Albuterol Improves Alveolar-Capillary Membrane Conductance in Healthy Humans

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ABSTRACT

BACKGROUND: Beta-2 adrenergic receptors (β_2ARs) are located throughout the body including airway and alveolar cells. The β_2ARs regulate lung fluid clearance through a variety of mechanisms including ion transport on alveolar cells and relaxation of the pulmonary lymphatics. We examined the effect of an inhaled β_2-agonist (albuterol) on alveolar-capillary membrane conductance (DM) and pulmonary capillary blood volume (V_C) in healthy humans.

METHODS: We assessed the diffusing capacity of the lungs for carbon monoxide (DLCO) and nitric oxide (DLNO) at baseline, 30 minutes, and 60 minutes following nebulized albuterol (2.5 mg, diluted in 3 mL normal saline) in 45 healthy subjects. Seventeen subjects repeated these measures following nebulized normal saline (age = 27 ± 9 years, height = 165 ± 21 cm, weight = 68 ± 12 kg, BMI = 26 ± 9 kg/m^2). Cardiac output (Q), heart rate, systemic vascular resistance (SVR), blood pressure, oxygen saturation, forced expiratory volume at one-second (FEV$_1$), and forced expiratory flow at 50% of forced vital capacity (FEF$_{50}$) were assessed at baseline, 30 minutes, and 60 minutes following the administration of albuterol or saline.

RESULTS: Albuterol resulted in a decrease in SVR, and an increase in Q, FEV$_1$, and FEF$_{50}$ compared to saline controls. Albuterol also resulted in a decrease in V_C at 60 minutes post albuterol. Both albuterol and normal saline resulted in no change in DLCO or DM when assessed alone, but a significant increase was observed in DM when accounting for changes in V_C.

CONCLUSION: These data suggest that nebulized albuterol improves pulmonary function in healthy humans, while nebulization of both albuterol and saline results in an increase in DM/V_C.

KEYWORDS: sodium channels, lung fluid, lung diffusion, CFTR

Introduction

Beta-2 adrenergic receptors (β_2ARs) are located throughout the body and have been localized to the vascular, cardiac, and pulmonary tissues.[1–3] In the lungs, the β_2ARs are densely distributed along the airways from the trachea to the alveoli and are also located on the pulmonary lymphatics.[4–6] Stimulation of the β_2ARs from endogenous or exogenous sources results in a cascade of events that ultimately increases 3′,5′-cyclic adenosine monophosphate levels. This increase in 3′,5′-cyclic adenosine monophosphate leads to bronchial and vascular smooth muscle relaxation, and stimulates alveolar fluid clearance along with the dilation of the pulmonary lymphatics, both of which can clear fluid from the lungs.[7–11]

The β_2ARs are important in lung fluid regulation by the activation of epithelial Na$^+$ channels (ENaC) on alveolar cells. The stimulation of ENaC can result in the following:

(1) an increase in the number of ENaC; (2) an increase in the probability of an ENaC being open on the apical side of alveolar epithelial cells; or (3) an increase in the likelihood of an interaction with the cystic fibrosis transmembrane conductance regulator (CFTR) on alveolar cells. The interaction with the CFTR results in an augmentation in the chloride (Cl$^-$) channel function, also on the apical portion of alveolar cells.[12,13] This results in the movement of salt from the apical to the basolateral side of the cell and associated osmotic movement of fluid. In addition, stimulation of the β_2ARs may play a direct role in regulating Na$^+$K$^+$-ATPase on the basolateral portion of these cells, and likely results in smooth muscle relaxation of the pulmonary lymphatics, which can augment lung fluid clearance.[14,15]

While it is understood that certain disease states or environmental factors, such as acute lung injury, heart failure, or exposure to high-altitude, can lead to lung fluid accumulation,

which can become life-threatening, less is known about basal lung water movement within the airspaces of healthy humans. Kerem et al.[16] found that subjects with pseudohypoaldosteronism (a loss of function mutation of the ENaC) demonstrate increased fluid accumulation in the airways when compared to individuals without this mutation, suggesting that even healthy subjects with normal ventricular function and pulmonary arterial pressures exhibit a fluid flux into the airways. In addition to the active movement of ions across alveolar epithelial cells, aquaporins mediate the movement of water based on the difference in osmolarity between the intracellular space and the alveolar lumen in order to maintain ion and fluid homeostasis.[17] We have previously demonstrated an unexpected reduction in lung water following overnight exposure to normobaric hypoxia in healthy humans,[18] which suggests that the basal level of fluid in the lungs can be reduced following endogenous stimulation; however, the mechanism by which fluid was cleared from the lungs remains unclear. We have also previously demonstrated that rapid fluid loading increases lung water in healthy humans. Both the decrease in lung water with hypoxia and the increase in lung water with rapid fluid loading are dependent on the genetic variation of the β_2AR.[19] Additionally, the decrease in lung water with hypoxia is related to β_2AR density on lymphocytes.[20] Collectively, our previous work suggests an influence of this receptor in lung fluid balance in healthy subjects.[19,21] Paolillo et al.[22] observed that administration of a non-selective β_1 and β_2 blocker significantly decreased the alveolar-capillary membrane conductance (DM), a common index of lung fluid, whereas a selective β_1 blocker did not have an effect on DM, thereby further supporting the role of the β_2AR in lung fluid balance. Although there was early promise for β_2AR stimulation on lung fluid balance in patients with acute respiratory distress syndrome, more recent studies have demonstrated that this stimulation seems to worsen the condition, rather than improve the condition.[23–25] Because of inconsistency on previous work regarding lung fluid clearance in models of disease, it is important to first characterize this stimulation, using inhaled albuterol, in the healthy, intact lung.

Therefore, the focus of this study was to determine the influence of β_2AR stimulation (using nebulized albuterol, a short-acting β_2-selective agonist) on alveolar-capillary membrane conductance in healthy subjects. We hypothesized that the administration of albuterol would increase alveolar-capillary conductance, even when corrected for changes in pulmonary capillary blood volume, which would suggest an increase in lung fluid clearance.

Materials and Methods

To determine the effect of β_2AR stimulation on DM in healthy humans, we simultaneously assessed the diffusing capacity of the lungs for carbon monoxide and nitric oxide (DLCO and DLNO, respectively) and calculated the pulmonary capillary blood volume (V_C) and DM at baseline and at 30 and 60 minutes for one hour following the administration of nebulized albuterol. The study was reviewed and approved by both the Mayo Clinic and University of Arizona Institutional Review Boards (Mayo Clinic: IRB# 05-004402, University of Arizona: IRB# 08-0855-01). All aspects of the study were performed according to the principles of the Declaration of Helsinki.

Subjects

Forty-five healthy subjects were recruited from both Rochester, Minnesota, and Tucson, Arizona for the study to receive nebulized albuterol. Seventeen subjects returned for a second visit and served as a saline control group. All subjects provided written informed consent and had no exclusion criteria (known cardiopulmonary abnormalities, regular use of an inhaled β-agonist, β-blockade, smoking history, anemia, or pregnancy).

Protocol

The study involved one albuterol visit to our laboratory, which included screening tests and physiologic testing and, for a subset of subjects (n = 17), one saline visit with physiologic testing. Screening tests included a complete blood count (for the assessment of hemoglobin) and, in women, a pregnancy test. Physiologic testing included measures of DM and V_C, airway function, and cardiac output (Q) before and 30 and 60 minutes following the administration of albuterol or saline.

Administration of Albuterol

Albuterol sulfate (0.083%, 2.5 mg diluted in 3 mL normal saline; RiteDose Pharmaceuticals, Colombia, SC) was administered by nebulization over 12–15 minutes. Subjects wore a nose clip during the nebulization and were instructed to breathe normally throughout the nebulization, taking a full inspiration every two minutes to further distribute the drug. Each subject breathed the nebulized albuterol until the distribution cup was emptied. A Drive Power Neb II nebulizer was used for both saline and albuterol administration (model #18002, Port Washington, NY) with a Drive disposable nebulizer kit (NEB KIT 500R-12). For the saline arm, 3 mL of normal saline was administered in the same manner.

Data Collection

Assessment of DM and V_C. Measurement of DM and V_C have previously required the use of at least two, but preferably three oxygen tensions.[26] Tamhane et al.[27] demonstrated that measuring the disappearance of nitric oxide in concert with carbon monoxide provides an accurate assessment of V_C and DM using just one oxygen tension. Triplicate maneuvers of DLNO and DLCO were performed before the administration of albuterol and at 30 and 60 minutes following administration. The DLCO and DLNO were assessed using the rebreathe technique with gases sampled on a mass spectrometer (Perkin-Elmer, 1100) and NO analyzer (Sievers Instruments, Boulder, CO) using custom analysis software as described previously.[19,21,28]

Assessment of cardiovascular function and oxygen saturation. Heart rate, blood pressure, and oxygen saturation (SaO_2) were assessed and recorded at baseline, every three minutes during the nebulization and every 15 minutes following the administration of the β-agonist. Cardiac output was assessed at baseline and for every 15 minutes following nebulization and was determined using a previously validated rebreathe technique by measuring the disappearance of acetylene with the same bag containing the diffusion mixtures including the addition of 0.7% acetylene and 9% helium.[29,30] Gases were sampled using a mass spectrometer, which was integrated with custom analysis software for the assessment of Q̇. Heart rate was assessed using a 12-lead electrocardiogram (Marquette Electronics, Milwaukee, WI), blood pressure was determined using the auscultatory method, and oxygen saturation was monitored via pulse oximetry with a finger sensor (Nellcor N-600 Pulse Oximeter, Boulder, CO). Mean arterial pressure (MAP) was calculated using the equation: MAP = diastolic blood pressure (DBP) + 1/3(systolic blood pressure (SBP) – DBP). Systemic vascular resistance was calculated using the formula: $SVR = 80 \times (MAP - 10)/\dot{Q}$, where 10 is the estimated central venous pressure (mmHg). We also measured pulmonary arterial pressures before and following albuterol in eight healthy subjects using tricuspid regurgitant velocity from echocardiography to estimate right ventricular systolic pressure (RVSP).[31]

Assessment of pulmonary function. To assess the airway function, the subjects performed inspiratory capacity maneuvers followed by a maximal expiratory flow maneuver to residual volume (MEFV). From the MEFV maneuver, forced vital capacity (FVC), forced expiratory volume after one-second (FEV_1), and maximal expiratory flow after 50% of the FVC had been expired (FEF_{50}) were determined. All subjects were carefully instructed on how to perform the MEFV maneuver with special emphasis on taking a gradual but maximal inspiration prior to forced exhalation. The MEFV maneuver was performed on the same mouthpiece as the DLCO and DLNO measures. Flow and volume signals were digitized at a rate of 100 samples per second and stored for later analysis to determine changes in airway function using custom analysis software.

Data Analysis

All statistical analyses were two-tailed and performed using the SPSS statistical software package (v.21.0). A repeated measures analysis of variance (ANOVA) was used to determine the influence of albuterol on Q̇, HR, SBP, DBP, MAP, SVR, FVC, FEV_1, FEF_{50}, DLCO, DM, V_C, and DM/V_C over time. Prior to each ANOVA, the data were assessed for normalcy using a Levene's test. An alpha level of 0.05 was used for each ANOVA to determine significance. Post-hoc analysis specific to time were used to compare groups at the three time points (baseline, 30, and 60 minutes post-nebulization) when the ANOVA demonstrated a significant group interaction.

Results

Subjects' characteristics are presented in Table 1. Following the administration of albuterol, Q̇ increased significantly from baseline (Q̇ = −0.8% ± 19% vs. 14% ± 20%, 2% ± 17% vs. 12% ± 22%, saline vs. albuterol percent change from baseline at 30 and 60 minutes, respectively) and SVR decreased significantly from baseline (SVR = 2% ± 14% vs. −14% ± 17%, 1% ± 15% vs. −10% ± 17%, saline vs. albuterol percent change from baseline at 30 and 60 minutes, respectively), but there was no significant change in blood pressure (SBP, DBP, MAP), HR, or SaO_2 in either group (Table 2). Albuterol resulted in no change in FVC but resulted in an improvement in FEV_1 and FEF_{50} compared to the saline control group (FEV_1 = −2.4% ± 3.8% vs. 4.1% ± 7.1%, −3.2% ± 4.6% vs. 3.7% ± 7.0%; FEF_{50} = −1.0% ± 7.7% vs. 18.7% ± 21.6%, −1.2% ± 0.2% vs. 20.2% ± 18.6%, saline vs. albuterol percent change from baseline at 30 and 60 minutes, respectively, Table 3).

Albuterol resulted in a significant decrease in DLCO at 60 minutes post nebulization when compared to 30 minutes post nebulization (P-ANOVA < 0.05, df = 2, F = 3.302; Fig. 1, panel A). The decrease in DLCO was primarily driven by a drop in V_C, which was significant at 60 minutes when compared to both baseline and 30 minutes post nebulization (P-ANOVA < 0.05, df = 2, F = 7.241; Fig. 1, panel B). There were no changes in DM in either group when considered alone (P-ANOVA = 0.443, df = 2, F = 0.823; Fig. 1, panel C), but DM was significantly higher than baseline when accounting for changes in V_C (DM/V_C, P-ANOVA < 0.05, df = 2, F = 7.667; Fig. 1, panel D). With saline, we found no change in the pulmonary function or in DLCO, DM, or V_C when considered independently; however, DM/V_C with the nebulization of 3 mL normal saline did increase at 30 minutes and 60 minutes post saline administration (P-ANOVA = 0.140, df = 2, F = 2.090; Figure 1, panel D). We also found that the administration of albuterol resulted in a trend toward a reduction in RVSP (P = 0.09 at 30 minutes), which may help to explain the reduction in V_C, if there is a reduction in pulmonary vascular tone (Fig. 2).

Discussion

We found that the administration of albuterol and saline increased the alveolar-capillary membrane conductance when

Table 1. Subject characteristics.

	ALBUTEROL	SALINE
N (% female)	45 (56)	17 (59)
Age (years)	28 ± 7	27 ± 9
Height (cm)	169 ± 17	165 ± 21
Weight (kg)	72 ± 16	68 ± 12
Body Mass Index (kg/m²)	26 ± 8	26 ± 9

Note: Values are presented as mean ± SD.

Table 2. Physiologic parameters at baseline and after nebulization.

TIME	BASELINE		POST-NEBULIZATION (MINUTES)				P-ANOVA
			30		60		
GROUP	ALBUTEROL	SALINE	ALBUTEROL	SALINE	ALBUTEROL	SALINE	
Heart rate (beats/min)	73 ± 10	74 ± 10	76 ± 11	72 ± 9	75 ± 10	73 ± 10	0.720
Systolic BP (mmHg)	108 ± 10	105 ± 10	106 ± 11	104 ± 11	106 ± 11	106 ± 10	0.313
Diastolic BP (mmHg)	71 ± 8	69 ± 6	69 ± 9	69 ± 8	71 ± 9	70 ± 6	0.236
Mean arterial pressure (mmHg)	83 ± 8	81 ± 7	81 ± 8	81 ± 9	83 ± 9	82 ± 7	0.138
Cardiac output (L/min)	4.2 ± 1.3	3.7 ± 1.1	4.6 ± 1.3*	3.6 ± 0.8	4.5 ± 1.1*	3.6 ± 0.7	0.167
SVR (dynes*sec/cm^5)	1527 ± 424	1616 ± 365	1303 ± 345*	1663 ± 433	1365 ± 354*	1618 ± 317	**0.015**
Oxygen saturation (%)	99 ± 2	99 ± 1	99 ± 1	99 ± 1	99 ± 1	99 ± 1	0.319

Notes: Values are presented as mean ± SD. *$P < 0.05$ when compared to saline.
Abbreviations: BP, blood pressure; *P*-ANOVA, ANOVA included baseline and each time point post nebulization *by condition*.

corrected for pulmonary capillary blood volume in healthy humans. With albuterol administration, we found a significant drop in DLCO at 60 minutes post albuterol administration that was primarily due to a drop in pulmonary capillary blood volume. There was a particularly large improvement in DM/V_C. With saline alone, we found no improvement in DLCO, DM, or V_C, but we did find an increase in DM/V_C. As expected, albuterol, but not saline, improved the pulmonary function as assessed by FEV$_1$ and FEF$_{50}$. Because there was no increase in Q or improvement in pulmonary function with saline alone, it is clear that the improvement in DM/V_C does not lie in the delivery of the drug, as the drug had predictable effects that were not seen with saline. Previous research has demonstrated that the administration of Na$^+$ to the apical portion of alveolar cells decreases the net activity of ENaC.[32] In addition, alterations in apical Cl$^-$ can have an effect on ENaC activity.[33] In the present study, we hypothesize that the increase in DM/V_C with saline was likely secondary to alterations in ion and cation regulation due to the administration of Na$^+$Cl$^-$ on the airway surface. This removal of Na$^+$Cl$^-$ from the apical side of the cell to the basolateral side of the cell likely resulted in paracellular removal of water as well. We have recently shown that rapid fluid loading challenges the ability of the lungs to handle fluid and that this effect was dependent on genetic variation of the β$_2$AR.[19] In addition, we have shown that short-term hypoxic exposure reduced lung water in healthy humans and

that this reduction in lung fluid was related to the density of β$_2$AR on lymphocytes.[20,21] Taken together, two studies[18,20] demonstrated that there is a basal level of lung fluid that can be cleared from healthy lungs and confirm the results of the previous work that has demonstrated an important influence of the β$_2$AR in lung fluid regulation.[6,10,16,29,34,35]

β$_2$ARs Influence Lung Fluid Balance in States of Health and Disease

There are several clinical and environmental conditions that challenge the ability of the lungs to handle fluid, including acute lung injury, heart failure, and exposure to high altitude. Therapies that aim to reduce lung fluid accumulation (either through decreasing pulmonary pressure and capillary leakage, or through increasing active fluid clearance) are key to attenuate pulmonary edema. At birth, the fluid in the airspace of the lungs is cleared following the stimulation of the β$_2$ARs by circulating catecholamines.[8] In addition, over-expression of the β$_2$ARs increases alveolar fluid clearance in mice, and the administration of salmeterol has been shown to decrease the susceptibility for developing high-altitude pulmonary edema in healthy humans who travel to high altitudes.[7,36] More recently, work in animals has demonstrated the importance of β$_2$AR-mediated fluid clearance in acute lung injury and in a model of heart failure.[7,34,37–40] Although there is clearly a role for β$_2$AR-mediated fluid clearance in acute lung injury, and early

Table 3. Pulmonary function before and for one hour following the administration of albuterol.

TIME	BASELINE		POST-NEBULIZATION (MINUTES)				P-ANOVA
			30		60		
GROUP	ALBUTEROL	SALINE	ALBUTEROL	SALINE	ALBUTEROL	SALINE	
FVC (L)	4.6 ± 1.1	4.3 ± 1.2	4.5 ± 1.1	4.2 ± 1.2	4.5 ± 1.1	4.1 ± 1.2	**0.015**
FEV$_1$ (L)	3.8 ± 0.8	3.5 ± 0.8	3.9 ± 0.9*	3.4 ± 0.9	3.9 ± 0.9*	3.4 ± 0.9	0.503
FEF$_{50}$ (L/min)	4.3 ± 1.0	3.9 ± 1.0	5.0 ± 1.4*	3.9 ± 1.0	5.1 ± 1.4*	3.9 ± 1.1	**0.002**

Notes: Values are presented as mean ± SD. *$P < 0.05$ when compared to saline.
Abbreviations: FVC, forced vital capacity; FEV$_1$, forced expiratory volume at one-second; FEF$_{50}$, forced expiratory flow at 50% of the FVC.
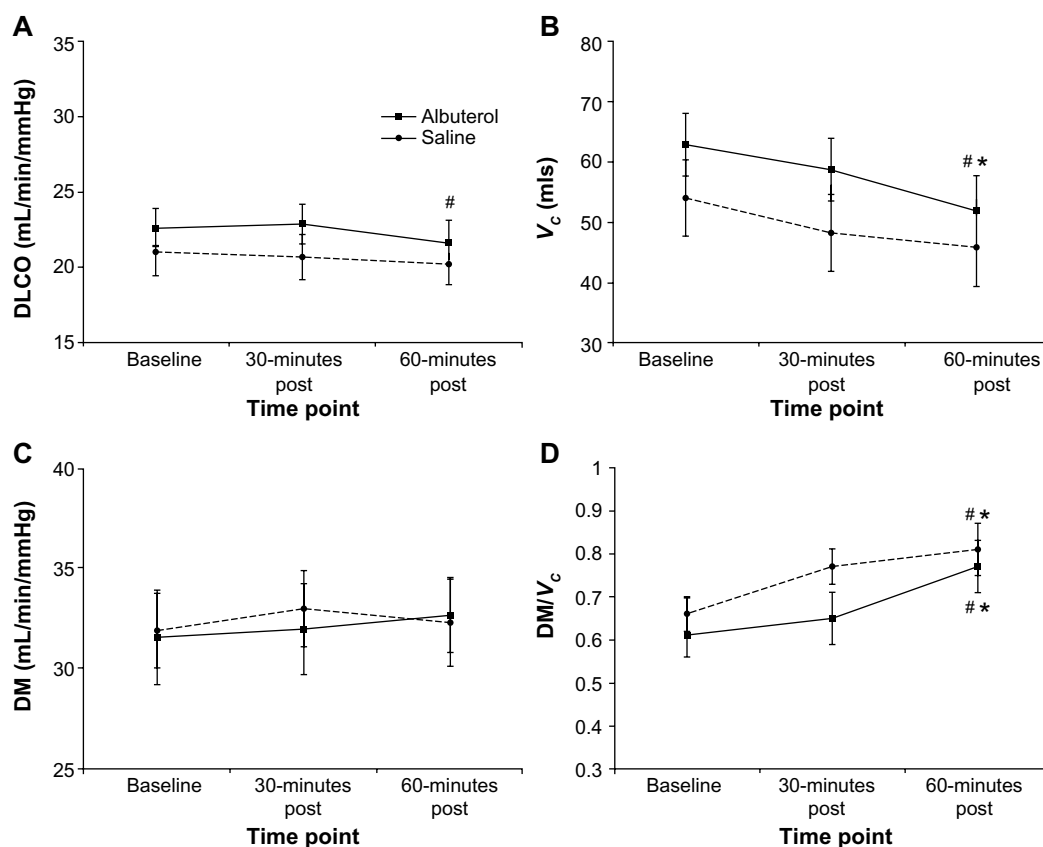
Figure 1. Change in the diffusing capacity of the lungs for carbon monoxide (DLCO) and the components of DLCO following the administration of albuterol or saline. The broken line and circle data points represent the saline arm and the solid line with the square data points represent the albuterol arm. The *x* axis represents the time point, the *y* axis represents DLCO (panel **A**), pulmonary capillary blood volume (V_C, panel **B**), alveolar capillary membrane conductance (D_M, panel **C**), and alveolar-capillary membrane conductance corrected for pulmonary capillary blood volume (D_M/V_C, panel **D**). **Notes:** [#]$P < 0.05$ vs. 30 minutes post treatment within group; [*]$P < 0.05$ vs. baseline within group.
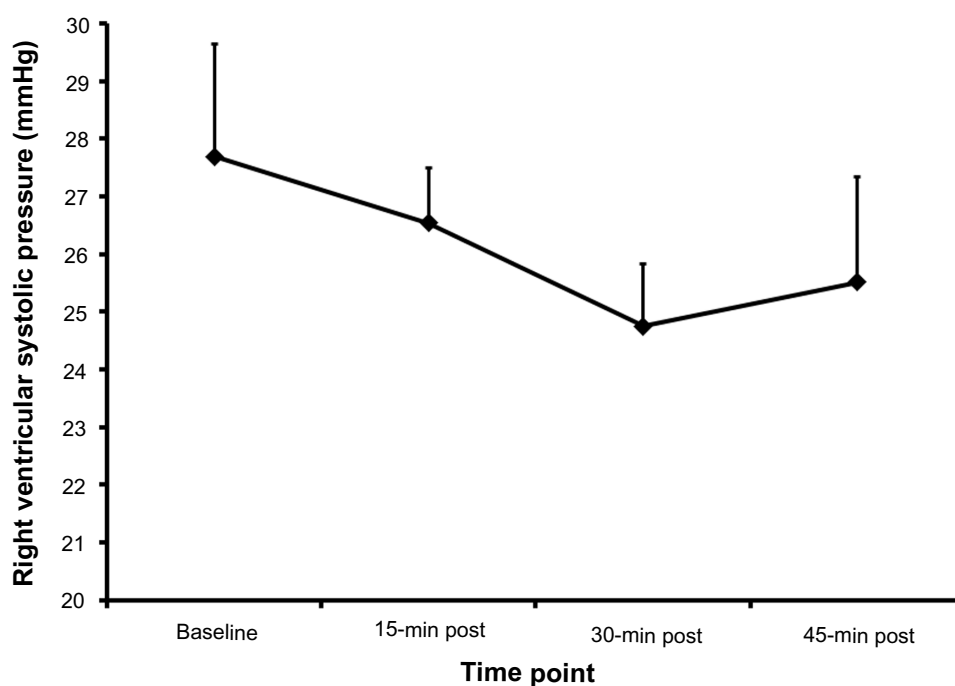

Figure 2. Right ventricular systolic pressure at baseline and following the administration of albuterol. The *y* axis represents right ventricular systolic pressure in a sub-group of subjects (n = 8). The *x* axis represents the time point of the measure baseline, 15 minutes post albuterol, 30 minutes post albuterol, and 45 minutes post albuterol.

trials demonstrated some benefit to these patients,[25] recent trials have demonstrated no clinical benefits from treatment with albuterol in humans with acute lung injury.[23,41] However, despite strong evidence about the role of the β_2ARs in the role of lung fluid clearance in times of fluid overload or stress, less is known about the regulation of lung water in healthy subjects without exposure to physiological or environmental stressors. The findings of the present study are the first, to our knowledge, that demonstrate stimulation of lung fluid clearance and a resultant increase in alveolar-capillary membrane conductance with an inhaled β-agonist under baseline conditions in healthy humans. However, normal saline also resulted in an increase in alveolar conductance (when corrected for pulmonary capillary blood volume), suggesting a possible direct role of Na^+ and Cl^- ion on ion channel function.

In addition, this study helps to define a possible mechanism by which short-term hypoxic exposure resulted in a decrease in lung water in healthy humans in a previous study by our group, where we proposed the possible influence of the β_2ARs in the decline in lung fluid following 17 hours of hypoxic exposure.[21] In this previous study, there was an observed 34% increase in venous epinephrine. Similar to albuterol, epinephrine is a β-agonist, suggesting a homologous mechanism of lung fluid clearance. In a follow-up study, we demonstrated that the decrease in lung water was related to β_2AR density; however, there are also non-β_2AR-mediated mechanisms, which could have resulted in the loss of lung water as observed in this previous study, such as the increased lymphatic flow due to increases in ventilation and greater fluctuations in intrathoracic pressure that is common with hypoxic exposure.[35] In this same study, we found an increase in V_C with exposure to hypoxia (a well-established result of hypoxic pulmonary vasoconsitriction) at the same time as we demonstrated a reduction in lung water. Future studies could take our assessment of V_C in the present study and further explore if the increase in V_C, which has been demonstrated with hypoxia, is abolished with inhalation of albuterol.

β_2AR Stimulation and Pulmonary Capillary Blood Volume

Interestingly, we demonstrated a reduction in V_C following the administration of albuterol but not saline. Although this was unexpected, we hypothesize that this may be due to the stimulation of the β_2AR on the pulmonary vessels, resulting in a loss of post-capillary vascular tone and a reduction in the number of recruited pulmonary capillaries. This is supported through the observed drop in systemic vascular resistance, suggesting dilation of the peripheral vessels. It is also likely that there was a simultaneous pulmonary vascular dilation and a drop in pulmonary vascular pressures, which could decrease pulmonary vascular tone and result in a lower V_C. In agreement with this finding, previous work has demonstrated that pulmonary vasodilation (using nitroprusside) results in an increase in intrapulmonary shunting, despite increases in cardiac output,

which suggests a drop in the number of pulmonary capillaries in contact with ventilated alveoli.[42,43]

Limitations

Although we did complete a placebo aim for the study, this was not performed in a randomized double-blind manner; it is not possible for the subjects to determine whether they are inhaling nebulized saline with albuterol or nebulized saline alone. Previous work has demonstrated that saline alone does not influence several pulmonary function parameters (exhaled nitric oxide, FVC, FEV_1) when compared to drugs diluted with saline; however, inhaled saline alone has been shown to improve symptoms in patients with chronic obstructive pulmonary disease.[44–46] Future studies should focus on placebo-controlled studies using albuterol administration (vs. placebo) with metered-dosed inhalers.

Conclusions

This study is the first to demonstrate an improvement in alveolar-capillary membrane conductance with a nebulized β-agonist in healthy humans. These findings confirm previous *in vitro* and *in vivo* work that has demonstrated the importance of the β_2ARs on lung fluid clearance; however, the role of the application of Na^+Cl^- itself on lung water regulation must further be elucidated in humans. The results of this study could become particularly important in future work exploring lung fluid clearance using an inhaled β-agonist in conditions of alveolar flooding such as heart failure.

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Author Contributions

EMS, TPO, SL, and BDJ all helped with data collection, analysis, and manuscript preparation. NET and SEB helped with data analysis and manuscript preparation. All authors reviewed and approved of the final manuscript.

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