.402
	HF-2	76.6 (67.5-86.1)	83.2 (74.3-90.4)	75.4 (70.5-82.5)		
GEDVI (mL/m^2)	HF-1	726 (621-787)	690 (570-852)	748 (551-858)	0.410	0.892
	HF-2	767 (624-867)	759 (615-916)	831 (661-971)		
ITBVI (mL/m^2)	HF-1	908 (776-983)	862 (712-1065)	935 (689-1073)	0.399	0.874
	HF-2	959 (780-1083)	948 (768-1144)	1038 (826-1213)		
PVPI	HF-1	2.3 (1.9-2.8)	2.5 (2.1-3.6)	2.7 (2.0-3.2)	0.047	0.417

	HF-2	2.9 (2.5-3.3)	2.2 (1.8-2.8)	2.8 (2.1-3.2)		
CFI (min^{-1})	HF-1	6.4 (5.1-8.8)	6.9 (4.5-7.9)	6.8 (4.7-8.2)	0.056	0.247
	HF-2	5.8 (4.1-7.6)	5.7 (3.5-7.4)	6.4 (4.7-7.6)		
SVRI (dyn x sec x cm^{-5}x m^2)	HF-1	1428 (958-1991)	1270 (1015-1788)	1374 (1191-1965)	0.210	0.780
	HF-2	1315 (866-1875)	1398 (1143-2077)	1246 (886-1771)		
CVP (mmHg)	HF-1	11.0 (8.0-15.5)	9.5 (8.0-14.5)	11.0 (7.3-13.8)	0.113	0.224
	HF-2	11.5 (10.0-16.7)	11.5 (10.3-16.0)	11.5 (7.3-15.0)		
Noradrenaline infusion (µg/kg/min)	HF-1	0.07 (0.05-0.19)	0.12 (0.04-0.25)	0.14 (0.05-0.26)	0.360	0.188
	HF-2	0.13 (0.05-0.24)	0.11 (0.06-0.32)	0.19 (0.06-0.34)		
Fluid balance (mL)*	HF-1	385 (55-858)	395 (116-1008)	185 (-337-1025)	0.633	0.260
	HF-2	435 (78-1030)	385 (148-1045)	375 (100-1060)		

Values are median (interquantile range). HF-1, patients initially underwent 8 hours of Conventional Mechanical Ventilation (CMV) followed by 8 hours of High Frequency Oscillatory (HFO) ventilation combined with Tracheal Gas Insufflation (TGI); HF-2, patients initially underwent 8 hours of HFO-TGI followed by 8 hours of CMV; MAP, mean arterial pressure; HR, heart rate; CI, cardiac index; SVI, stroke volume index; $ScvO_2$, central venous oxygen saturation; GEDVI, global end diastolic volume index; ITBVI, intrathoracic blood volume index; PVPI,pulmonary vascular permeability index; CFI, cardiac function index; SVRI, systemic vascular resistance index; CVP, central venous pressure.

"HFO-TGI dependent" p values (p_{hd}) <0.03 indicate effects of HFO-TGI administration. "HFO-TGI independent" p values (p_{hi}) <0.03 indicate HFO-TGI independent effects.

* Fluid balance refers to the 8-hour period of HFO-TGI or CMV ventilation. Baseline fluid balance refers to the 8-hour period preceding baseline measurements.

ELECTRONIC SUPPLEMENTARY MATERIAL

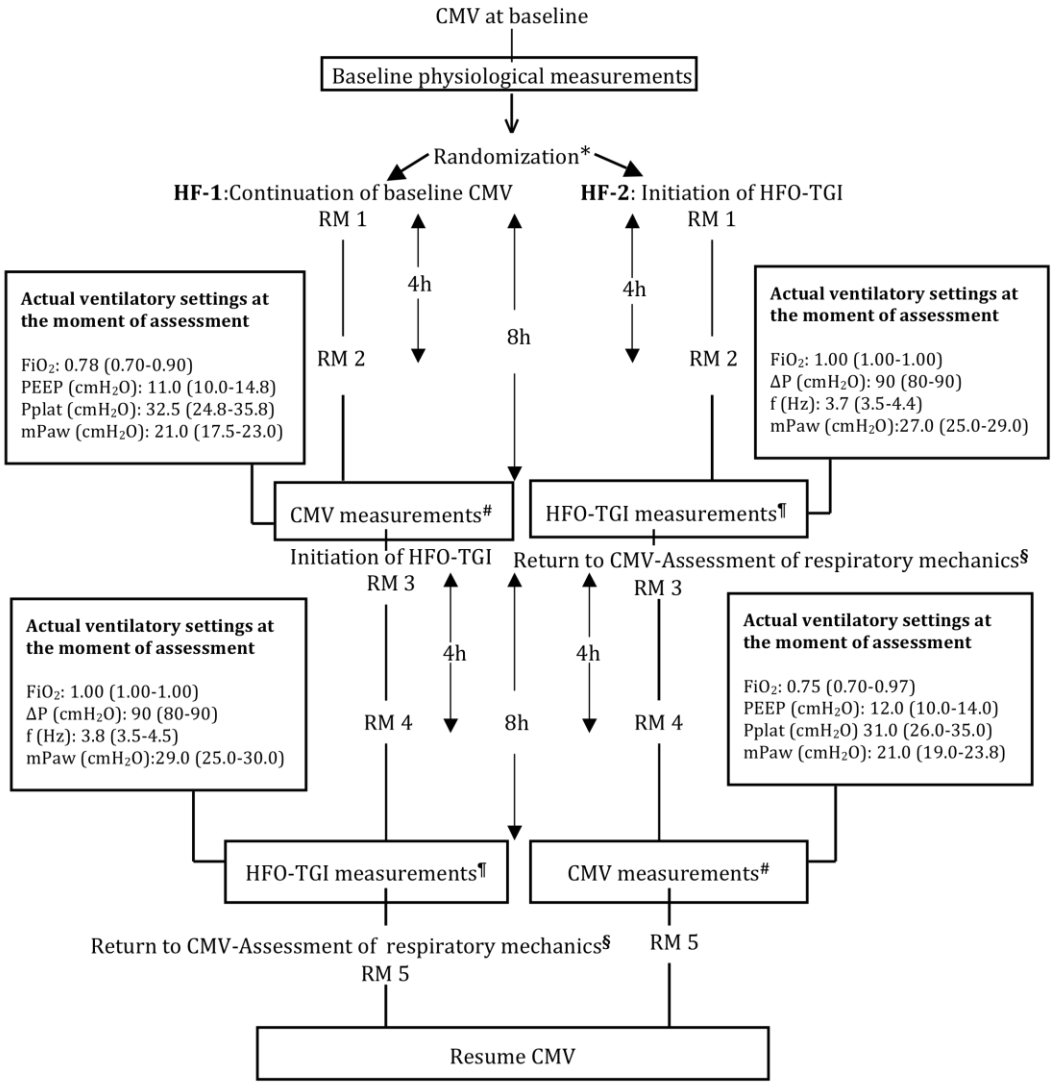

Figure E1

Schematic presentation of the study protocol. During high frequency oscillation with tracheal gas insufflation (HFO-TGI), recruitment meneuvers (RMs) were performed with TGI turned off and the tracheal tube cuff inflated. Time-points of physiological measurements and actual ventilatory settings at the moments of assessment are in boxes. Data are presented as median (interquantile range).

***** For each patient odd number sessions (1st and when applicable 3rd) were randomly assigned to HF-1 or HF-2 groups. Even numbered sessions (2nd and when applicable 4th) were assigned to the opposite group from the preceding session.

[#] Physiological measurements were performed after the end of the 8-hour treatment period and required 10-15 minutes, during which the patient was still on CMV ventilation.

[¶] Physiological measurements were performed after the end of the 8-hour treatment period and required 10-15 minutes, during which the patient was still on HFO-TGI ventilation.

[§] Respiratory mechanics were assessed 1-5 minutes after return to CMV.

CMV, conventional mechanical ventilation; FiO_2, fraction of inspired oxygen; PEEP, positive end-expiratory pressure; Pplat, end expiratory (plateau) airway pressure; mPaw, mean airway pressure; ΔP, oscillatory pressure amplitude; f, oscillation frequency.

Table 1. Baseline ventilation settings and physiological measurements

	HF-1	HF-2
Sessions (n)	16	16
Tidal volume (mL/kg PBW)[a,b,c]	6.73 (6.01-7.27)	7.17 (6.22-7.76)
Respiratory rate (breaths/min)[b,c]	24 (24-28)	27 (23-31)
Minute ventilation (L/min)[b,c]	12.3 (11.6-15.3)	14.4 (12.1-15.3)
Inspiratory-to-expiratory time ratio[b,c]	1:2	1:2
Positive end-expiratory pressure (cmH_2O)[b,c]	12 (11-12)	12 (11-16)
FiO_2[b,c]	0.7 (0.7-0.8)	0.7 (0.7-0.9)
PaO_2/FiO_2 (mmHg)[b,c]	130 (94-187)	131 (90-181)
$PaCO_2$ (mmHg)[b,c]	42.3 (39.5-53.2)	39 (35.6-45.3)
Oxygenation index[b,c,d]	18.2 (10.3-22.3)	16.8 (10.5-18.7)
End-inspiratory plateau airway pressure (cmH_2O)[b,c]	28.5 (24-31)	26.5 (25.7-30.2)
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Table(s)

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"HFO-TGI dependent" p values (p_{hd}) <0.03 indicate effects of HFO-TGI administration. "HFO-TGI independent" p values (p_{hi}) <0.03 indicate HFO-TGI independent effects.

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Figure(s)

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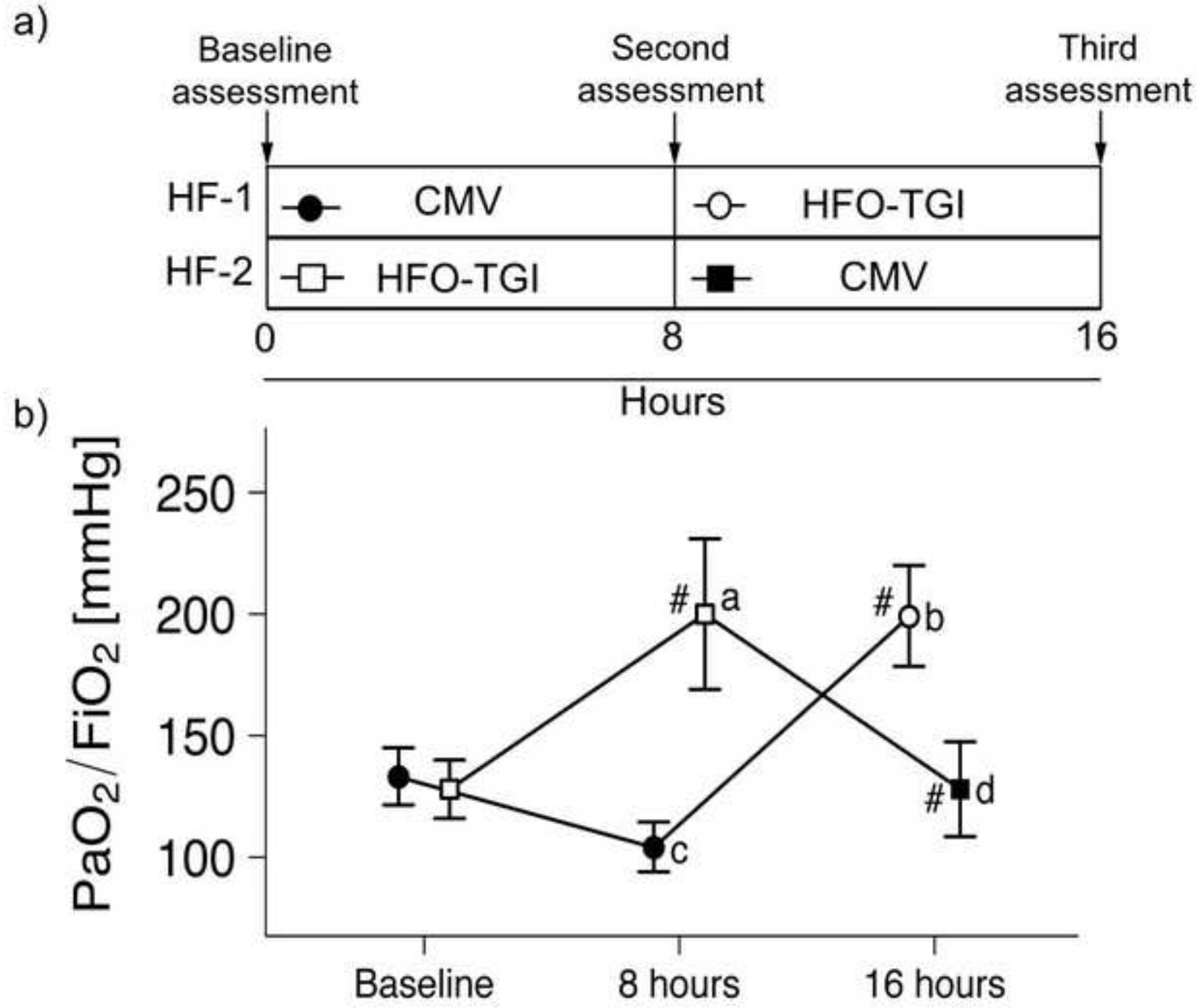

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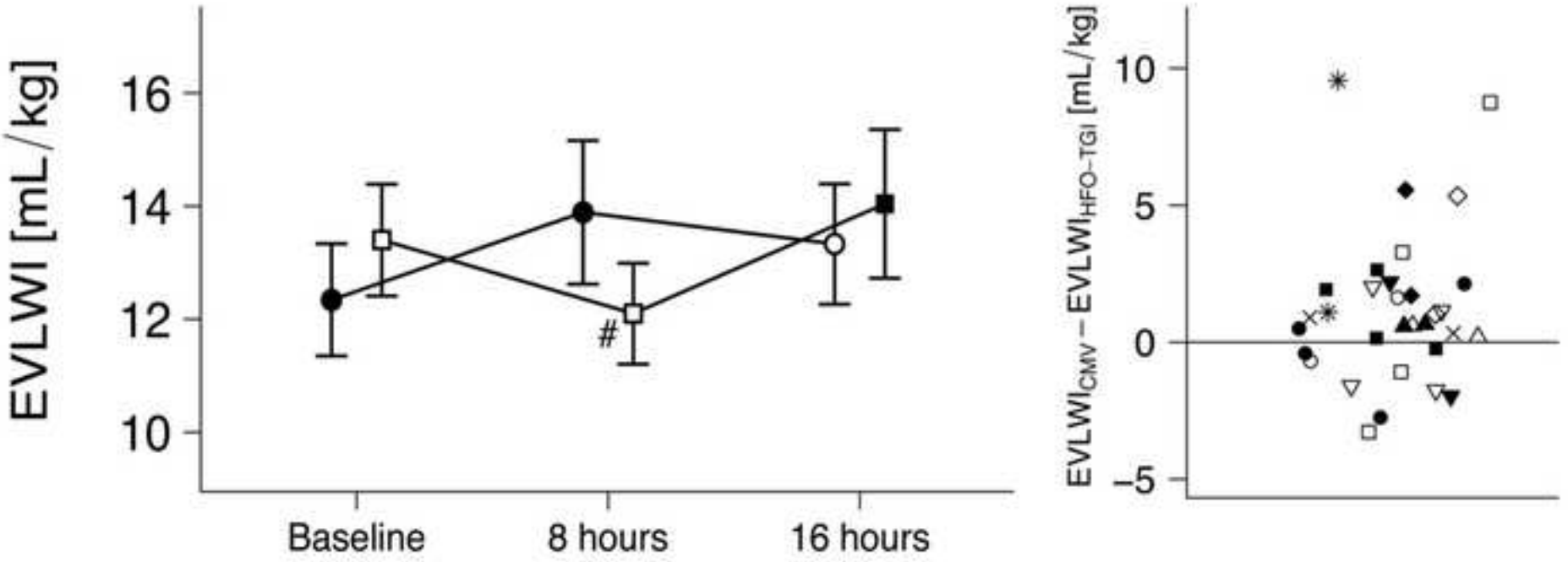

Figure(s)

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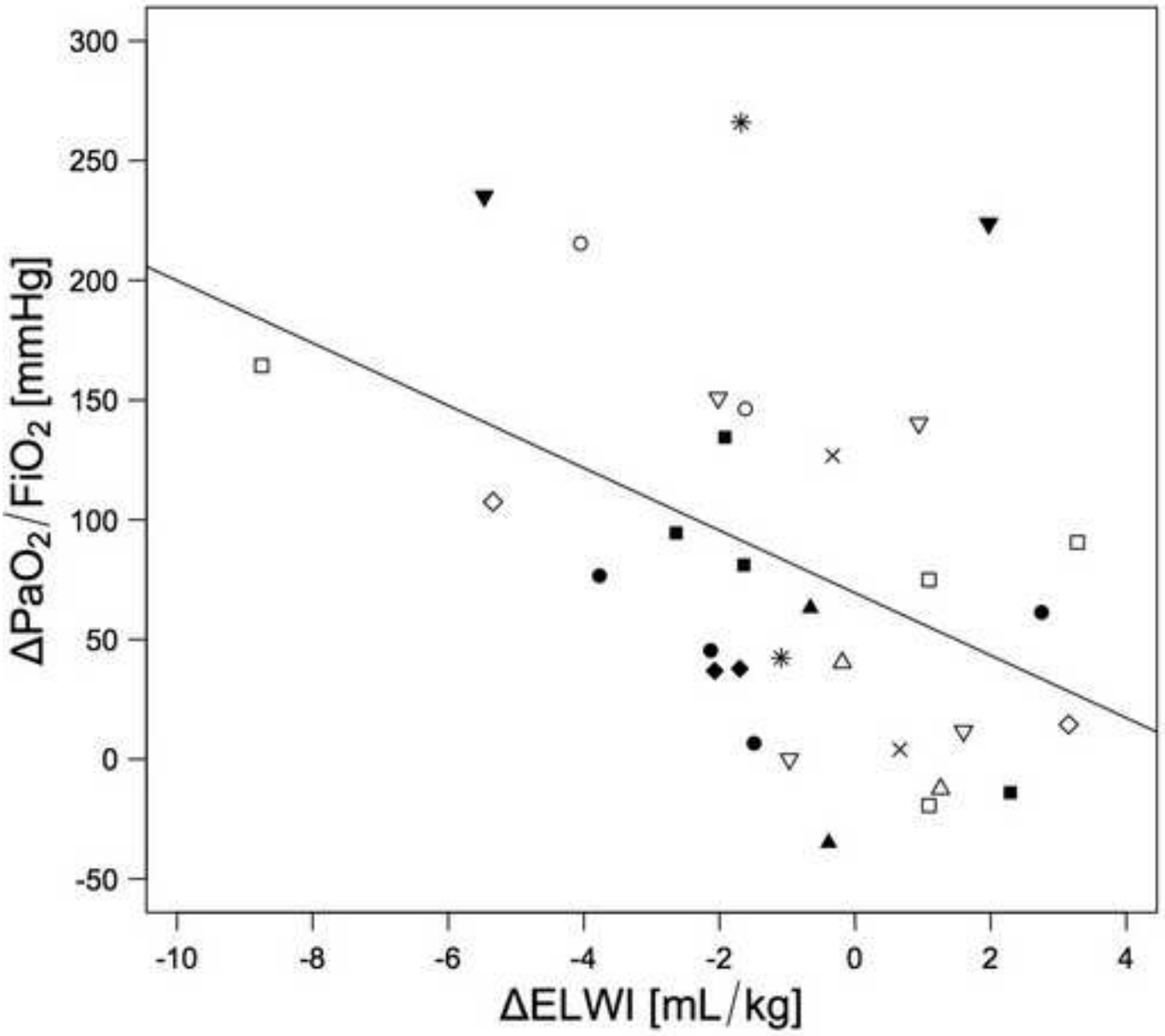

Response to reviewers

We would like to thank the reviewers for carefully reading the manuscript and providing insightful comments. Our detailed responses to the reviewers' comments follow below.

Abbreviations: ESM=electronic supplementary material.; HFO= high-frequency oscillation; TGI=tracheal gas insufflation; CMV=conventional mechanical ventilation. Revision-related text changes are highlighted in red script.

Reviewers' comments:

Reviewer 1:
Specific comments
#1. Throughout the manuscript : the proper timing of the measurements should be better defined: "following" is used at many places, but the methods section refers to the end of the treatment period. The figures seem to indicate they were performed shortly (?) after the end of the treatment period.
Response: Besides baseline, there were two time points of physiologic measurements in each ventilation session (Figure E1). One after 8 hours of CMV ventilation and one after 8 hours of HFO-TGI ventilation. These measurements were taken while the patient was still on CMV or HFO-TGI ventilation respectively, and lasted 10-15 minutes. Since we could not assess respiratory mechanics on HFO-TGI ventilation, this assessment was performed within 5 minutes after return to CMV. The timing of each measurement is now better defined as suggested by the reviewer:
- The last sentence of the Materials and Methods paragraph in the Abstract now reads: "Arterial/central venous blood gas analysis and measurement of hemodynamic parameters and EVLW were performed at baseline and after each 8-hour period using the single-indicator thermodilution technique."
- In the main manuscript, the first sentence of the *Measurements* paragraph in the Patients and Methods section now reads:"Patients underwent three assessments in every session: at baseline, after 8 hours of CMV and after 8 hours of HFO-TGI (Fig. 1a, Fig. E1, ESM). Each assessment lasted 10 to 15 minutes, while the patient was still on CMV or HFO-TGI ventilation respectively"
-The phrasing in the results section has also been changed to "after using HFO-TGI" and "after 8 hours of HFO-TGI" instead of "following HFO-TGI".
-The legend to Figure 2b now reads: "Individual differences in EVLWI between the two ventilation strategies. Each symbol corresponds to one session and represents the difference in EVLWI after 8 hours of CMV minus EVLWI after 8 hours of HFO-TGI…." and in figure 3 "Scatter plot for values of the change in extravascular lung water index (ΔEVLWI) and in PaO_2/FiO_2 ($\Delta PaO_2/FiO_2$) over the 8-hour HFO-TGI period…."
-In the ESM the duration of each assessment is now denoted with symbols:

Physiological measurements were performed after the end of the 8-hour treatment period and required 10-15 minutes during which the patient was still on CMV ventilation.
¶ Physiological measurements were performed after the end of the 8-hour treatment period and required 10-15 minutes during which the patient was still on HFO-TGI ventilation.
§ Respiratory mechanics were assessed 1-5 minutes after return to CMV.

#2. The summary should indicate the method of measuring EVLW (PiCCO).
Response: The requested information has been added to the last sentence of the *Materials and Methods* paragraph of the Abstract which now reads: "Arterial/central venous blood gas analysis and measurement of hemodynamic parameters and EVLW were performed at baseline and after each 8-hour period using the single-indicator thermodilution technique".

#3. Patients and methods : ejection fraction : I suppose it is left ventricular (it should be mentioned).
Response : This is correct, thank you. "Left ventricular ejection fraction" is now mentioned in the Patients and Methods section.

#4. Results : "SAPS scores were enrolled": some words are missing
Response: The beginning of the first paragraph of the Results section now reads: "Of 20 consecutive patients (15 males) assessed for eligibility, 12 patients (10 males) were enrolled in the study; [age 45.0 (31.3-47.5) years; body mass index 25.0 (23.0-26.9) kg/m^2; predicted body weight 72.7 (66.8-80.2) kg; Simplified Acute Physiology Score II 35.0 (28.0-40.9)]."

#5. Table : how was the fluid balance recorded ?
Response: Fluid balance (i.e. fluid intake minus fluid output comprising urine and any drain output if present) is documented hourly by the nursing staff in the ICU. The reported values refer to the cumulative 8-hour fluid balance at each assessment point. We have added this information in the Patients and Methods section, at the end of the first paragraph of *Measurements* which now reads as follows:
"EVLW, hemodynamic parameters, respiratory system mechanics, arterial and central venous blood gases and the cumulative fluid balance over the preceding 8 hours (8-hour fluid intake minus 8-hour fluid output) were documented for each assessment."
The legend to Table 2 now includes the statement: " * Fluid balance refers to the 8-hour period of HFO-TGI or CMV ventilation. Baseline fluid balance refers to the 8-hour period preceding the baseline measurements."

Minor comments
1. The P/F ratio does not need a decimal
2. GVEDVI and ITBVI : the "V" are missing
3. FiO2 could be abbreviated
Response:
1. We omitted the decimal from the P/F ratio values in Table1.

2. Global End Diastolic Volume and Intra Thoracic Blood Volume Index are now abbreviated GEDVI and ITBVI respectively in the main manuscript and Table 2.
3. We abbreviated FiO_2 throughout the main manuscript, Tables and Figures.

Reviewer 2:

#1: The length of time each patient was exposed to HFO is rather short and thus differences between the two groups although interesting is not necessarily indicative of any lasting affect. This should probably be discussed in the "limitations" section.

Response: We now address this point in the limitations section as follows: "Longer HFO-TGI sessions could have resulted in a larger and more uniform reduction in EVLWI. Notably, in the prone position the time required to observe a significant reduction in EVLWI compared to CMV was 18 hours [35]. Although our physiological data do not describe a potentially lasting effect of HFO-TGI, we have previously shown a progressively sustained improvement in oxygenation and lung mechanics with repetitive (for up to 10 days) HFO-TGI sessions [3]."

#2: Statistical analysis of the data: I don't know how well the Wilcoxon-matched paired test, although recommended given the lack of washout-period, truly compensates for this absence. More concerning, however, is that some of the patients had multiperiod crossover trials. It is my understanding that for patients who receive multiperiod treatments (ABAB, for example-where A is the first treatment and B is the second), their data should be analyzed differently than those who undergo the standard AB treatment (1. Jones B, Kenward MG 2nd edition, Boca Raton: Chapman & Hall/CRC; 2003. Design and analysis of cross-over trials).

Response:

Regarding the choice of test: Given the absence of a wash-out period, the analysis proposed by Tudor and Koch (Review of nonparametric methods for the analysis of cross-over trials, Statistical Methods in Medical Research (1994), 3:345-381) using the Wilcoxon matched paired test is recommended, and does not suffer from the disadvantages of preliminary testing for carry-over (Jones-Kenward, Design and analysis of cross-over trials (2003), CRC, §2.12.6). However, we do not claim that the method of analysis itself *compensates* for the lack of wash-out period. The absence of a carry-over effect is an assumption based on the fact that measurements were taken 8h after the cross-over and, to some extent, corroborated by the negative testing for carry-over. A different design with four groups (AA/BB/AB/BA) would have allowed a better estimation of the difference in treatment effect, if a carry-over effect were present. On the other hand, the analysis based on the design we have followed is more robust to departures from normality, as well as assumptions on the covariance structure of the variables (Jones & Kenward §5.7.2).

We have added the following comment to the limitations section: "The crossover 2x2 design without a washout period introduces some limitations in the interpretation of the data due to the possibility of a carry-over effect even if statistical tests to detect it are negative. This limitation applies, to some extent, to any crossover trial [34]." The book by Jones & Kenward suggested by the referee has also been added to the references.

Regarding the analysis of patients with multiperiod treatments: What we actually did was randomizing the patients again to a treatment group if they were eligible to receive more than two sessions, and treating the subsequent sessions as new cases. This is described in the first paragraph of the *Protocol* section, as well as in the Legend to Figure E1 (supplementary material). Naturally, this may raise some concerns regarding the interpretation of the results, as we may no longer assume that different cases come from independent observations. However, it is not hard to check that, under mild assumptions, cross-over differences over different sessions of the same patient are uncorrelated (e.g. a uniform covariance structure would suffice).

In principle, we could also assume that all patients underwent multiperiod sessions, but for some of them data from the last periods are missing. We could then proceed with a Bayesian analysis (as in Jones & Kenward §2.9) that can handle missing data effectively. However, this would require a different set of assumptions, and it would make the analysis much more technical.

Figure 2b and Figure 3 essentially depict our raw data on EVLWI. Each symbol corresponds to one session and represents the difference in EVLWI at the end of CMV minus EVLWI at the end of HFO-TGI. Ventilation sessions of the same patient are represented by the same kind of symbol. One reason for providing this detailed information is to show that patients with four sessions (□, ▽, ■ and ●) had variable responses to HFO-TGI, and they do not seem to introduce a bias in the interpretation of the results.

#3. More information regarding the study subjects, specifically: A. Had all the patients diagnosed with ARDS within the preceding 96H of study entry been mechanically ventilated during those 96H (your separating these two criteria imply they may not have been). B. What does "volume loading" mean? How much volume did each patient actually receive prior to the initiation of vasopressors? C. How did you define "chronic therapy"? Was this just regular use of albuterol or did this also mean patients who are on mono-therapy with one agent in addition to albuterol or patients who are on LABA/ICS and albuterol or patents who are on LABA/ICS, LAMA with albuterol?

Response: A. Patients included in the study were diagnosed with ARDS within the preceding 96 hours of study entry but they might have been ventilated for a longer period of time. Pre-enrollment ventilation duration was variable, and we added this information to the first paragraph of the Results section: "The time of mechanical ventilation prior to study enrollment was 108 (66-120) hours." B. Patients with low blood pressure received 20-30 ml/Kg bolus crystalloid targeting a CVP of 12 prior to the initiation of noradrenaline. In the Patients and Methods section, the first paragraph of *Study Subjects* regarding hemodynamic instability as exclusion criterion now reads: "2) severe hemodynamic instability (systolic arterial pressure <90 mmHg despite volume loading with up to 30 mL/kg crystalloid targeting a central venous pressure (CVP) of 12 mmHg and norepinephrine infusion ≥0.5 μg/kg/min)". C. We defined chronic therapy for COPD/asthma patients as chronic corticosteroid therapy of any kind (inhaled or oral). These eligibility criteria were also applied in *Mentzelopoulos SD, Malachias S, Zintzaras E, et al: Intermittent recruitment with high-frequency oscillation/tracheal gas insufflation in acute respiratory distress syndrome. Eur*

*Respir J 2012; 39:635–647.*Exclusion of COPD patients is now defined as: "significant chronic obstructive pulmonary disease (COPD) or asthma (previous hospital admissions for COPD/asthma, chronic corticosteroid therapy, oral or inhaled, for COPD/asthma, and/or documented chronic CO_2 retention with baseline $PaCO_2$ >45 mmHg);"

#4. There are a few grammatical errors (which I will leave to the editors) and a couple word-choice errors: A) I think you would prefer to write "bronchoscopy" instead of "endoscopy'" on page 6. B) I think you mean barotrauma instead of biotrauma on page 12.
Response: We did our best to eliminate grammatical errors. A) we changed "endoscopy" to "bronchoscopy" as suggested by the referee in the last paragraph of page 6 which now reads: "correct positioning of tracheal tube tip (~4 cm above the carina) was verified by chest radiography, and tracheal tube patency was confirmed by a ≤10 sec lasting bronchoscopy." B) At this point we were actually referring to biotrauma, so the phrasing has now been changed to better convey the message that: "avoidance of repeated opening and collapsing of affected alveoli with HFO, prevents ventilator induced inflammatory response, promotes effective tissue repair, and ameliorates alveolar and interstitial edema."

Reviewer 3:
#1: Unfortunately, in the rationale of the study and in the discussion, the investigators fail to mention the recent study of Ferguson et al (N Engl J Med. 2013 Feb 28;368(9):795-805) where it was reported that high-frequency oscillatory ventilation may increase in-hospital mortality of patients with ARDS. In other words, the investigators must strongly defend the role - if any - of HFO-TGI in clinical practice. By not providing a robust defence of potential uses of HFO-TGI then the current study could be interpreted by a potential reader as an academic exercise.
Response: We would like to thank the reviewer for giving us the opportunity to elaborate. The protocol we use for HFO-TGI ventilation is totally different than the protocol used in the study by Ferguson et al. In the recent large randomized control trial by Ferguson et al. showing worse outcomes with HFO in early ARDS, high mPaws were used. This could potentially contribute to hemodynamic compromise by directly affecting right ventricular afterload and end organ failure, thus leading to an increased need for vasopressors in the HFO group [Guervilly et al. Crit Care Med 2012; 40(5):1539-45, Ferguson et al. N Engl J Med 2013; 368:795-805]. The present study's combination of HFO-TGI with short lasting RMs and cuff leak was almost identical to the previously employed HFO-TGI/RMs protocol that resulted in substantial physiological benefit without hemodynamic compromise and improved survival when it was applied intermittently during lung protective CMV and targeting improved oxygenation [Mentzelopoulos et al. Eur Respir J 2012; 39:635–647]. This protocol was also successfully applied as rescue ventilation in patients with ARDS and traumatic brain injury and succeeded to control $PaCO_2$ [Vrettou et al. Crit Care 2013; 17(4):R136]. Possible mechanisms contributing to hemodynamic stability during HFO-TGI include a) recruitment of the dependent lung units with the addition of TGI to HFO, which might decrease pulmonary vascular resistance and thus

reduce the risk of right ventricular dysfunction [Mentzelopoulos et al. Intensive Care Med 2011; 37:990-999, Guervilly et al. Crit Care Med 2012; 40(5):1539-45], and b) enhanced CO_2 elimination with the use of TGI and cuff leak, hence further protecting right ventricular function [Mekontso et al. Intensive Care Med 2009; 35(11): 1850-8]. Moreover the intermittent use of HFO-TGI may have prevented long term HFO-related adverse effects.
The last paragraph of the Discussion section now reads as follows:

"The protocol we use for HFO-TGI ventilation is totally different from the protocol used in the study by Ferguson et al. [30]. In this recent large randomized controlled trial showing worse outcomes with HFO in early ARDS, high mPaws were used [30]. This could potentially contribute to hemodynamic compromise by directly affecting right ventricular afterload, thus leading to an increased need for vasopressors in the HFO group and end organ failure [30,31]. The present study's combination of HFO-TGI with short lasting RMs and cuff leak was almost identical to the previously employed HFO-TGI/RMs protocol that resulted in substantial physiological benefit without hemodynamic compromise and improved survival when it was applied intermittently during lung-protective CMV, with a target of improved oxygenation [3]. This protocol was also successfully applied as rescue ventilation in patients with ARDS and traumatic brain injury [32]. Possible mechanisms contributing to hemodynamic stability during HFO-TGI include a) recruitment of the dependent lung units with the addition of TGI to HFO, which may decrease pulmonary vascular resistance and reduce the risk of right ventricular dysfunction [4,31], and b) enhanced CO_2 elimination with the use of TGI and cuff leak, hence further protecting right ventricular function [33]. Moreover the intermittent use of HFO-TGI may have prevented long term HFO-related adverse effects. Table 2 shows that parameters of right ventricular function such as the CVP were similar in the two ventilation strategies without a need for excess fluid volume administration or vasopressor dose escalation."

Specific Comments
#1: Which criteria were used to define/identify the presence of ARDS?
Response: We have followed the American-European Conference definition of ARDS (1994) that was the broadly used definition at the time of study design. We have added the appropriate reference in the main manuscript [16].

#2: The investigators must better stress/discuss that in one third of the HFO-TGI sessions extravascular lung water were not lower with HFO-TGI as compared with conventional mechanical ventilation.
Response: We now stress this point in the legend to Figure 2 " … Those below the zero line correspond to sessions where EVLWI was lower following CMV (9/32)" and in the end of the first paragraph of the Discussion section: "Figure 2b shows that although EVLWI was significantly lower following HFO-TGI/RM ventilation, this response was not uniform neither among patients nor within sessions of the same patient." The addition we made to the limitations section, requested by Reviewer 2 is also relevant: "Longer HFO-TGI sessions could have resulted in a larger and more uniform reduction in EVLWI. Notably, in the prone

position the time required to observe a significant reduction in EVLWI compared to CMV was 18 hours [35]. "

Reviewer 4:

#1: The authors have used the so-called 40/40 rule as their recruitment manoeuvre for HFOV. However, from a physiological perspective this is not the most optimal method. Although time-consuming, a stepwise incremental-decremental titration of the CDP allows for a better lung volume, better oxygenation and oscillation on the deflation limb of the pressure volume loop. Do the authors think their results would have been different if they would have used such an approach?

Response: To the best of our knowledge, the optimal method for lung recruitment has not yet been established. However, the method you suggest is obviously physiologically sound. Nevertheless, we cannot speculate on the effect of such a method on our results. Therefore, we added in the 4th paragraph of the Discussion section the following:

"We applied RMs by using the 40/40 rule during CMV and HFO-TGI. Another physiologically sound approach could be to apply a stepwise incremental-decremental titration of the continuous distending pressure [29]. However, we cannot speculate on how this approach would have affected our results."

#2: After their recruitment on HFOV, the authors turned down the CDP to approximately 6 - 8 cmH_2O above the mean airway pressure during conventional mechanical ventilation. Such an approach does not make sense to me. Can the authors elaborate on why they did this? One can speculate that such an approach would lead to serious derecruitment.

Response: We adopted this approach based on physiological measurements obtained in previously published studies published by our group in patients with ARDS of similar severity. Indeed, following a recruitment meneuver, we performed inspiratory and expiratory pressure-volume curves and found that the point of maximal curvature (PMC) of the expiratory limb had mean value 25-26 cmH_2O [Mentzelopoulos SD et al. Crit Care Med 2007; 35:1500–1508]. Therefore, in the current study by setting the mPaw during HFO-TGI at approximately 6-8 cmH_2O above the mPaw of the preceding CMV, we expected to have a mPaw at the level or slightly above the PMC where lung recruitment is maintained. Indeed, mPaw during HFO-TGI in the current study was 27-29 cmH_2O, a pressure rather unlikely to lead to serious derecruitment.

#3a: Regarding Figure 2, can the authors explain why there was no effect on extravascular lung water at 16 hours in patients who crossed over from CMV to HFOV?

Response: Statistical analysis of a 2X2 cross-over trial involves the cross-over differences (EVLWI in HFO-TGI - EVLWI in CMV) in both groups. Restricting the analysis in group HF-1 does not necessarily yield statistically significant results, possibly due to the small sample size.

#3b: Am I right by interpreting that the effect of HFOV+TI is only short-lived?

Response: Indeed, the effect of HFO-TGI ventilation on EVLW may be short-lived. In fact, we have implicitly assumed the short lived effect of a single, 8-hour

HFO-TGI period in our study design, when we assumed that 8-hour intervals would suffice to minimize the carry-over effect of the previous treatment. However, repetitive periods of HFO-TGI have previously resulted in a progressively sustained respiratory physiological benefit [3]. We have added a relevant comment to the first paragraph of the Limitations section: "Although our physiological data do not indicate a potentially lasting effect of HFO-TGI, we have shown a progressively sustained improvement in oxygenation and lung mechanics with repetitive (for up to 10 days) HFO-TGI sessions [3]."

Kind regards,

CS Vrettou, SG Zakynthinos, S Malachias, SD Mentzelopoulos.

ELECTRONIC SUPPLEMENTARY MATERIAL

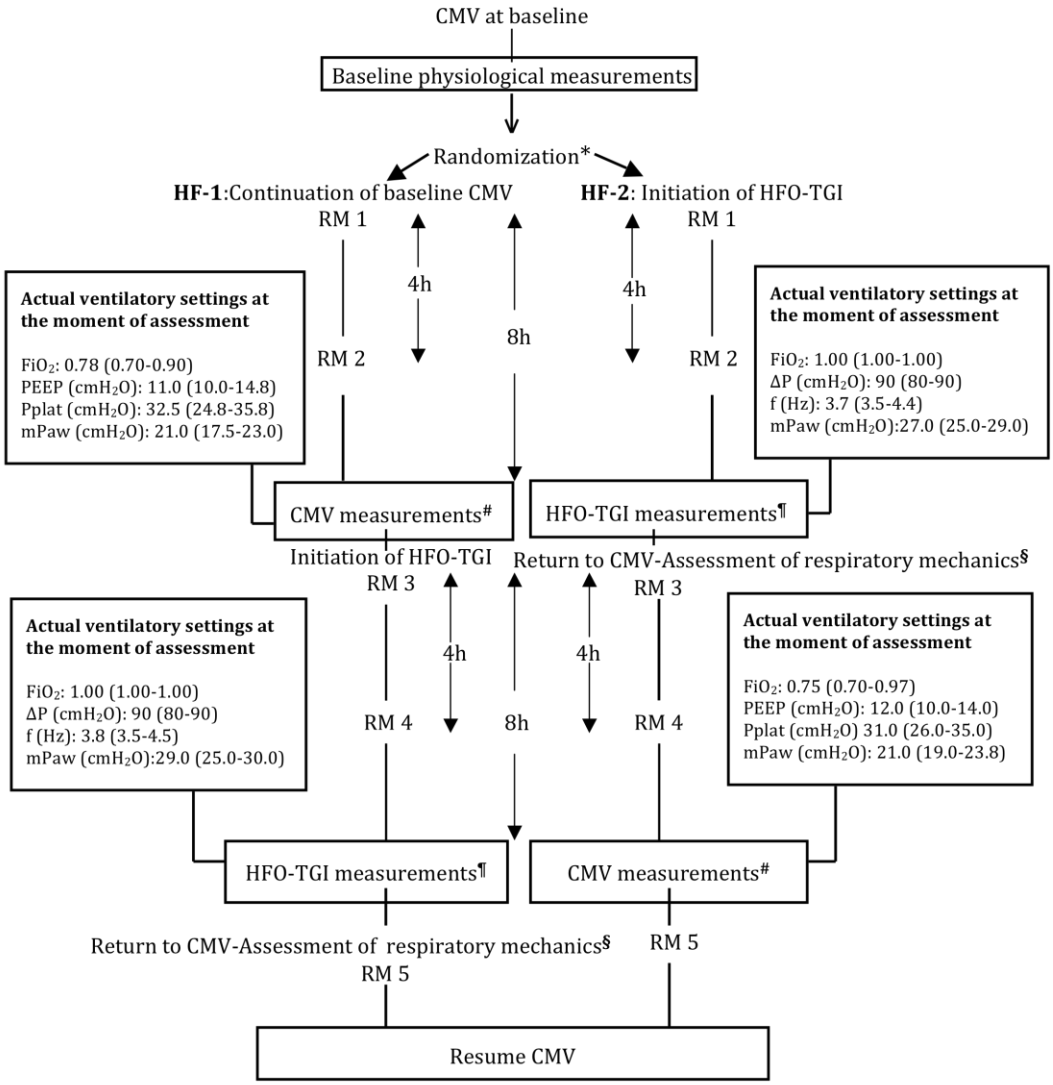

Figure E1

Schematic presentation of the study protocol. During high frequency oscillation with tracheal gas insufflation (HFO-TGI), recruitment meneuvers (RMs) were performed with TGI turned off and the tracheal tube cuff inflated. Time-points of physiological measurements and actual ventilatory settings at the moments of assessment are in boxes. Data are presented as median (interquantile range).

***** For each patient odd number sessions (1st and when applicable 3rd) were randomly assigned to HF-1 or HF-2 groups. Even numbered sessions (2nd and when applicable 4th) were assigned to the opposite group from the preceding session.

[#] Physiological measurements were performed after the end of the 8-hour treatment period and required 10-15 minutes, during which the patient was still on CMV ventilation.

[¶] Physiological measurements were performed after the end of the 8-hour treatment period and required 10-15 minutes, during which the patient was still on HFO-TGI ventilation.

[§] Respiratory mechanics were assessed 1-5 minutes after return to CMV.

CMV, conventional mechanical ventilation; FiO_2, fraction of inspired oxygen; PEEP, positive end-expiratory pressure; Pplat, end expiratory (plateau) airway pressure; mPaw, mean airway pressure; ΔP, oscillatory pressure amplitude; f, oscillation frequency.