

RESEARCH ARTICLE

Effects of superimposed pressure oscillations on a chronic sensitized airways mouse model

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Roos KL, Al-Jumaily AM. Effects of superimposed pressure oscillations on a chronic sensitized airways mouse model. *Am J Physiol Lung Cell Mol Physiol* 318: L900–L907, 2020. First published February 26, 2020; doi:10.1152/ajplung.00348.2019.—The hyperconstriction of airway smooth muscle (ASM) is the main driving mechanism during an asthmatic attack. The airway lumen is reduced, resistance to airflow increases, and normal breathing becomes more difficult. The tissue contraction can be temporarily relieved by using bronchodilator drugs, which induce relaxation of the constricted airways. In vitro studies indicate that relaxation of isolated, precontracted ASM is induced by mechanical oscillations in healthy subjects but not in asthmatic subjects. Further, short-term acute asthmatic subjects respond to superimposed pressure oscillations (SIPO) generated in the range of 5–15 Hz with ~50% relaxation of precontracted sensitized airways. Mechanical oscillations, and specifically SIPO, are not widely characterized in asthmatic models. The objective of this in vivo study is to determine the effects of a range of oscillation patterns similar to our previous acute study differing from normal breathing. Both healthy and sensitized mice were observed, with their responses to SIPO treatments measured during induced bronchoconstriction resulting from acetylcholine (ACh) challenge. SIPO-generated results were compared with data from treatments using the bronchorelaxant isoproterenol (ISO). The study shows that SIPO in the range of 5–20 Hz induces relaxation in chronic sensitized airways, with significant improvements in respiratory parameters at SIPO values near 1.7 cmH₂O irrespective of the frequency of generation.

chronic asthma; mouse model; pressure oscillation; sensitized airways

INTRODUCTION

Asthma is a respiratory disease that manifests as intermittent shortness of breath, coughing, tightness of the chest, and wheezing, with additional persistent bouts of breathlessness (50). Physiologically, asthmatic airways are different from healthy airways (10, 18, 36) and constrict in response to multiple factors. Inflammatory responses, bronchospasm, and airway hyperreactivity combine to create a life-threatening condition in worst-case scenarios. Mechanical constriction of asthmatic airway smooth muscle (ASM) is the predominant factor in severe reduction of measured respiratory parameters through the airways (24, 33, 39, 41).

Asthmatic individuals respond differently to allergen challenges compared with those with healthy airways (7, 13). Healthy airways fail to collapse in a manner indicative of inflammatory, bronchospastic, or hyperreactive tissues of asth-

matics. Asthmatic airway remodeling is seen as a distinct structural change to the airway tissue (4) resulting from long-term efforts to overcome disease indicators. Proliferation of epithelial cells, thickening of the reticular basement membranes, and subepithelial fibrosis combine to decrease the compliance of the airway (5). The increases in vascular flow to support this proliferation also aid in the accumulation of smooth muscle mass surrounding the airways.

Anti-inflammatory and bronchodilator medications are used in asthma therapies to relieve airflow challenges (46). The implementation of alternative methods of treatment is aimed at decreasing the amounts of drugs used in suppressing symptoms. Given that healthy and asthmatic individuals respond to differential breathing patterns (7, 37), stretch and ASM mechanics are areas of focused research intent on alleviating airway constriction (26, 32). Tidal oscillations (TO) and deep inspiration (DI) therapies are ineffective as treatments for precontracted asthmatic ASM relative to bronchodilator options (7, 37). However, inhibition of active force and stiffness by imposing parameters that differ from traditional TO and DI values has been shown (7, 15, 37, 43, 48), supporting the impact of applied oscillations on relieving ASM contractions in the presence of bronchoconstrictors (11, 48).

Sensitization of mice by using allergens emulates immunological, inflammatory, and respiratory responses of chronic asthma (1, 14, 20, 25, 29, 42). Assessment of the effects of length oscillations, introduced in the form of applied superimposed pressure oscillations (SIPO), over spontaneous breathing is a novel approach to overcoming chronic asthmatic airway constriction. Pretreatment of airways with ACh chemical challenges mimics airway hyperresponsiveness (AHR), and post-challenge treatment with isoproterenol (ISO) as the reference bronchodilator provides context for the resistive and compliance effects obtained from SIPO values utilized in this study.

MATERIAL AND METHODS

Mice. Female BALB/c mice (8–16 wk of age) were supplied by the Auckland University of Technology Animal Facility (Auckland, New Zealand). BALB/c mice are notably reactive to sensitizations (35) and are therefore appropriate for this study. Mice were housed with free access to food and water ad libitum. All protocols were approved by the University of Auckland Animal Ethics Committee and experiments were conducted at the Auckland University of Technology Animal Facility in accordance with the Animal Welfare Act 1999.

Animal model and sensitization. A mixture of three allergens obtained from Greer Laboratories (Lenoir, NC) was prepared in 0.9% saline solution to sensitize the subjects: dust mite (*Dermatophagoides farinae*, 0.3 µg/µL), ragweed pollen (*Ambrosia artemisiifolia*, 3.3 µg/µL), and Aspergillus mold (*Aspergillus fumigatus*, 0.3 µg/µL)

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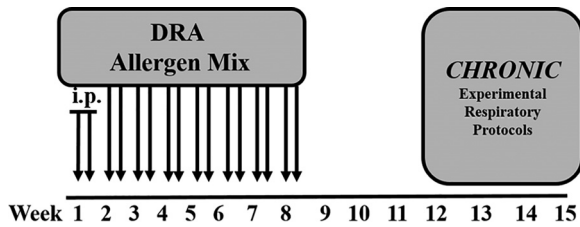


Fig. 1. Sensitization protocol to generate long-term chronic asthmatic model. An initial twice-weekly intraperitoneal injection of allergen, followed by twice-weekly inhaled treatments for a period of 8 wk, established the model. Healthy controls received 0.9% saline in place of DRA allergen mix. All subjects were rested for 3 wk prior to respiratory protocols.

were combined and stored at 4°C until use. The methods used for long-term sensitization were similar to those described in Goplen et al. (17), given the obtained asthmatic symptoms of airway tissue hypertrophy and hyperplasia and increased numbers of eosinophils, mast cells, and molecular markers, along with AHR.

For the chronic asthma group (CHR), mice were initially sensitized with an intraperitoneal injection of DRA allergen mix suspended in 0.9% saline. The DRA allergen mix was administered via intraperitoneal injection on 2 days of the first week of sensitization, followed by nebulization [inhaled (inh.)] 2 days each week for weeks 2–8. A 3-wk rest period preceded experimental protocols (Fig. 1). Control animals received only 0.9% saline as intraperitoneal and inh. treatments. Baseline saline aerosol as well as the acetylcholine (ACh) and isoproterenol (ISO) drug treatments were delivered to study animals by a Harvard aerosol jet nebulizer apparatus (P/N 73-1963).

Assessment parameters. Airway hyperresponsiveness (AHR) in this study was determined by measurements of two primary pulmonary health parameters utilizing tracheal intubations and esophageal cannulation in conjunction with plethysmography. Lung resistance (R_L) and dynamic compliance (C_{dyn}) were measured in response to concentrations of nebulized bronchoconstrictor (ACh, 10^{-4} M) and bronchorelaxant (ISO, 10^{-6} M). In line with linear single-compartment calculations, primary determinants of the parameters include

tidal volume (T_v), flow rate (Q), peak inspiratory pressure (PIP), positive end-expiratory pressure (PEEP), and transpulmonary pressure (P_{tp} ; Fig. 2, *a*). R_L and C_{dyn} equations assume that P_{tp} is equal to the sum of the pressure required to overcome resistive and elastic forces (29, 38, 45).

$$R_L = [P_{tp} - (T_v / C_{dyn})] / Q \quad (1)$$

$$C_{dyn} = T_v / (PIP - PEEP) \quad (2)$$

In vivo ASM length oscillations were produced using applied oscillating pressure as previously described (28). Briefly, a waveform generator (Hewlett Packard) for frequency and amplitude, power amplifier (LDS PA25E, Ling Diagnostic System LTD), and oscillating shaker (model V203, Ling Diagnostic System LTD) generated the SIPO values of the study. DRA allergen mix and drugs were delivered as aerosol droplets (Jet Nebulizer, no. 731963, Harvard Apparatus). Measurement of raw breathing parameters was accomplished using two differential pressure transducers along with their amplifiers. Data acquisition was performed using PowerLab 16/30 in conjunction with its LabChart software (ADInstruments).

The experimental layout for this study used a combination of component and auxiliary systems (Fig. 2, *b*). The data collection surface (with warming blanket) for animal placement during protocols was maintained at 37°C. The pneumotachometer was connected to the tracheal cannula by connective tubing. Separate tubing also supplied the nebulized solutions to the subjects from the nebulizer. The differential low-pressure transducer signal attached to the pneumotachometer was run through the TAM-A transducer amplifier module. Transpulmonary pressures were detected through the Honeywell pressure transducer-linked esophageal cannula. Transducer signals were carried to the 16-channel data acquisition board (PowerLab component), and data were processed using LabChart software.

Experimental protocol. Prior to placement of tracheal and esophageal cannulas, mice were anesthetized with intraperitoneal injections of ketamine (40 μ g) and xylazine (10 μ g)/g weight. Data acquisition of (spontaneously breathing) pressure, volume, and flow-related information was sent directly from sensors associated with the cannulas

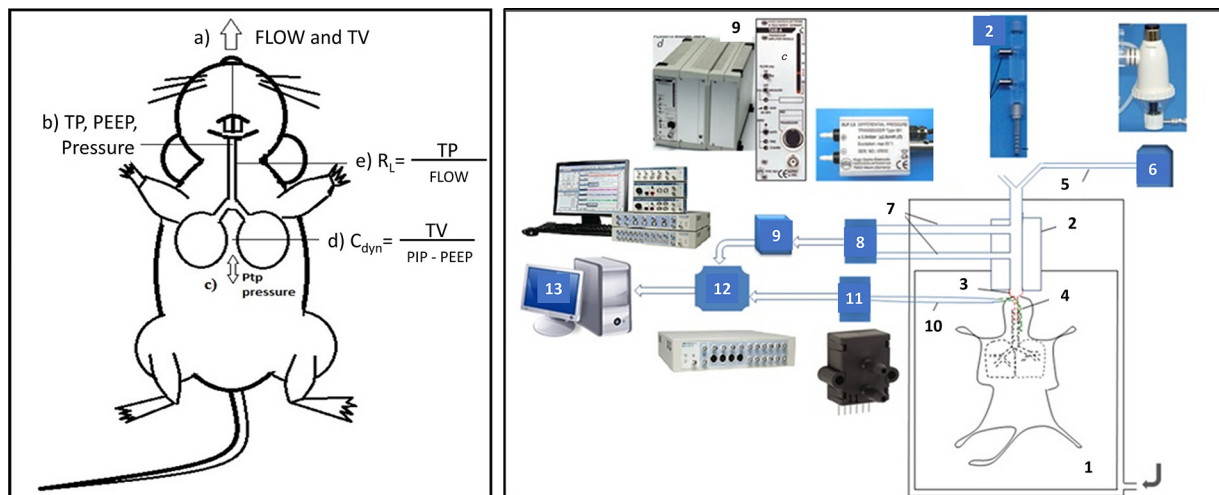


Fig. 2. *Left*: representative map of respiratory parameter measurements. Components used to gather data for the parameters are the pneumotachometer, tracheal cannula, esophageal cannula, and associated differential pressure transducer elements (refer to *b*). The pneumotachometer measures flow and tidal volume (T_v) data (*a*) as well as tracheal pressure (*b*). Transpulmonary pressure, P_{tp} (*c*), is measured from the esophageal cannula's midthorax placement between the lungs, allowing for indirect calculation of dynamic compliance, C_{dyn} . C_{dyn} is directly calculated from T_v , peak inspiratory pressure (PIP), and positive end-expiratory pressure (PEEP) data (*d*), while lung resistance, R_L , is calculated from transpulmonary pressure (T_p) and flow values (*e*). *Right*: the experimental layout for respiratory parameter protocols in this study. Components and auxiliary system combinations are labeled as follows: 1) data collection surface (with warming blanket) for animal placement during protocols; 2) pneumotachometer; 3) tracheal cannula; 4) esophageal cannula; 5) tube supplying the nebulized solutions; 6) nebulizer; 7) connection tubes from pneumotachometer to transducer; 8) differential low-pressure transducer; 9) TAM-A transducer amplifier module (*c*) and PLUGSYS Minicase (*d*); 10) connection pipe from catheter to transducer; 11) Honeywell pressure transducer; 12) 16-channel data acquisition board (PowerLab component); and 13) computer with data acquisition (LabChart software).

Table 1. ACh-ISO challenge and treatment responses

	R_L , $\text{cmH}_2\text{O} \cdot \text{mL}^{-1} \cdot \text{s}^{-1}$		C_{dyn} , $\mu\text{L}/\text{mmH}_2\text{O}$	
	HEA	CHR	HEA	CHR
ACh	2.1 ± 0.1	5.3 ± 0.4	15.2 ± 0.6	6.5 ± 0.6
ISO	1.7 ± 0.1	3.5 ± 0.2	18.9 ± 1.2	9.5 ± 0.8

Values are means $\pm$ SE. Healthy (HEA) and chronic (CHR) long-term sensitized mouse respiratory parameters were measured and compared as lung resistance (R_L) and dynamic compliance (C_{dyn}) values, $n = 7$. Significant increases in R_L and decreases in C_{dyn} were observed when comparing CHR parameters against HEA controls. ISO, isoproterenol.

to PowerLab and subsequently processed. Each set of respiratory challenges is separated from any other test by a rest period where no manipulations are carried out.

Baseline (saline) and applied treatments. Baseline readings were taken during exposure to nebulized saline solution for a period of 2 min, followed by a 1-min rest period. A 1-min ACh challenge preceded a 1-min treatment with either ISO or SIPO. The order in which the ISO or oscillation challenge was applied to a given study animal was taken from a randomized list to ensure that linear progression through the applied pressures was avoided. A 2-min rest period separated the end of one test from the next test starting with saline aerosol. Testing for any subject was concluded when respiratory parameters associated with calculating R_L (Eq. 1) did not recover from zero (terminal) values.

Superimposed pressure oscillations. Applied SIPO values (ranging from 0.4 cmH_2O to 3.6 cmH_2O) in this study have shown effectiveness in both in vitro and in vivo studies (28) and are similarly characterized by their frequency and amplitude. Four amplitudes, increasing stepwise by 100 mV to increase the generated pressures, were coordinated with each frequency tested. Five-hertz frequency and 100–400 mV amplitudes defined 4 of the SIPO applications (0.8, 1.6, 2.6, and 3.6 cmH_2O). Additionally, 10 (0.7, 1.5, 2.4, and 3.3 cmH_2O), 15 (0.4, 1.1, 1.7, and 2.4 cmH_2O), and 20 Hz (0.4, 1.1, 1.7, and 2.3 cmH_2O) groups provided the remaining pressures.

Statistical analysis. The software package GraphPad Prism 7 (GraphPad Software, Inc., La Jolla, CA) was used for data analysis of R_L and C_{dyn} results. Results are shown as the means $\pm$ SE with $n = 7$ for saline, ACh, and ISO data sets; SIPO data sets are generated from $n = 5$. Statistically significant differences are considered for values of $P < 0.05$. For the purpose of this study, because of its small sample sizes with outcomes that are not normally distributed, the t test, nonparametric comparison, and one- and two-way ANOVA applications were used for analysis. Additionally, postanalysis Dunnett testing was used for identifying differences in data obtained from the protocols utilized in this study.

RESULTS

ACh-ISO challenge and treatment responses. The R_L of sensitized mice to ACh was 157% higher than healthy mice, and the C_{dyn} of healthy (HEA) mice treated with ISO was 134% higher than CHR mice receiving the same treatment (Table 1). Analysis of R_L and C_{dyn} values showed a significant increase in the sensitized group's response to ACh-induced bronchoconstriction and significant decrease in the sensitized group's response to ISO treatments for bronchodilation, providing evidence for the presence of AHR in the airways of the sensitized mice. The R_L and C_{dyn} values of ACh and ISO in this study are presented in figures as comparative measures for all applied SIPO frequency groups.

SIPO treatment responses. Precontracted airways, stimulated by ACh (10^{-4} M) to mimic an asthmatic attack, were

treated with SIPO values to ascertain respiratory response parameters. Measurements of R_L and C_{dyn} calculations from HEA and CHR subjects were compiled and evaluated against ACh and ISO reference values. Figures 3 and 4 show the effects of SIPO generated from 5, 10, 15, and 20 Hz (with 100–400 mV amplitudes) on R_L and C_{dyn} values, respectively. Units of SIPO are presented as applied pressure values of cmH_2O .

The 5-Hz sensitized R_L values range from 3.37 $\text{cmH}_2\text{O} \cdot \text{mL}^{-1} \cdot \text{s}^{-1}$ for 200 mV treatments up to a high of 5.29 $\text{cmH}_2\text{O} \cdot \text{mL}^{-1} \cdot \text{s}^{-1}$ with ACh challenge. The lowest pulmonary resistance value for sensitized subjects was achieved from the 1.6 cmH_2O SIPO set, which was also comparable to ISO treatment. Healthy group R_L values range from 1.66 $\text{cmH}_2\text{O} \cdot \text{mL}^{-1} \cdot \text{s}^{-1}$ for ISO treatments up to a high of 2.06 $\text{cmH}_2\text{O} \cdot \text{mL}^{-1} \cdot \text{s}^{-1}$ with ACh challenge. The lowest pulmonary resistance value for healthy subjects was achieved from the ISO and 3.6 cmH_2O SIPO sets. Application of the 5 Hz-generated SIPO waveforms resulted in a significant decrease in the R_L of the sensitized airway for all treatments. The most significant decrease (36%) was found in the 1.6 cmH_2O set, where the resistance was akin to treatment with ISO. The remaining three treatments (0.8, 2.6, and 3.6 cmH_2O) were comparable to one another, and while still significantly lower than ACh, none of the treatments was equivalent to ISO. The 5-Hz sensitized C_{dyn} values range from 6.5 $\mu\text{L}/\text{mmH}_2\text{O}$ for ACh challenges up to a high of 9.5 $\mu\text{L}/\text{mmH}_2\text{O}$ with ISO treatment. The 200 mV (1.6 cmH_2O) treatment is also comparable to ISO. Healthy group C_{dyn} values range from 15.2 $\mu\text{L}/\text{mmH}_2\text{O}$ for ACh challenge up to a high of 18.9 $\mu\text{L}/\text{mmH}_2\text{O}$ with ISO treatments. Application of the 5 Hz-generated SIPO waveforms resulted in 2 of 4 treatments having a significant increase in the C_{dyn} of the sensitized airways. The most significant increase (45%) was found in the 200 mV (1.6 cmH_2O) set, where the dynamic compliance was statistically equivalent to treatment with ISO.

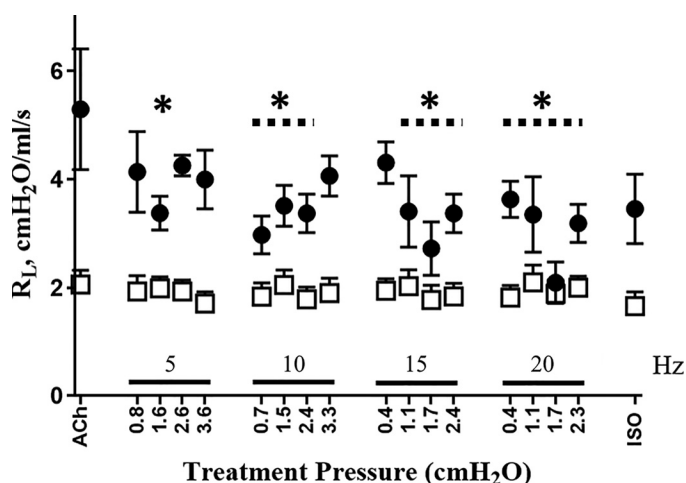


Fig. 3. Comparison of lung resistance (R_L) between asthmatic and healthy mice. The y-axis corresponds to the value of R_L observed with different experimental conditions presented on the x-axis [$n = 7$ for all healthy and chronic ACh and isoproterenol (ISO) data, healthy ($\square$) superimposed pressure oscillation (SIPO) data, $n = 5$ for all chronic ($\bullet$) SIPO data]. Statistical significance is indicated (* $P < 0.05$). The x-axis data sets are arranged in order of frequency groups of 5-, 10-, 15-, and 20 Hz. Increasing pressure values in each group are the result of amplitudes increasing from 100 mV to 400 mV as described.

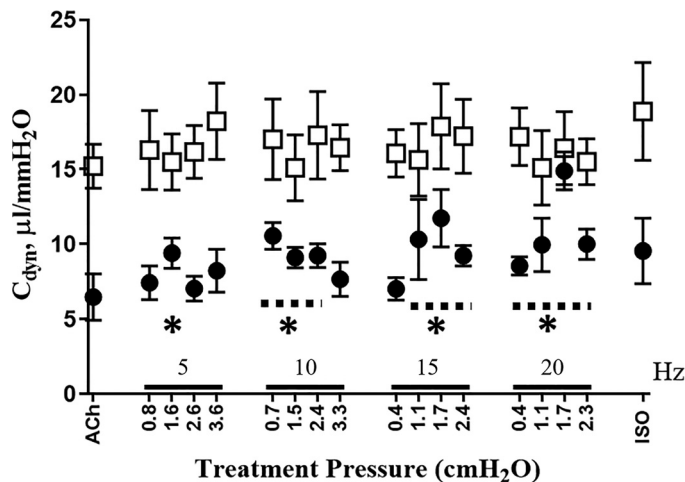


Fig. 4. Comparison of dynamic compliance (C_{dyn}) between asthmatic and healthy mice. The y-axis corresponds to the value of C_{dyn} observed with different experimental conditions presented on the x-axis [$n = 7$ for all healthy and chronic ACh and isoproterenol (ISO) data, $n = 5$ for all healthy (□) superimposed pressure oscillation (SIPO) data, $n = 5$ for all chronic (●) SIPO data]. Statistical significance is indicated (* $P < 0.05$). The x-axis data sets are arranged in order of frequency groups of 5, 10, 15, and 20 Hz. Increasing pressure values in each group are the result of amplitudes increasing from 100 to 400 mV as described.

The 400 mV (3.6 cmH₂O) set increased C_{dyn} by 26% compared with ACh, while the remaining two treatments (0.8 and 2.6 cmH₂O) were comparable to one another and with ACh challenge, indicating no benefit toward offsetting the mimicked asthma attack from the standpoint of compliant airways.

The 10-Hz sensitized R_L values range from 2.97 cmH₂O·mL⁻¹·s⁻¹ for 100 mV treatments up to a high of 5.29 cmH₂O·mL⁻¹·s⁻¹ with ACh challenge. The lowest pulmonary resistance value for sensitized subjects was achieved from the 0.7 cmH₂O SIPO set. Healthy group R_L values range from 1.66 cmH₂O·mL⁻¹·s⁻¹ for ISO treatments (comparable to 100 and 300 mV treatments) up to a high of 2.06 cmH₂O·mL⁻¹·s⁻¹ with ACh challenge, which is also comparable with the 1.5 and 3.3 cmH₂O SIPO sets. The lowest pulmonary resistance value for healthy subjects was achieved from the ISO, 0.7, and 2.4 cmH₂O SIPO sets. Application of the 10 Hz-generated SIPO waveforms also resulted in significant decrease in the R_L of the sensitized airway for all treatments, especially when using an amplitude of 100 mV. The most significant decrease (44%) was found in the 0.7 cmH₂O set, where the resistance was significantly lower than ISO. The remaining three treatments (1.5, 2.4, and 3.3 cmH₂O) were significantly lower than ACh, with the first two groups comparable to ISO, and the 3.3 cmH₂O set still significantly higher than R_L values of ISO. The 10 Hz sensitized C_{dyn} values range from 6.5 μL/mmH₂O for ACh challenges up to a high of 10.5 μL/mmH₂O with 100 mV (0.7 cmH₂O) treatment. Healthy group C_{dyn} values range from 15.1 μL/mmH₂O for the 200 mV treatment (the ACh challenge is statistically equivalent at 15.2 μL/mmH₂O) up to a high of 18.9 μL/mmH₂O with ISO treatments. Application of the 10 Hz-generated SIPO waveforms resulted in significant increases in all 4 C_{dyn} values in sensitized airway treatments, especially when using an amplitude of 100 mV. The most significant increase (61%) was found in the 0.7 cmH₂O set, where the dynamic compliance was higher than ISO by 15%. The re-

maining three treatments (200–400 mV) were significantly higher than ACh, with the first two groups (1.5 and 2.4 cmH₂O) comparable to ISO and the 3.3 cmH₂O group significantly higher than C_{dyn} values of ACh but not comparable to ISO.

The 15-Hz sensitized R_L values range from 2.72 cmH₂O·mL⁻¹·s⁻¹ for 300 mV treatments up to a high of 5.29 cmH₂O·mL⁻¹·s⁻¹ with ACh challenge. The lowest pulmonary resistance value for sensitized subjects was achieved from the 1.7 cmH₂O SIPO set. Healthy group R_L values range from a low of 1.66 cmH₂O·mL⁻¹·s⁻¹ for ISO treatments (comparable to 300 and 400 mV treatments) up to a high of 2.06 cmH₂O·mL⁻¹·s⁻¹ with ACh challenge, which is also comparable to the 0.4, 1.1, and 2.4 cmH₂O SIPO sets. The lowest pulmonary resistance values for healthy subjects were achieved from the ISO, 1.7, and 2.4 cmH₂O SIPO sets. The standard error calculation from 2.4 cmH₂O SIPO treatments is enough to overlap low and high R_L values in the narrow range of healthy subjects' data. Application of the 15 Hz-generated SIPO waveforms resulted in significant decreases in the R_L of the sensitized airway for all treatments, especially when using an amplitude of 300 mV. The most significant decrease (48%) was found in the 1.7 cmH₂O set, where the resistance was significantly lower than ISO. The remaining three treatments (0.4, 1.1, and 2.4 cmH₂O) were significantly lower than ACh, with the initial group (0.4 cmH₂O) having the smallest percentage (18%) of improved R_L below ACh values. Both 1.1 and 2.4 cmH₂O sets were statistically comparable to each other and with ISO but not significantly better than ISO. The 15-Hz sensitized C_{dyn} values range from 6.5 μL/mmH₂O for ACh challenges up to a high of 11.7 μL/mmH₂O with 300 mV treatment (1.7 cmH₂O). Healthy group C_{dyn} values range from 15.2 μL/mmH₂O for the ACh challenge up to a high of 18.9 μL/mmH₂O with ISO treatments. Application of the 15 Hz-generated SIPO waveforms resulted in significant decreases in the C_{dyn} of the sensitized airway in 3 of the 4 treatments, particularly when using an amplitude of 300 mV. The second-most significant C_{dyn} increase (80%) of the entire study was found in these waveforms' 1.7 cmH₂O set, where the C_{dyn} was significantly higher than ISO. The remaining two treatments (200 and 400 mV) that were significantly higher than ACh were also comparable to ISO. The 100 mV (0.4 cmH₂O) group was comparable to ACh challenge, indicating no benefit in overcoming the modeled asthmatic attack.

The 20-Hz sensitized R_L values range from 2.09 cmH₂O·mL⁻¹·s⁻¹ for 300 mV treatments up to a high of 5.29 cmH₂O·mL⁻¹·s⁻¹ with ACh challenge. The lowest pulmonary resistance value for sensitized subjects was achieved from the 1.7 cmH₂O SIPO set. Healthy group R_L values range from a low of 1.66 cmH₂O·mL⁻¹·s⁻¹ for ISO treatments (comparable to 100 mV treatments) up to a high of 2.10 cmH₂O·mL⁻¹·s⁻¹ from the 1.1 cmH₂O SIPO set, which is also comparable to ACh 300 and 400 mV treatments. The lowest pulmonary resistance values for healthy subjects were achieved from the ISO and 0.4 cmH₂O SIPO sets. Application of the 20 Hz-generated SIPO waveforms resulted in significant decreases in the R_L of the sensitized airway for all treatments, and the group also contains the greatest relaxation values (the 300 mV set) from among all of the 19 distinct treatments in the study. SIPO treatments defined by 20 Hz/300 mV (1.7 cmH₂O) had a mean $R_L = 2.09$ cmH₂O·mL⁻¹·s⁻¹, equivalent to a 60% reduction in

R_L values of ACh treatment. The remaining three treatments (0.4, 1.1, and 2.3 cmH₂O) had significantly lower R_L values than ACh, with all sets' R_L values comparable to ISO, though none of them are significantly different from the reference treatment. Of note, 0.4 and 2.3 cmH₂O sets are significantly different from each other in the sensitized airway tests, with the lower SIPO application resulting in a 31% decrease of R_L compared with the ACh value and the latter of the two sets eliciting a 40% decrease. The 20-Hz sensitized C_{dyn} values range from 6.5 μ L/mmH₂O for ACh challenges up to a high of 14.9 μ L/mmH₂O with 300 mV treatment (1.7 cmH₂O). Healthy group C_{dyn} values range from 15.1 μ L/mmH₂O for the 200 mV treatment (the ACh challenge is statistically equivalent at 15.2 μ L/mmH₂O) up to a high of 18.9 μ L/mmH₂O with ISO treatments. Application of the 20 Hz-generated SIPO waveforms resulted in significant increases in all of the C_{dyn} means measured for treatments of the sensitized airways. The group also contains the greatest improvement for a C_{dyn} value (129%, in the 300 mV set) among all of the 38 (19 each for healthy and sensitized) distinct treatments in the study. The 100, 200, and 400 mV groups are all comparable to ISO values. The 0.4 cmH₂O group is the lowest of the four SIPO treatments, improving 32% over ACh. The 1.1 and 2.3 cmH₂O groups are equivalent with each other, aside from SE values, and improve C_{dyn} by 54% over ACh values.

DISCUSSION

Contraction of ASM (and subsequent reduction in the airway lumen) is recognized as the predominant mechanism of asthmatic attacks (24, 33, 39, 41). Lung resistance is based on measuring airflow and respiratory pressures, with the impact of this relationship found in decreasing flow values driving the rise in resistance. Dynamic compliance accounts for the impact of peak inspiratory pressure values and end-expiratory pressures on the tidal volume of each breath. Together, R_L and C_{dyn} are mechanical indicators of the respiratory system.

Responses of asthmatic tissue can be either less than that observed in normal tissue, or there is no effect from tidal oscillations and deep inspiration oscillations at all (47, 49). Of direct interest to this study, tidal and deep breathing mechanics in asthmatics are limited to a subset of airway tissues (34) and present unique treatment challenges. Such findings have generated a need to identify reasons for the lack of response by the tissues as well as drive investigations into alternative (SIPO) oscillations that do elicit responses in precontracted, sensitized ASM. Studies that replicate normal breathing conditions (those found in deep inspirations and tidal oscillations)—as well as those that introduce alternative oscillations—and apply them to healthy and asthmatic airways are the main basis for generating past and current airway responsiveness theories.

This study has focused on assessing healthy and DRA-sensitized (chronic) asthmatic airways' responses to SIPO applications from 0.4 to 3.6 cmH₂O. Detection of breathing parameters was continuous with no observed stoppages in airflow due to either blocked airway or pneumothorax tube. The observed responses of the healthy and asthmatic airways to the range of applied pressures are both beta agonist independent yet markedly different from each other despite a common preceding ACh-instigated asthmatic event. Baseline respiratory parameters in asthmatic subjects were characterized by signif-

icantly lower compliance and higher resistance relative to controls. The established conditions are ascribed to changes in the ASM basal tone and rearrangement of internal cellular structures as well as overall change to the cellular composition of asthmatic ASM (20, 24, 30, 31, 38, 44).

Historically, precontracted healthy ASM is relaxed after application of ISO alone, normal breathing oscillations alone, and ISO combined with normal breathing oscillations (3, 6, 7, 8, 9, 20–22, 45, 47). Findings in this study are consistent with relaxation of healthy ASM based on application of either ISO alone or SIPO combined with spontaneous breathing. The preferential effects of bronchorelaxant treatments due to physiological differences in healthy tissues are also consistent with the observed differences in SIPO treatment outcomes across healthy and diseased states in this study.

Based on healthy airways' responses to applied length oscillations, Fredberg et al. (16) proposed a model of crossbridge perturbation to explain the affected contractile behavior, wherein the equilibrium of the system is disrupted by the applied oscillations. A temporal rearrangement of ASM contractile elements has also been observed as a result of applied length changes to the tissue (30). Fredberg et al.'s proposal as well as confirmed temporal rearrangement of contractile machinery are consistent with earlier crossbridging microscopy study results from this institute (2). Within the context of this study, the observed increase in C_{dyn} , along with decreases in R_L , of SIPO-treated chronic asthmatic airways is β -agonist (ISO) independent, providing a novel approach to bronchoprotective treatment of precontracted airways. Current assessments of respiratory parameters in this chronic model of asthma further address postulates and hypotheses regarding perturbation of actinomyosin crossbridge cycling through use of such alternative oscillations applied to the mechanics of airway smooth muscle.

Given that the identity of the crossbridge is maintained regardless of healthy or diseased states, it is therefore plausible that crossbridge activity changes with the state of the disease. Evidence for this differential tissue response is found in application of similar oscillations to differing states of healthy and sensitized ASM. Adaptation of ASM over the course of disease progression is a valid characterization of changes to chemical and mechanical ASM identities. In fact, the adaptability of the tissues is consistent with observed outcomes between healthy and chronic asthma data sets of this study. The observed effects of SIPO in a single strain of mice across different disease states allow for the assumption that the crossbridge remains the same in terms of components but changes in its functional capabilities to effect force over the distance of its structures.

The basal tone of ASM tissue is characterized by both healthy and control asthmatic respiratory parameters. Basal tones are physiological results of the periodic function of actinomyosin interactions within the cellular framework. R_L and C_{dyn} values are parameters that extrapolate respiratory conditions resulting from changing basal tone. Changes in the rate of crossbridge cycling are then expected to change the basal tone of the tissue and are expected to influence the degree of AHR to external challenges and treatments, i.e., ACh, ISO, and SIPO in this study. Relaxation of precontracted ASM due to applied SIPO can then be viewed as a disruption to the intrinsic disease state cycling of the actinomyosin crossbridge.

Rates of myosin attachment and detachment are therefore viewed as being perturbed. The consistency of the theory is found in the fact that in healthy tissues, the perturbation of the crossbridge is possible with TO and DI applications (7, 15, 16, 23), while in chronic asthmatic ASM, with a cycling rate expected to be different from the healthy rate, the successful “healthy” interference pattern from similar applications does not occur because the template cycle is no longer present in the crossbridge of diseased tissues.

The frequencies and amplitudes used in this study to generate applied pressures resulted in reduction of R_L and increase in C_{dyn} (see Figs. 2 and 3) following ACh challenges of sensitized airways. These effects are likely due to the rate at which crossbridge cycling occurs and its response to applied length oscillations. The rate of crossbridge cycling is a difficult value to estimate with accuracy, as it is dependent on chemical and physiological factors. Future investigations involving the identified significant SIPO frequencies and amplitudes in this study may assist in estimating this rate.

The utility of SIPO is attributed to regional activity within the airway, followed by possible dissipation of the effects in lower airway regions within the lungs (27). This regional utility, however, does not equate with an overall panacea of bronchoconstrictive parameters due to SIPO treatments alone. Indeed, heterogeneous remodeling of asthmatic airways results in differential physiological responses to ASM contractile stimuli (and dilatory mechanisms) (34). Within an acute model of asthma the low oscillating pressures applied were able to overcome flow resistance but not elastic resistance (33). This study demonstrates that a chronic model responds with lower R_L and greater C_{dyn} at midrange oscillating pressures, overcoming both flow and elastic resistance. While the SIPO findings provide impetus for future studies to assess the significance of identified and postulated frequency and amplitude combinations for airway (flow and elastic) resistance relief across a range of diseased states, the access of inhaler-delivered diffusion treatments remains relevant. Therefore, a combination of SIPO along with bronchorelaxant treatments is also of interest.

The forced oscillations technique (FOT) currently evaluates in vivo healthy and asthmatic human airway performance. The range of frequencies (4–30 Hz) and amplitudes (less than 1 cmH₂O) (40) are similar to those used in this SIPO study. FOT oscillations in humans and SIPO oscillations in mice are applied to different breathing patterns and physiologies. It will be necessary to identify a correction factor to the findings of this study for human utility of the applications. Additionally, the range of SIPO frequencies and amplitudes will likely differ from the findings of this murine model in that periodicity of spontaneous healthy and asthmatic breathing are dissimilar between species.

Conclusions. Investigation of the effects of SIPO on a long-term chronic asthma model has determined airway responses to treatments ranging from 0.4 to 3.6 cmH₂O. Comparison of the CHR values of the study against HEA subjects indicates that applied SIPO, in line with the theory that ASM length oscillations interact with the actin and myosin contraction processes, disrupts the crossbridge cycling process (12, 15, 19). Findings from this chronic asthmatic model further add to our understanding regarding physiological actions of frequencies and amplitudes of the generated mechanical oscillations,

in particular with those responsible for beneficial bronchodilator indications. ASM relaxation achieved from such treatments supports the need for further investigations in animal and human clinical settings.

Analysis of R_L and C_{dyn} values obtained for ACh, ISO, and SIPO treatments has indicated the presence (and treatment-dependent decrease) of AHR in sensitized mice as well as changes in respiratory responses in the airways of healthy mice. Applications of bronchorelaxant methods in the form of SIPO treatments improve respiratory conditions of both healthy and sensitized mice in terms of decreasing pulmonary resistance and increased dynamic compliance caused by ACh exposure. Greater numbers of statistically significant decreases in R_L (as well as increases in C_{dyn}) are found in sensitized mice compared with healthy mice. Future work combining SIPO and bronchorelaxant treatments in parallel will be of benefit to further understanding the ability of SIPO treatments to approximate pharmacology-dependent observations. Both healthy and sensitized mice respond most favorably to SIPO sets from distinct ranges of pressures, indicating that diseased and healthy airways respond to similar treatments in different fashions. Stated another way, the response of airway tissues’ pulmonary resistance to SIPO treatments has at least a partial dependence on the state of health or disease in the mouse.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

A.M.A.-J. conceived and designed research; K.L.T.R. performed experiments; K.L.T.R. and A.M.A.-J. analyzed data; K.L.T.R. and A.M.A.-J. interpreted results of experiments; K.L.T.R. prepared figures; K.L.T.R. and A.M.A.-J. drafted manuscript; A.M.A.-J. edited and revised manuscript; A.M.A.-J. approved final version of manuscript.

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