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Early Effect of Tidal Volume on Lung Injury Biomarkers in Surgical Patients with Healthy Lungs

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Abstract

Background—The early biological impact of short-term mechanical ventilation on healthy lungs is unknown. We aimed to characterize the immediate tidal volume (V_T)-related changes on lung injury biomarkers in patients with healthy lungs and low risk of pulmonary complications.

Methods—Twenty-eight healthy patients for knee replacement surgery were prospectively randomized to volume-controlled ventilation with V_T 6 (V_T6) or 10 (V_T10) mL/kg predicted body weight. General anesthesia and other ventilatory parameters (positive end-expiratory pressure 5 cmH_2O, FiO_2 0.5, respiratory rate titrated for normocapnia) were managed similarly in the two groups. Exhaled breath condensate (EBC) and blood samples were collected for nitrite, nitrate, tumor necrosis factor α, interleukins-1β, 6, 8, 10, 11, neutrophil elastase (NE), and Clara Cell protein 16 (CC16) measurements, at the onset of ventilation and 60 min later.

Results—No significant differences in biomarkers were detected between the V_T groups at any time. The coefficient of variation of EBC nitrite and nitrate decreased in the V_T6 but increased in the V_T10 group after 60-min ventilation. Sixty minute ventilation significantly increased plasma NE levels in the V_T6 (35.2 ± 30.4 *vs.* 56.4 ± 51.7 ng/mL, $P = 0.008$) and CC16 levels in the V_T10 group (16.4 ± 8.8 *vs.* 18.7 ± 9.5 ng/mL, $P = 0.015$). EBC nitrite correlated with plateau pressure ($r = 0.27$, $P = 0.042$) and plasma NE ($r = 0.44$, $P = 0.001$). Plasma CC16 correlated with compliance ($r = 0.34$, $P = 0.014$).

Conclusion—No tidal volume-related changes were observed in the selected lung injury biomarkers of patients with healthy lungs after 60-min ventilation. Plasma NE and plasma CC16 might indicate atelectrauma and lung distention, respectively.

INTRODUCTION

Large tidal volumes (V_T) contribute to and worsen the acute respiratory distress syndrome (ARDS) in Intensive Care Unit (ICU) patients after hours or days of ventilation[1–8]. Recent studies suggest intraoperative ventilation settings affect postoperative pulmonary outcomes[1,9–13]. Many surgical patients undergo short-term ventilation with large V_T (greater than 10 mL/kg predicted body weight [PBW])[12,14] without negative consequences. These observations reinforce the lack of translation of ICU protective ventilation strategies with low V_T (6 mL/kg PBW)[7] into the perioperative setting. It is not known if widely used V_T 10mL/kg PBW[12,14] triggers any immediate inflammatory changes in healthy lungs. Understanding the early inflammatory changes triggered by different V_T in healthy lungs, and the relationship of these changes with ventilatory parameters, may help identify injurious pulmonary insults and susceptible individuals. This knowledge may complement recently developed risk scores for predicting ARDS[15–19] or postoperative pulmonary complications[13,20] in their goal of early detection and prevention of lung inflammation.

Several V_T-associated injury biomarkers have been identified. The nitrite and nitrate levels in exhaled breath condensate (EBC), representing the metabolism of nitric oxide in the lung, have been measured frequently for assessing lung injury in patients breathing spontaneously or ventilated in the ICU[2,6,21,22] and after cardiothoracic surgery[23–25]. Nitrite concentration in the EBC showed a positive correlation with V_T in ICU patients with or without ARDS[6], and with the degree of lung overdistention in chronic obstructive pulmonary disease (COPD) patients[26]. Increasing nitrite and nitrosylated proteins in the bronchoalveolar lavage (BAL) may have a prognostic value suggestive of lung injury progression in ARDS[27]. In humans, cytokines such as tumor necrosis factor α (TNFα), interleukin (IL)-1β and IL-6 in plasma and BAL were increased in ARDS patients ventilated with greater V_T and lower positive end-expiratory pressure (PEEP), compared to those receiving smaller V_T and greater PEEP[28]. The levels of TNFα and IL-8 in BAL also increased in ICU patients without ARDS ventilated with V_T 10–12 mL/kg PBW for 12 h[3] compared to those ventilated with V_T 5–7mL/kg PBW and similar PEEP. The antiinflammatory cytokine IL-10 was affected by ventilatory settings and ventilation duration in brain-injured patients[2] and used for functional repair of human donor lungs[29]. IL-11 has a protective role against murine hyperoxia-induced DNA fragmentation and lung injury[30,31]. The plasma concentration of neutrophil elastase (NE) is an indicator of alveolar recruitment[32] and activation of neutrophils during the development of lung injury[33]. Finally, plasma Clara cell protein 16 (CC16), an antiinflammatory protein secreted by the Clara cells of the distal respiratory epithelium, is a marker of acute epithelial lung injury[34,35] and increases in ventilated preterm neonates[36] and after 5-h ventilation during abdominal surgery in adults[37].

We hypothesized that mechanical ventilation induces early V_T-related changes in selected lung injury biomarkers in surgical patients with healthy lungs and low risk of pulmonary complications. Healthy patients undergoing knee replacement surgery under general anesthesia were randomized to volume-controlled ventilation with V_T 6 (V_T6) or 10 (V_T10) mL/kg PBW. The levels of selected biomarkers in EBC and plasma and their time or tidal volume dependent changes were analyzed.

MATERIALS AND METHODS

The experimental protocol was approved by the University of Colorado Multiple Institutional Review Board (Aurora, Colorado) before conducting the study.

Experimental protocol

After obtaining informed consent, 30 patients scheduled to receive elective orthopedic surgery for total knee replacement under general anesthesia were prospectively randomized to receive a tidal volume (V_T) of 6 or 10 mL/kg predicted body weight (PBW) during mechanical ventilation. Exclusion criteria for study patients included: American Society of Anesthesiologists (ASA) class 4; age ≥70 yr; emergency procedure; status post pneumonectomy; diagnosed COPD, emphysema, asthma, pulmonary hypertension, sleep apnea or any other respiratory disease; oxygen-therapy during last month; tobacco use in the last 5 yr; severe obesity (body mass index [BMI] ≥35 kg/m^2); immunosupression within 3 months prior to the procedure; diagnosed infection; or shock. Preoperatively, all patients

received sciatic and femoral regional blocks for postoperative analgesia. The predetermined general anesthesia management included intravenous propofol for induction and maintenance (2–2.5 mg/kg and 0.05–0.2 mg/kg/min, respectively), to avoid potential differences of cytokine-induction between different anesthetic drugs [38,39], fentanyl (1–2 mcg/kg initially, then 0.7–10 mcg/kg as needed) and rocuronium (0.6–1.2mg/kg for intubation, 0.1–0.2 mg/kg as needed when 25% recovery of T1 in train-of-four neuromuscular monitoring). Induction was performed in all patients while breathing FiO2 1.0. Immediately after confirmation of adequate endotracheal tube placement, mechanical ventilation was started with a volume control ventilation mode with V_T of either 6 (V_T6) or 10 (V_T10) mL/kg PBW as randomly selected. To set up the appropriate V_T the PBW was calculated based on the following formulas[7]: PBW-Males = 50 + 0.91(centimeters of height - 152.4); PBW-Females = 45.5 + 0.91(centimeters of height - 152.4). The respiratory rate was titrated for eucapnia (end-tidal carbon dioxide partial pressure [E_TCO_2] 30–40 mmHg), and all patients received the same following ventilatory settings: inspiratory: expiratory (I:E) ratio 1:2, inspiratory pause 5 %, fresh gas flow 2 L/min, FiO_2 0.5, PEEP 5 cmH_2O. Ventilatory parameters, except the respiratory rate, were unchanged during the study. Withdrawal criteria from the study were established as: 1) V_T needed to be changed after randomization for any clinical or provider-related reason; 2) airway plateau pressure could not be managed to remain below 30cmH_2O; or 3) for any other reason at the discretion of their anesthesia providers.

Immediately after starting mechanical ventilation (0-min time point), before the surgical incision, we initiated the collection of EBC. During the EBC collection, ventilatory and hemodynamic physiology parameters were recorded, and a sample of venous blood for analysis of biomarkers and an arterial blood gas sample were also obtained. At 60 min after the initiation of mechanical ventilation we repeated the sample and data collection (60-min time point).

Demographics, physiology and outcomes

Age, gender, ASA classification, height, weight and BMI were recorded for all patients. Patients' PBW was calculated preoperatively as described above for V_T calculation.

Physiology parameters were recorded simultaneously with the arterial blood gas sample collections, including: hemodynamics (heart rate, respiratory rate, mean blood pressure), temperature, gas exchange (saturation of oxygen by pulse-oximetry or Sat_pO_2, and E_TCO_2 as described before) and ventilation parameters (exhaled tidal volume or V_T, respiratory rate, minute volume ventilation, peak and plateau airway pressures and respiratory system compliance). Compliance was calculated as exhaled V_T/(plateau pressure − PEEP). Measurement of the functional dead space was attempted for calculating the alveolar minute ventilation, but it was impossible due to imperfect capnography calibration in several patients (*i.e.*, equal or nonphysiological E_TCO_2-$PaCO_2$ differences).

Any complications occurring intraoperatively were recorded (*i.e.*, high airway pressure, hypoxemic event, need for withdrawal). Any postoperative respiratory complications were also recorded, as well as length of hospital stay and any in-hospital complications.

Exhaled breath condensate and blood sample collection

EBC samples were collected with an Rtube™ breath condensate vial (Respiratory Research, Inc., Austin, TX) inserted in the expiratory limb of the ventilatory circuit for 20 minutes. This Rtube™ consists of a sterile polypropylene collection tube with a one-way valve trap that is kept cooled with an outer chilled aluminum sleeve (−80°C) during the collection period to condense the breath at the inner wall of the tube. No humidification was added to the ventilatory circuit, and the heat moisture exchange filter adjacent to the Y connector was removed. After 20 min, EBC samples were immediately placed in regular ice, volume measured and aliquoted into vials prewashed with deionized water and frozen at −80C until analysis.

Venous blood were obtained at each time point in EDTA vials and immediately transported in regular ice to the laboratory. Samples were centrifuged at 2,000 rpm for 10 min, plasma volume aliquoted and frozen at −80C until analysis.

Arterial blood was obtained from the radial artery at each time point in heparinized syringes and immediately processed for arterial blood gas analysis.

Inflammatory and oxidative stress biomarkers

Nitric oxide metabolites—All samples were maintained frozen until analysis to minimize stability changes from freezing/thawing cycles and ambient contamination[40]. The collection and storage manipulation were strict because of concerns of nitric oxide metabolites instability and contamination from ambient air[41,42]. Concentration of nitrite and nitrate were independently measured in duplicates by a dedicated HPLC system (ENO-20, Eicom, San Diego, CA)[43]. Before measurement, plasma samples were mixed 50:50 v/v with methanol, vortexed, and centrifuged to remove fat and protein. The clarified supernatants were used for measurement. EBC samples were run without pretreatment. Nitrite and nitrate concentrations were calculated based on authentic standards. Nitrite and nitrate concentrations were summed to reflect the Total Nitric Oxide (NOx) levels.

Cytokines—Concentrations of cytokines TNFα, IL-1β, IL-6, IL-8, and IL-10 were measured in EBC and plasma samples with the Fluorokine® MAP Human High Sensitivity Cytokine Base Kit (cat# LHSC000 R&D Systems, Inc., Minneapolis, MN). IL-11 concentration was analyzed in EBC and plasma samples with the Quantikine® Human IL-11 Immunoassay (cat#D1100 R&D Systems, Inc.). Average concentrations from duplicates were used if variation between them was less than 20%, and results excluded from the analysis if variability greater than 20% after analysis repetition.

Neutrophil elastase—Plasma samples were measured in duplicates in 1:50 dilution samples using the Human PMN Elastase Platinum ELISA assay (Cat# ALX-850-265, Enzo® Life Sciences, Inc., Farmingdale, NY).

Clara cell protein 16—Clara cell protein concentrations was measured in duplicates in 1:25 diluted plasma samples with the Human Clara Cell Protein ELISA assay (Cat# RD191022200, BioVendor LLC, Candler, NC).

All analyses were performed in duplicated samples following the specific manufacturer's instructions.

Correlation analysis was performed between the V_T-related ventilatory parameters such as plateau pressure or compliance and lung injury biomarkers. Other ventilatory parameters were not included in the correlation analysis if they were intrinsically dependent on our study design (*i.e.*, V_T, respiratory rate and administered minute ventilation) or were not available (*i.e.*, transpulmonary pressure or other measurements of lung stretch/strain). Association between the different biomarkers was also examined with correlation analysis to find common factors influencing the observed biomarker changes.

Statistical analysis

This pilot study was based on the linear correlation between EBC nitrite levels and V_T in ventilated ICU patients with no lung injury ($R^2 = 0.79$) reported by Gessner *et al.*[6] Based on this finding, our pilot study was designed to detect a correlation coefficient of 0.79, with the null being 0.12 (two-tailed, alpha = 0.05, and power = 0.8). Using these parameters, the estimated sample size was 11, which we increased to 15 in each group to account for any lack of information on non-ICU patients and on the other biomarkers.

All recorded parameters (physiology, biomarkers, outcomes) were graphically depicted and summarized using means and standard deviations. All biomarker measurements were examined for normality. If not normally distributed, they were logarithmically transformed for comparison between groups. If not normally distributed and showing a sigmoid distribution, the zxphysiology parameters by Pearson correlation. All continuous variables were statistically compared within the V_T groups (same subjects, different time points) with a paired *t*-test. The change scores of continuous variables from 0min to 60-min time points were compared between the two V_T groups using an independent *t*-test. Chi-square test was used for comparing categorical variables. Because of the high variability in nonnormally distributed biomarkers in different groups and time points, we used the coefficient of variation (CV), calculated as a ratio of the standard deviation and the mean. All statistical analyses were two-tailed and performed with SPSS version 21 (IBM, New York, NY). Significance was accepted at $p < 0.05$.

RESULTS

Demographics, physiology and outcomes

Thirty patients who fulfilled the inclusion and exclusion criteria were enrolled and consented for the study. One patient from each V_T group was removed from the study because of a nondisclosed steroid course within 10 days before surgery and previously undiagnosed sleep apnea symptoms. Only the remaining 28 patients (14 per group) were included in the final analyses. No significant differences were found between the two groups in terms of age, gender, comorbidities, ASA classification, height, weight, BMI, PBW, ASA classification and comorbidities. No respiratory complications were detected and there was no significant difference in hospital length of stay between the two groups (table 1).

Hemodynamic parameters, temperature and oxygenation were comparable at the 0-min time point in both V_T groups (table 2). E_TCO_2 was within the targeted range (30–40 mmHg) in both groups. At the 0-min time point, as it was expected from the study design, the V_T10 group had lower respiratory rate and greater exhaled V_T, minute volume ventilation, peak and plateau airway pressures and compliance. The arterial blood gas analysis showed a lower $PaCO_2$ and higher pH in the V_T10 compared to the V_T6 group.

From 0-min to 60-min time points, both groups experienced a small but statistically significant decrease in oxygenation in terms of Sat_pO_2 ($P = 0.045$ for both). Only in the V_T10 group there was a reduced but statistically significant decrease from 0 min to 60 min in heart rate ($P = 0.030$), respiratory rate and minute volume ventilation ($P \leq 0.001$ for both), an increase in plateau pressure ($P = 0.010$) and a reduction in compliance ($P = 0.023$). The decrease of compliance in the V_T10 group was related to a small but significant increase in plateau pressure from 17.8 ± 3.1 to 18.6 ± 2.9 cmH_2O, while tidal volumes and PEEP were unchanged. The observed decrease in PaO_2 in both groups only reached statistical significance in the V_T10 group ($P = 0.013$). Similarly, the decrease in Sat_aO_2 observed in both groups only reached statistical significance in the V_T6 group ($P = 0.040$).

After 60 min of mechanical ventilation, the E_TCO_2 remained within the target range in both groups. From 0 min to 60 min there was a similar decrease in oxygenation in both groups. The decrease over time observed in minute ventilation, bicarbonate concentrations and base excess was significantly greater in the V_T10 group than in the V_T6 group.

There were no complications or withdrawals related to the study. No respiratory complications (respiratory failure for any reason requiring reintubation, noninvasive ventilation or oxygen therapy needed at hospital discharge) were observed in the early postoperative period and during the rest of the hospital stay. The hospital length of stay was not different between the two groups (2.9 ± 0.9 days in the V_T6 group *vs.* 2.6 ± 0.5 days in the V_T10 group, $P = 0.445$).

Effect of mechanical ventilation with different tidal volumes on measured biomarkers

Nitric oxide metabolites—Nitrite and nitrate concentrations were detectable in EBC and plasma samples from all ventilated patients. At 0 min, the average EBC concentrations of nitrite and nitrate were similar in patients from both groups. There was a slight decrease from 0 min to 60 min of EBC nitrite in the V_T6 group and an increase of EBC nitrate in the VT10 group, but no changes reached statistical significance in the EBC or plasma concentrations of nitric oxide metabolites between time points or between the V_T groups (fig. 1). The variability of EBC nitrite, nitrate and Total NOx (nitrite + nitrate) concentrations, measured with the CV was comparable in both groups at 0 min (fig. 2). At 60min, a few individuals in the V_T10 group doubled their respective baseline levels of EBC nitrite and EBC nitrate. No individuals in the V_T6 group showed this magnitude of increase in EBC nitrite or nitrate or Total NOx. Thus, the CV of EBC nitrite and nitrate, and therefore of Total NOx, decreased in the V_T6 group (by 47.1%, 17.1%, and 32.6%, respectively) but increased in the V_T10 patients (by 4.2%, 75.1%, and 61.9%, respectively) (fig. 2).

Cytokines—Only a few EBC samples (out of the total 56) showed detectable levels of TNFα (n = 8), IL-1β (n = 21) and IL-10 (n = 19). Most of the measured concentrations were lower than the detection limit and not reliable for quantitative comparison. No significant differences were observed in plasma cytokine concentrations from 0-min to 60-min time points, or between the change scores of the V_T groups (fig. 3A).

Neutrophil elastase—Neutrophil elastase was not detectable in the EBC samples. Plasma NE levels from all patients combined did not significantly increase from 0-min to 60-min time points. The mean concentration of NE from the V_T6 patients significantly increased from 0 min to 60 min ($P = 0.008$), but not in the V_T10 group. There were no significant differences between the change scores of the V_T groups (fig. 3B).

Clara cell protein 16—CC16 was not detectable in the EBC samples. CC16 concentrations from all patients significantly increased from 0-min to 60-min time points. It significantly increased in the V_T10 group ($P = 0.015$) but not in the V_T6 group ($P = 0.081$). There were no significant differences between the change scores of the V_T groups (fig. 3C).

Correlation between biomarkers and ventilatory parameters

Nitric oxide metabolites—Measurements of EBC nitrite concentration, but not of EBC nitrate or total NOx, logarithmically correlated with the plateau pressure measurements in the pooled samples from all patients and time points (r = 0.27, $P = 0.042$) (fig. 4A).

EBC nitrite concentrations showed a significant positive logarithmic association with plasma neutrophil elastase when all measurements were pooled for analysis (r = 0.44, $P = 0.001$) (fig. 4B). Levels of EBC nitrate or total NOx did not present any significant association with neutrophil elastase measurements.

Cytokines—Plasma levels of these parameters did not correlate with any ventilatory parameters.

Neutrophil elastase—Plasma concentrations of NE did not correlate with any ventilatory physiology parameters. Plasma concentrations of NE, as mentioned above, showed a positive logarithmic correlation with EBC nitrite levels.

Clara cell protein 16—Plasma levels of CC16 showed a significant positive correlation with compliance (r = 0.34, $P = 0.014$) (fig. 4C).

DISCUSSION

Our prospective randomized pilot study examined the early changes of selected lung injury biomarkers induced by 60-min mechanical ventilation with either 6 or 10 mL/kg PBW V_T in surgical patients with healthy lungs and low risk of pulmonary complications.

Nitrite or nitrate concentrations in EBC did not significantly change after 60-min ventilation in our patients, and changes were not significantly different between the V_T groups. Nitrite and nitrate levels in EBC, individually or combined, are often used as biomarkers of lung

nitrosative stress. Increased nitric oxide metabolites reflect activation of macrophages, neutrophils or other various lung cell types[27,44,45] through the inducible or endothelial nitric oxide synthase[46,47,48]. Nitrite in EBC and nitric oxide in exhaled air are accepted by the American Thoracic Society as complementary but not equivalent measurements of nitrosative stress[42,49,50]. Nitrite and nitrate are relatively stable metabolites of nitric oxide in aqueous EBC samples[51]. In physiological conditions nitric oxide can be oxidized to nitrite by lung epithelial cells, making nitrite measurable in the EBC of healthy individuals[52]. Nitrite can be reduced to nitric oxide, if decreased availability of oxygen, or further oxidized to nitrate[53]. Human cells are not able to reduce nitrate to nitrite; thus nitrate is either exhaled or diffuses into the circulation[53]. Multiple questions remain about the biological role of nitric oxide metabolites in pulmonary function. For example, EBC nitrite significantly increases in healthy recreational runners in a time-dependent manner without change in lipid peroxidation[54], suggesting an increased nitric oxide production independent of significant lung injury. Nitrite level increases with lung distention in the EBC of COPD or ventilated patients with or without ARDS[6,26] as well as with high V_T ventilation of isolated rabbit lungs[55]. EBC nitrate reflects the severity of asthma better than nitrite or exhaled nitric oxide[56]. Our observed nitrite and nitrate concentrations in EBC were comparable to those found in healthy humans[23,54,57], suggesting healthy lung conditions during our study period. An insufficient time or intensity of the mechanical insult may explain the absence of significant changes in EBC nitrite or nitrate in our study patients.

The coefficients of variation of EBC nitrite and nitrate were noticeably different in the V_T groups after 60 minutes of ventilation, decreasing in the V_T6 group while increasing in the V_T10 group. This difference reflected a few patients in the V_T10 group who had at least a two-fold increase of their initial EBC nitrite or nitrate levels. These patients did not differ from the rest of the group in physiology parameters or other biomarkers. Low V_T ventilation attenuates the increases in the concentration of other biomarkers (cytokines, CC16 and procoagulants)[58–62]. However, the interpretation of the increased variability of EBC nitrite or nitrate as an early sign of individual susceptibility to ventilator-induced lung injury deserves caution. The additional alveolar space ventilated with larger V_T could contribute to the increased nitric oxide metabolites without any associated lung injury. Furthermore, we cannot exclude a role of ambient contamination in this variability, since our study lacked of controlling for inspiratory nitric oxide metabolites[41].

EBC nitrite levels positively correlated with ventilatory plateau pressures in our study. Gessner *et al.*[6] reported a positive correlation between EBC nitrite concentrations and lung distention parameters (V_T, mean airway pressure, and PEEP) in ICU patients, ventilated for at least 24 h for pneumonia or COPD exacerbation. The relationship between EBC nitrite and V_T had a steeper correlation coefficient in ARDS patients than in patients with mild, or without, lung injury. The same authors described that EBC nitrite levels correlated logarithmically with residual volume, total lung capacity and intrathoracic gas volume, but not with parameters of expiratory flow or EBC cytokine levels, in spontaneously breathing COPD patients[26]. Our findings suggest that plateau pressure, rather than volume parameters, might influence nitrite changes in EBC of healthy ventilated patients. The time and pressure

dependence of nitrite generation in healthy lungs during mechanical ventilation needs further investigation.

EBC nitrite concentrations correlated with plasma NE levels in our study patients, which may suggest a shared process between pulmonary nitrite production and plasma NE. Concurrent increases in exhaled nitric oxide and NE activity have been observed in a murine oleic acid-induced lung injury model[63]. Release of NE by activated neutrophils may reflect ongoing alveolar neutrophil migration[32] during mechanical ventilation. Plasma NE has been evaluated as a marker and predictor of ARDS development[33,64]. In our study, plasma NE levels significantly increased after 60-min ventilation in the V_T6, but not in the V_T10 group. A greater atelectrauma in the V_T6 compared to the V_T10 group, despite the same PEEP levels, might contribute to this finding[65,66]. One patient from the V_T10 group had greater than 200 ng/mL NE values (level usually measured in ARDS[33]) at 0 min and 60 min without any sign of lung injury suggesting that the role of high NE levels in healthy individuals needs clarification.

Cytokines have been measured in EBC samples[2,21,26]. Our low detection rate is similar to findings in other studies in healthy patients ventilated during surgery[2] and reinforces the normal pulmonary status of our study patients. Plasma cytokine concentrations did not significantly change in relation to surgical trauma or greater V_T. Possible contributing factors to this finding were the short study duration, lack of lung injury, or preoperative regional anesthesia[67,68]. However, the inhibitory effect of regional anesthesia on systemic inflammatory response after orthopedic surgery is still unproven[69,70].

Plasma CC16 significantly increased after 60 minutes of mechanical ventilation in all patients and in the V_T10 group, but not in the V_T6 group. CC16 is a small protein secreted by epithelial Clara cells and an accepted biomarker of alveolar-capillary permeability[34]. Leakage of CC16 into the circulation is observed after lipopolysaccharide inhalation in healthy humans[71], lung contusion[72] and mechanical ventilation in preterm neonates[36] or animal models[73]. Plasma CC16 concentrations are increased in ventilated ARDS patients[58] and predict poor outcomes such as greater mortality and fewer ventilator-free days[35]. Increased plasma CC16 has been observed in surgical patients after 5 h of mechanical ventilation[37]. The observed increase in plasma CC16 levels in our patients could be related to passive leak due to positive pressure ventilation or to up-regulation of protein synthesis. Plasma CC16 concentrations from all patients correlated with lung compliance. The V_T10 patients received almost double insufflating lung volumes (≈620 mL in V_T10 compared to ≈350 mL in V_T6) with a small difference (≈3 cmH_2O) in drive pressure, and therefore they had greater measured compliance. Thus, compliance was more affected by volume (exhaled V_T) than drive pressure (plateau pressure – PEEP) change. Greater passive leakage of CC16 might reflect greater lung end-inspiratory volume and possible increased lung strain[74]. Plasma CC16 levels however, were not significantly different between the V_T groups after one hour in our study or after 5 h in Determann *et al.*'s study[37]. Our findings could therefore represent an immediate response of plasma CC16 to short-term positive pressure ventilation that might be influenced by lung distention with unknown clinical relevance.

The impact of the selected and often used V_T[1,12] on respiratory physiology or clinical outcomes of our healthy patients was minimal after 60-min ventilation. The clinically insignificant worsening of oxygenation and compliance observed in our patients may reflect an under-recruitment phenomenon from ventilation within the lower portion of the pressure/volume curve[65,66]. This explanation, however, cannot be confirmed with our study design.

Our relatively low number of patients and the short duration of ventilation constitute the major limitations of our study. However, our study was designed primarily to characterize the early effect of different V_T on biomarkers in patients with healthy lungs. Accordingly, we excluded patients with any respiratory or immune disease, or with a BMI ≥35 kg/m^2 because of the increased likelihood of obesity-related respiratory impairment. The effect of obesity or age on the lung production of nitrite and other biomarkers is unknown. We also excluded any surgery involving a nonsupine body position or restricted lung excursion, to avoid confounders as positioning or external thoracic restraints. Finally, the understanding of the influence of minute ventilation, alveolar ventilation or other ventilatory settings on the studied lung injury biomarkers during intraoperative ventilation is limited. Although we attempted to control for as many potential variables (PEEP, FiO_2) to focus on the V_T, there are still several factors that may have affected our findings. For practical reasons, the effects of minute ventilation or respiratory rate need to be addressed separately.

In conclusion, we studied the early changes of selected lung injury biomarkers after 60-min ventilation with V_T 6 or 10 mL/kg PBW in patients with healthy lungs. No significant changes in EBC nitrite or nitrate levels were observed. The variability of EBC nitrite and nitrate decreased in the V_T6 but increased in the V_T10 group after 60-min ventilation. We observed a significant increase in plasma levels of NE in the V_T6 group and CC16 in the V_T 10 group, which may represent the effect of atelectrauma and increased alveolar distention, respectively. Future studies in patients with higher risk for postoperative pulmonary complications may confirm if EBC nitric oxide metabolites, plasma NE or CC16 constitute early diagnostic or predictive biomarkers of lung inflammation.

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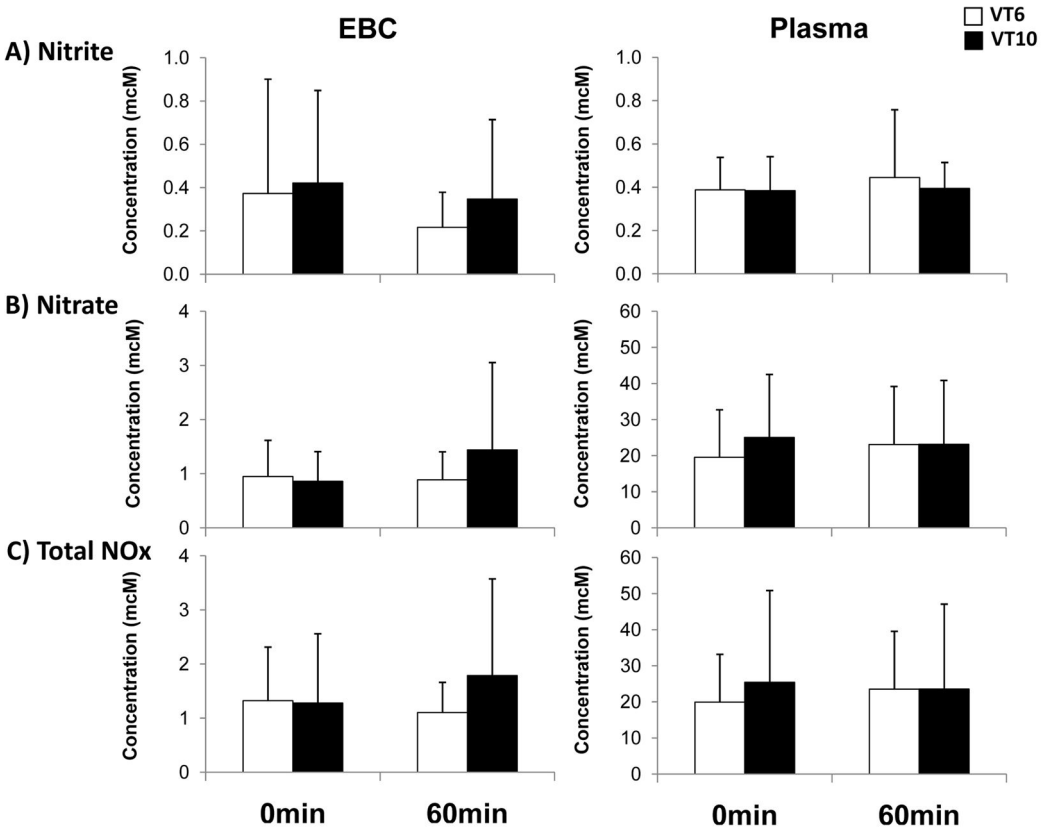


Figure 1. Nitrite, nitrate and Total nitric oxide (Total NOx = nitrite + nitrate) concentrations in exhaled breath condensate (EBC) and plasma

No statistical differences were found between the tidal volume (V_T) groups at any time point in either EBC or plasma concentrations of nitrite (**A**), nitrate (**B**) or Total NOx (**C**). Note the similar scale of nitrite in EBC and plasma and the approximately 20-fold concentrations of nitrate and Total NOx in plasma compared to EBC concentrations. Graphs represent mean and standard deviation (SD). Values from different time points within each group were compared with a paired t-test. The change scores of continuous variables from 0min to 60min time points were compared between the two V_T groups using an independent t-test. Significant p value was accepted as $p<0.05$.

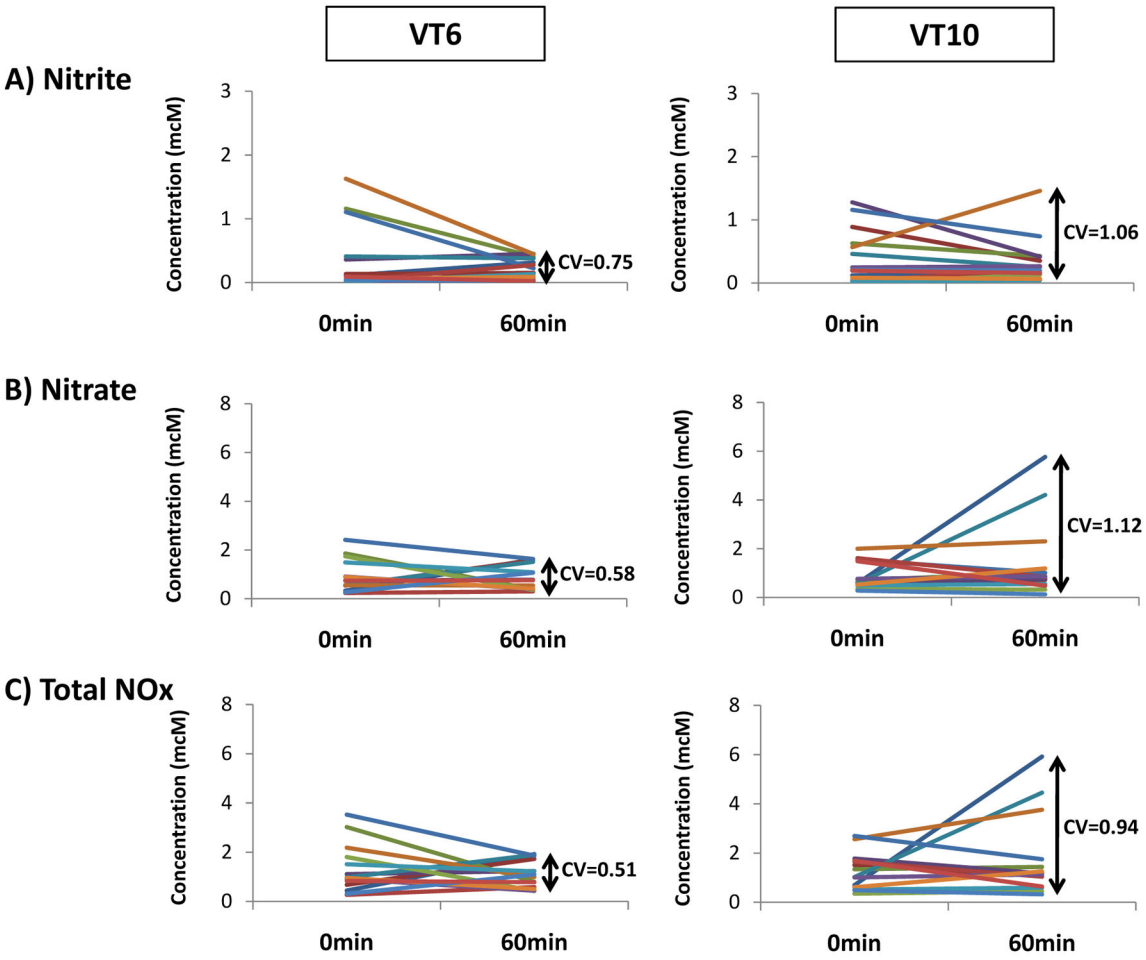
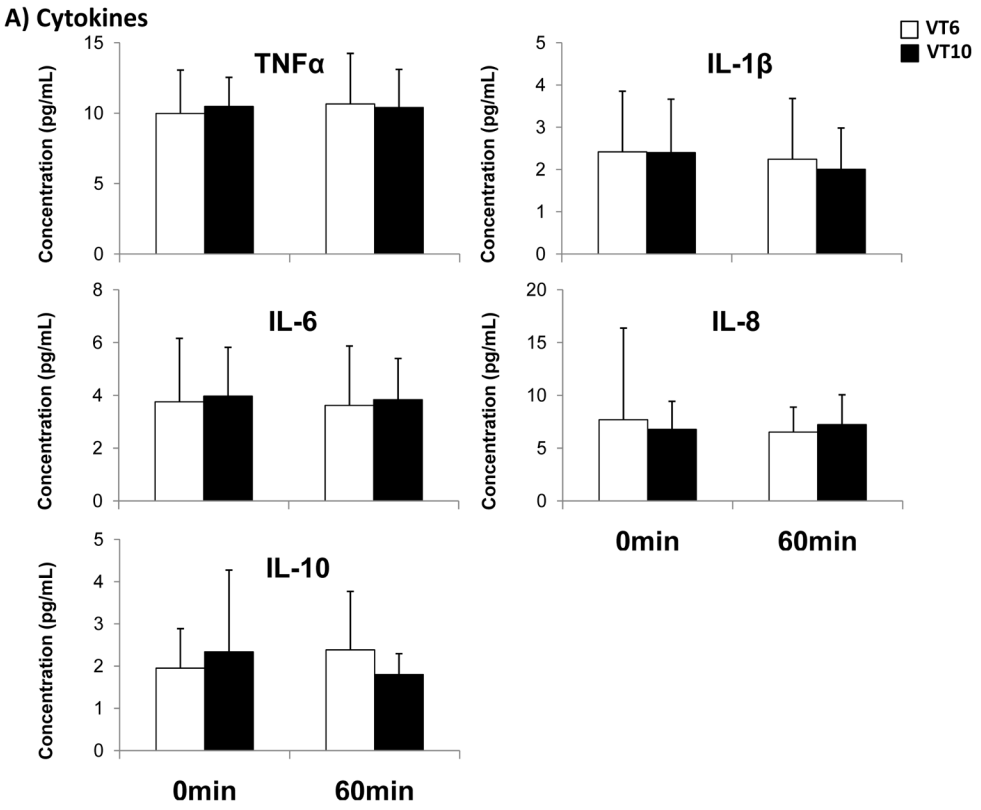


Figure 2. Nitrite and nitrate concentrations in exhaled breath condensate (EBC): Individual values

The range of values at the 0min time point for nitrite (**A**), nitrate (**B**) and total nitric oxide (Total NOx = nitrite + nitrate)(**C**) were comparable in both groups. At the 60min time point the variability of nitrite and nitrate concentrations, and therefore of Total NOx, measured as the coefficient of variation (standard deviation/mean) was greater in the tidal volume 10 (V_T10) group compared to the V_T6 group. The increased variability in Total NOx in the V_T10 group was due to the increase in 3 patients that presented a raise predominantly in nitrate concentration, with 1 of these patients showing a simultaneous 2.5-fold increase in nitrite concentration (note the different scales for nitrite and nitrate concentrations).



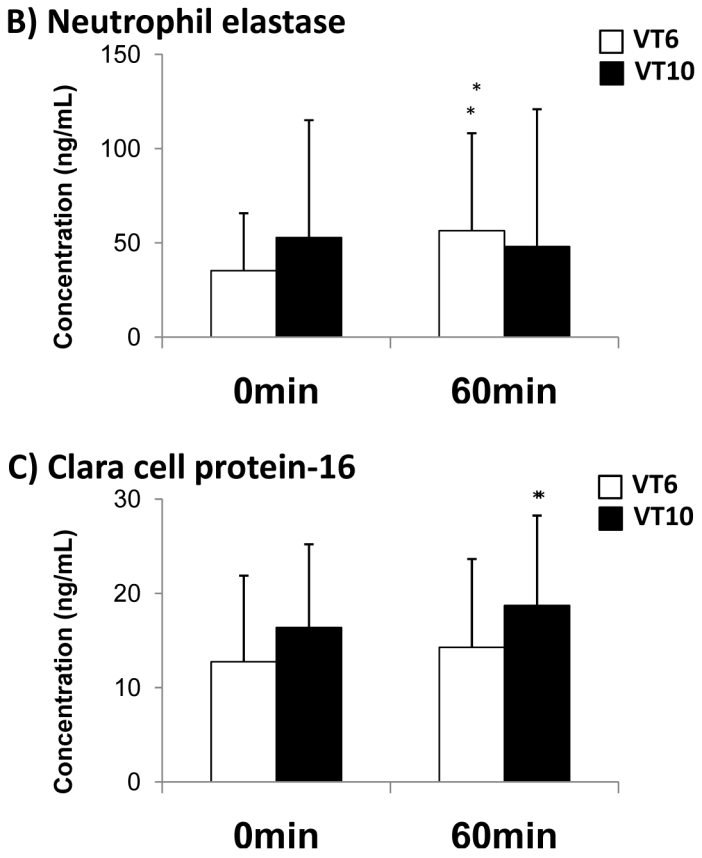


Figure 3. Plasma concentrations of cytokines, neutrophil elastase and Clara cell protein-16 (CC16)

The plasma concentrations of cytokines (**A**), neutrophil elastase (**B**) and CC16 (**C**) were plotted at 0min and 60min time points in both tidal volume (V_T) groups. No significant differences were observed in cytokine concentrations at any time point and between the V_T groups. The mean level of neutrophil elastase significantly increased from 0min to 60min in the V_T6 group. The mean concentration of CC16 increased from 0min to 60min in the V_T10 but not in the V_T6 group. Graphs represent mean and standard deviation (SD). Comparisons between different time points were performed with a paired t-test. The change scores of continuous variables from 0min to 60min time points were compared between the two V_T groups using an independent t-test. No significant difference was observed between the V_T groups. *$P < 0.05$. (TNFα = Tumor Necrosis Factor α; IL= Interleukin).

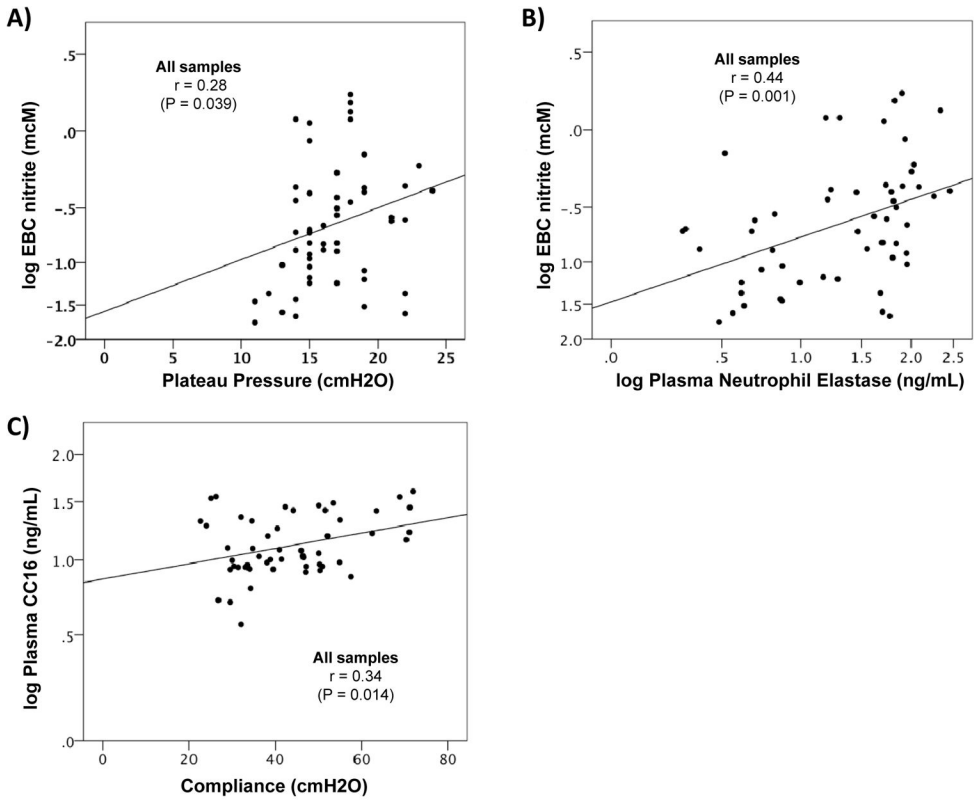


Figure 4. Correlation between lung injury biomarkers and ventilatory physiology parameters

Exhaled breath condensate (EBC) nitrite concentration measurements from all samples pooled together showed a significant and positive logarithmic correlation with the airway plateau pressure (**A**) and plasma neutrophil elastase levels (**B**). Plasma Clara Cell protein 16 (CC16) concentrations showed a positive correlation with compliance (**C**). r represents the Pearson correlation coefficient.

Table 1

Demographic Details and Clinical Outcomes of Study Patients

	All	V_T6	V_T10	*P*
Number	28	14	14	
Age (years)	61.7 ± 7.0	61.1 ± 5.5	62.2 ± 8.3	0.691
Gender distribution				
Male	8(26.7%)	3(21.4%)	5(35.7%)	0.678
Female	20(66.7%)	11(78.6%)	9(64.3%)	
Height (cm)	168.4 ± 8.9	166.8 ± 10.9	170.0 ± 6.4	0.354
Weight (kg)	79.7 ± 13.1	78.0 ± 11.6	81.4 ± 14.8	0.495
Body Mass Index (kg/m^2)	28.0 ± 3.3	27.9 ± 2.6	28.0 ± 4.0	0.934
Predicted Body Weight (kg)	61.3 ± 9.7	59.6 ± 11.6	63.0 ± 7.3	0.368
ASA classification				
1	1(3.3%)	0(0%)	1(7.1%)	0.171
2	22(73.3%)	13(92.9%)	9(64.3%)	
3	5(16.7%)	1(7.1%)	4(28.6%)	
Common comorbidities				
Mild/Moderate obesity (BMI 25–34.9)	22(73.3%)	12(85.7%)	10(71.4%)	0.648
Hypertension	11(36.7%)	5(35.7%)	6(42.9%)	1.000
Gastroesophageal reflux disease	7(23.2%)	3(21.4%)	4(28.6%)	1.000
Diabetes Mellitus	4(13.3%)	2(15.4%)	2(14.3%)	1.000
Clinical outcomes				
Respiratory complications	0(0%)	0(0%)	0(0%)	*a*
Hospital LOS (days)	2.7 ± 0.7	2.9 ± 0.9	2.6 ± 0.5	0.297

There were no significant differences between both study groups in any parameter. Data is expressed as Mean ± SD or n(%), and compared by two-tailed independent *t*-test or Pearson's chi-square (or Fischer's exact) test, where appropriate. *P* represents the statistical significance of the differences between the V_T6 and V_T10 groups.

ASA = American Society of Anesthesiologists classification; BMI = body mass index; LOS = length of stay; V_T = tidal volume).

a no statistics computed because all values in both groups were zero).

Table 2

Physiology Parameters and Blood Gas Analyses

	0 min	60 min	*P*	Change scores (0 min to 60 min)	*P*
Physiology data					
Heart rate (beats/min)					
V_T6	73.6 ± 14.2	69.9 ± 11.7	0.290	−3.7 ± 12.6	0.287
V_T10	75.9 ± 15.9	66.7 ± 8.5	0.030	−9.2 ± 14.1	
Mean Blood Pressure (mmHg)					
V_T6	77.6 ± 11.7	83.1 ± 11.4	0.084	5.5 ± 11.0	0.062
V_T10	81.9 ± 10.1	80.1 ± 7.2	0.455	−1.8 ± 8.7	
Temperature (°C)					
V_T6	35.7 ± 0.6	35.8 ± 0.7	0.637	0.1 ± 0.6	0.890
V_T10	35.8 ± 0.4	35.9 ± 0.3	0.694	0.1 ± 0.5	
Sat_pO_2 (%)					
V_T6	98.7 ± 1.1	98.1 ± 1.3	0.045	−0.6 ± 1.1	1.000
V_T10	98.3 ± 1.9	97.6 ± 2.0	0.045	−0.6 ± 1.1	
E_TCO_2 (mmHg)					
V_T6	36.6 ± 3.1	36.0 ± 3.9	0.493	−0.6 ± 3.4	0.677
V_T10	33.0 ± 2.7	32.9 ± 2.4	0.854	−0.1 ± 2.9	
Ventilatory data					
Exhaled tidal volume (mL)					
V_T6	346.0 ± 58.7	349.2 ± 61.6	0.439	3.2 ± 15.1	0.367
V_T10	618.6 ± 79.1	615.2 ± 80.7	0.581	−3.4 ± 22.2	
Exhaled tidal volume (mL/kgPBW)					
V_T6	5.8 ± 0.3	5.9 ± 0.4	0.462	0.1 ± 0.3	0.367
V_T10	9.8 ± 0.3	9.8 ± 0.4	0.572	−0.1 ± 0.4	

	0 min	60 min	*P*	Change scores (0 min to 60 min)	*P*
Respiratory rate (breaths/min)					
V_T6	13.7 ± 1.9	13.6 ± 2.2	0.816	−0.1 ± 2.3	0.053
V_T10	8.8 ± 0.6	7.3 ± 0.9	≤0.001	−1.5 ± 1.1	
Minute volume ventilation (L/min)					
V_T6	4.7 ± 0.9	4.7 ± 0.9	0.914	−0.0 ± 0.8	0.004
V_T10	5.4 ± 0.6	4.5 ± 0.7	≤0.001	−1.0 ± 0.8	
Peak Pressure (cmH_2O)					
V_T6	16.4 ± 2.1	16.7 ± 1.9	0.500	0.3 ± 1.5	1.000
V_T10	20.3 ± 3.1	20.6 ± 3.2	0.486	0.3 ± 1.5	
Plateau Pressure (cmH_2O)					
V_T6	14.8 ± 2.2	15.2 ± 2.0	0.321	0.4 ± 1.6	0.473
V_T10	17.8 ± 3.1	18.6 ± 2.9	0.010	0.8 ± 1.0	
Compliance (mL/cmH_2O)					
V_T6	37.1 ± 10.7	36.1 ± 12.8	0.633	−1.1 ± 8.1	0.367
V_T10	50.6 ± 12.2	47.3 ± 12.1	0.023	−3.4 ± 4.9	
Arterial blood gas analysis					
pH					
V_T6	7.40 ± 0.03	7.42 ± 0.06	0.294	0.02 ± 0.06	0.742
V_T10	7.44 ± 0.04	7.45 ± 0.02	0.145	0.01 ± 0.03	
$PaCO_2$ (mmHg)					
V_T6	35.7 ± 3.1	34.8 ± 5.1	0.577	−0.9 ± 6.1	0.710
V_T10	31.9 ± 3.1	30.1 ± 2.3	0.075	−1.6 ± 3.0	
PaO_2 (mmHg)					
V_T6	149.9 ± 41.6	124.6 ± 33.0	0.060	−25.4 ± 46.0	0.717
V_T10	166.0 ± 58.7	146.6 ± 44.3	0.013	−20.1 ± 25.0	

	0 min	60 min	*P*	Change scores (0 min to 60 min)	*P*
Sat_aO_2 (%)					
V_T6	97.2 ± 0.9	96.6 ± 1.5	0.040	−0.6 ± 0.9	0.453
V_T10	97.4 ± 1.2	97.2 ± 1.8	0.219	−0.3 ± 0.9	
CO_3H^- (mmHg)					
V_T6	21.9 ± 1.5	22.8 ± 2.0	0.118	0.9 ± 1.9	0.032
V_T10	21.6 ± 1.5	20.9 ± 1.9	0.136	−0.6 ± 1.4	
Base excess					
V_T6	−2.2 ± 1.7	−1.3 ± 2.1	0.055	0.1 ± 0.9	0.033
V_T10	−1.9 ± 1.7	−2.3 ± 1.6	0.333	−0.8 ± 0.9	

Physiology, ventilatory and blood gas data from both tidal volume (V_T) groups and time points expressed as Mean ± SD. Comparison within each group from different time points was performed with a paired *t*-test. The change scores of each variable from 0 min to 60 min time points were compared between the two V_T groups using an independent *t*-test. Significant *P* value was accepted as $P < 0.05$.

CO_3H^- = bicarbonate concentration; E_TCO_2 = end tidal carbon dioxide; $PaCO_2$ = arterial carbon dioxide partial pressure; PaO_2 = arterial oxygen partial pressure; PBW = predicted body weight; Sat_aO_2 = arterial saturation of oxygen; Sat_pO_2 = peripheral saturation of oxygen by pulse-oximetry; V_T = tidal volume.