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# Mechanopharmacology and Synergistic Relaxation of Airway Smooth Muscle

*Asthmatic airways are stiffer than normal. We have shown that the cytoskeletal passive stiffness of airway smooth muscle (ASM) can be regulated by intracellular signaling pathways, especially those associated with Rho kinase (ROCK). We have also shown that an oscillatory strain reduces the passive stiffness of ASM and its ability to generate force. Here, we investigated the combined effect of inhibiting the ASM contraction with $\beta_2$ agonist and decreasing the ASM cytoskeletal stiffness with ROCK inhibitor and/or force oscillation (FO) on the relaxation of contracted ASM. We hypothesize that the ASM relaxation can be synergistically enhanced by the combination of these interventions, because drug-induced softening of the cytoskeleton enhances the FO-induced relaxation and vice versa. Sheep tracheal strips were isotonically contracted to acetylcholine ($3 \times 10^{-5}$ M). At the plateau of shortening, $\beta_2$ agonist salbutamol ($10^{-7}$ M), ROCK inhibitor H1152 ($10^{-7}$ M), and FO (square wave, 1 Hz, amplitude 6% maximal active force) were applied either alone or in combination. After adjusting for nonspecific time-dependent variation, relengthening by individual interventions with low-dose salbutamol or H1152, or small amplitude FO was not significantly different from zero. However, significant relengthening was observed in all combination treatments. The relengthening was greater than the mathematical sum of relengthening caused by individual treatments thereby demonstrating synergistic relaxation. The ASM stiffness did not change with salbutamol or H1152 treatments, but was lower with FO in combination with H1152. The results suggest that the mechanopharmacological treatment can be an effective therapy for asthma.* [DOI: 10.1115/1.4042477]

## 1 Introduction

Deep inspiration (DI) has been known as the first line of defense against excessive airway narrowing. For nonasthmatics, a DI can reverse bronchoconstriction (bronchodilatory effect) [1,2], and when taken prior to bronchochallenge, DIs can attenuate the severity of airway narrowing caused by a subsequently administered constricting agent (bronchoprotective effect) [3]. These beneficial effects of DI are characteristically diminished or absent in asthmatics [3–5]. Lack of DI-induced bronchodilation and bronchoprotection are now recognized as consistent features of asthma, highlighting a basic difference between asthmatic and nonasthmatic airways, possibly as a result of asthmatic airways being stiffer than normal [6].

There are two known contributors to airway stiffness: airway smooth muscle (ASM) tone and altered airway wall thickness and/or mechanical properties due to airway remodeling. We have shown that the development of passive stiffness is separate from the development of active force in ASM [7]. The stiffness related to tone can be reversed by the use of bronchodilators that relax ASM [8], but the stiffness related to the passive components of the airways, including that associated with the extracellular matrix and the relaxed ASM, remains. A different therapeutic approach is needed for targeting passive stiffness. Studies from our laboratory revealed that a component of the passive ASM stiffness likely stems from its cytoskeleton [7]. We showed that this component is calcium sensitive and can be regulated by intracellular signaling pathways, especially those associated with Rho kinase (ROCK) [7,9,10].

In addition to ROCK inhibitor, we have also shown that oscillatory strain reduces the passive ASM stiffness and ASM ability to generate force [7]. The ASM functions in a mechanically dynamic environment. Oscillatory strain associated with tidal breathing and DIs is known to soften the cytoskeleton and reduce contractility in ASM [11]. The term softening refers to increased compliance. This mechanotransduction likely explains the long-recognized phenomenon that "breathing is good for breathing." Recently, it has been shown that superimposed pressure oscillation on continuous positive airway pressure (CPAP) is more effective than standard CPAP in treating obstructive sleep apnea [12]. This study provided an example of combined mechanical/medical treatment for a breathing disorder by superimposing a second oscillation through a device on top of that due to breathing alone to achieve amplified therapeutic benefits. Similarly, it has been shown that superimposed length oscillation on isolated mouse tracheal rings is more effective than "breathing" alone in inducing airway relaxation [13].

Based on these reports, we designed this study to investigate the effect of combined interventions on relaxing precontracted ASM by using $\beta_2$ agonist salbutamol to reverse ASM contraction and ROCK inhibitor H1152 as well as force oscillation (FO) that would be superimposed on top of the normal tidal breathing when

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used in vivo to target passive stiffness. We hypothesize that ASM relaxation can be synergistically enhanced by the combination of these interventions because drug-induced softening of the cytoskeleton enhances FO-induced relaxation which in turn facilitates further softening of the muscle. This may lead to the design of a new device that provides tolerable pressure fluctuations in the airways which then allows bronchodilators and/or ROCK inhibitors at low doses to achieve desired level of bronchodilation while minimizing their side effects.

## 2 Methods

**2.1 Muscle Preparation.** Ovine tracheal smooth muscle was used. All experimental procedures were approved by the Ethics Committee for Animal Care and the Biosafety Committee of the University of British Columbia and conformed to the guidelines set out by the Canadian Council on Animal Care. Ovine tracheas were obtained from a local abattoir, transported in ice-cold physiological saline solution (modified Krebs solution) to our laboratory, and stored in fresh Krebs solution at 4 °C. The experiments were performed within 4 days of obtaining the tracheas. The composition of the Krebs solution was: 118 mM NaCl, 4 mM KCl, 1.2 mM $NaH_2PO_4$, 22.5 mM $NaHCO_3$, 2 mM $MgSO_4$, 2 mM $CaCl_2$, and 2 g/l dextrose at *p*H 7.4. Smooth muscle strips devoid of epithelium and loose connective tissue were dissected from the posterior membranous portion of the trachea while being maintained at the in situ length (taken as a reference length, $L_{ref}$), i.e., the distance between the two insertion points of the muscle to the cartilage ring under relaxed condition. Aluminum clips were then attached to the ends of the muscle strip. Through these aluminum clips and silk thread, the muscle strip was attached to a force/length transducer (Duo mode, model 300 C, Aurora Scientific, Inc., Aurora, ON, Canada) with minimal passive resting tension and submerged in 37 °C Krebs solution aerated with 95% oxygen and 5% carbon dioxide. Indomethacin ($5 \times 10^{-5}$ M) was used to prevent the development of intrinsic tone.

**2.2 Experimental Protocol.** Figure 1 schematically illustrates the experimental protocol. Muscle strips were equilibrated with periodic electric field stimulation (60 Hz at selected voltage and current density sufficient to elicit maximal response from the muscle) at 5 min intervals until a steady isometric force was reached. The strips were then stimulated with acetylcholine (ACh, $3 \times 10^{-5}$ M). The resulting active isometric force ($F_{max}$) was recorded and used to calculate the isotonic load for the subsequent isotonic contractions elicited to evaluate the relaxing effects of mechanopharmacological interventions. During the isotonic contractions, muscle strips were allowed to shorten maximally and then relengthen while the load was kept constant. Relengthening was evaluated in the presence and absence of either single or combined relaxing interventions. Figures 1(*a*) and 1(*b*) show schematic diagrams of the experimental protocol describing the interventions without and with FO, respectively.

(1) *Isotonic shortening*: An isotonic load was preset at 30% $F_{max}$. Muscle strips were stimulated with ACh ($3 \times 10^{-5}$ M) and first contracted isometrically until the force reached the preset load when the muscle began to contract isotonically. About 4–5 min later, muscle shortening reached a plateau. The amount of shortening from $L_{ref}$ to plateau was defined as the maximal shortening (MS). After the plateau, while keeping the load constant, different interventions were applied and the length of the muscle strips was monitored for 60 min.

(2) *Force oscillation*: After the isotonic shortening reached a plateau, one of the interventions was FO, where the force was controlled to oscillate around the preset isotonic load. The wave form of FO was chosen to be symmetrical square waves. The frequency was chosen to be 1 Hz. Note, this frequency is not the breathing frequency, but meant to be the frequency of pressure oscillations that would be riding on top of the normal tidal breathing at a much lower frequency. The preliminary data (not shown) indicated that tidal breathing alone had no effect on ASM relaxation compared to time control. Also, in preliminary tests, we found that greater frequencies (2, 4, 5, 10, or 20 Hz) of superimposed pressure oscillation did not result in greater relengthening (data not shown). The amplitude was chosen to be 6% $F_{max}$ such that the amplitude of stretching force was less than what would be exerted with a deep inspiration (see more details in the Results and Discussion sections). Due to the intrinsic thread (which connects the muscle preparation to the measuring device) compliance, FO also resulted in oscillatory stretch of the thread. This length variation due to thread compliance would not affect the accuracy of the evaluation of muscle length hence relengthening, because it did not change the midline around which the length oscillation occurred. However, it would affect the stiffness calculation of the muscle during FO. To measure the resulting length oscillation of the thread, at the end of each experiment, the muscle strip was removed. In its place, the warm wet thread was connected to the stationary rod and the exact same FO was applied to the thread alone at the same preset load. The amplitude of length oscillation of the thread was measured and subtracted from the total length change in the calculation of actual amplitude of length change produced by FO in the muscle strip.

(3) *Interventions and relengthening*: At the plateau of an isotonic contraction, one of seven interventions (Figs. 1(*a*) and 1(*b*)) was randomly chosen and applied to a selected muscle strip. These interventions were: (1) $\beta_2$ agonist salbutamol ($10^{-7}$ M; Sigma-Aldrich, St. Louis, MO), (2) ROCK inhibitor H1152 ($10^{-7}$ M, Tocris, Avonmouth, Bristol, UK), (3) salbutamol ($10^{-7}$ M) + H1152 ($10^{-7}$ M), (4) FO (square wave, 1 Hz, amplitude 6% $F_{max}$), (5) FO + salbutamol ($10^{-7}$ M), (6) FO + H1152 ($10^{-7}$ M), and (7) FO + salbutamol ($10^{-7}$ M) + H1152 ($10^{-7}$ M). The relengthening was defined as the amount of recovered

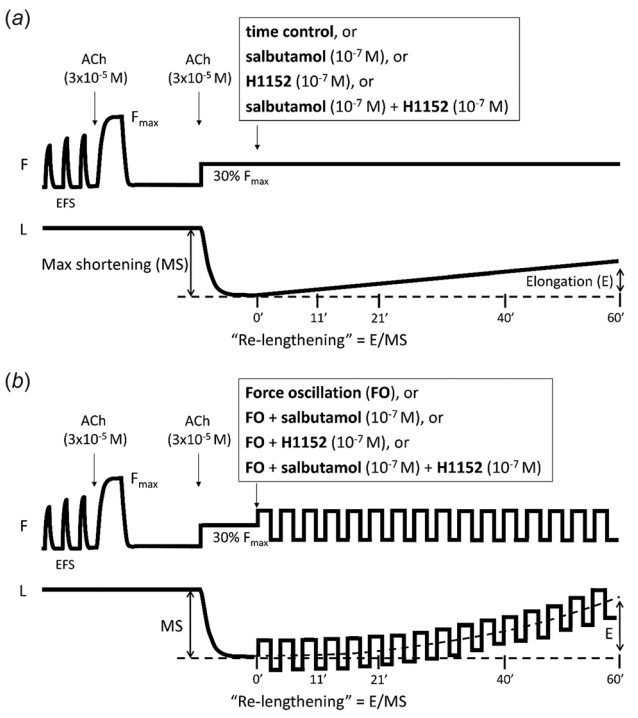


**Fig. 1 Schematics of experimental design and definition of relengthening: (*a*) interventions without force oscillation and (*b*) interventions with force oscillation**

length, or elongation (E), normalized by MS. The relengthening due to time alone was subtracted from the total relengthening to obtain the net relengthening due to intervention.

(4) *Evaluation of synergy*: Synergy is defined as the combined effect being greater than the mathematical sum of the individual effects. Relengthening due to combined interventions was compared to the mathematical sum of relengthening due to each individual intervention. For example, the net relengthening of combined salbutamol, H1152, and FO was compared to the mathematical sum of relengthening values due to salbutamol, H1152, and FO alone. This was evaluated at four arbitrarily selected time points (11, 21, 40, and 60 min) after application of intervention.

(5) *Measurement of stiffness*: Stiffness of the contracted muscle strip, with or without interventions, was calculated by the change in force divided by the corresponding change in length during FO. In interventions where FO was not involved, a brief period of FO (5 s) was applied to the strips at the four time points chosen to evaluate synergy; stiffness was calculated as the ratio of the force amplitude and the resulting length amplitude of the square waves (corrected for thread compliance).

**2.3 Statistical Analysis.** All data in the figures and Table 1 are presented as mean ± standard error (SE). One way repeated measure (RM) analysis of variance (ANOVA) was used to detect differences in active stress generation, the amount of maximal shortening, and the time course of muscle stiffness. Two way RM ANOVA was used for comparisons of relengthening and stiffness at the chosen time points between treated and time control and for comparison at these time points between mathematical sum of relengthening due to individual intervention and that due to combined intervention to detect the presence of synergy.

## 3 Results

**3.1 Contractility Was the Same Among All Tracheal Strips.** Eight strips from each of the five tracheas were tested. According to the interventions that they received, these strips formed eight groups. Before any intervention was applied, their force generation and shortening ability were compared (Table 1). The active stress was calculated as active force measured from the first isometric stimulation with ACh ($3 \times 10^{-5}$ M, $F_{max}$) normalized by the cross-sectional area of the strips. The maximal shortening was calculated from the second stimulation with ACh ($3 \times 10^{-5}$ M, MS) under constant load as the extent of shortening at plateau normalized to $L_{ref}$. Repeated measure one way ANOVA indicated that the eight groups of strips had the same force generating ability and shortening capacity before any treatment was applied.

**3.2 The Amplitude of Force Oscillation Was Lower Than the Predicted Amplitude of Pressure Oscillation Exerted by Deep Inspiration.** The $L_{ref}$ and the width of the muscle strips (mean ± standard deviation) were measured under microscope to be 5.9±0.7 and 1.4±0.3 mm, respectively. The thickness was estimated to be 0.25 mm as the thickness of the muscle sheet in comparable sizes of tracheas is rather constant from our previous morphological measurements [7,10]. By considering the muscle strip wrapped around end to end to form a cylinder with the muscle strip width as the height ($h$), we could calculate the tension along the length of the muscle, or the circumference of the cylinder, given the pressure inside the cylinder. According to LaPlace law, the tension ($T$) per segment $h$ in the wall of a hollow cylinder is directly proportional to the cylinder's radius ($r$) and the distending pressure ($P$) across the wall, i.e., $T = Pr/h$. We used values of the pressure across the wall as 5 cm$H_2O$ during tidal breathing and 40 cm$H_2O$ during DI [14]. We calculated for each muscle strip according to its dimensions what the tension would be during tidal breathing and during DI. This tension was compared with the amplitude of FO (6% $F_{max}$). A representative example is shown in Fig. 2(*a*). When compared to the tension during tidal breathing and DI, the amplitude of the FO that we applied to the muscle strips was 560±60% of the predicted tension during tidal breathing and 70±8% of the predicted tension during DI.

**3.3 Force Oscillation Resulted in Net Stretching of the Muscle Strips by About 0.5% of the Maximally Shortened Length.** Force oscillation resulted in oscillations in length. Considering that the compliance of the thread needs to be subtracted, we measured the length oscillation of thread alone with the same setting of the FO that was applied when the muscle strips were present. The displacement measured from the thread alone was subtracted from the total recorded length changes to obtain the actual fluctuations in length of the contracted muscle due to FO. The displacement of length due to thread compliance was about 49.5±2.4% of the total length displacement. A representative example of the total response (muscle + thread) and thread alone to FO is shown in Fig. 2(*b*). After subtracting the thread displacement, the corrected length stretch was 0.28±0.04% $L_{ref}$, or 0.5±0.05% of the current shortened length.

**3.4 Individual Interventions Were Not Different From Time Control.** Without any intervention, there was a certain degree of relengthening. As shown in Fig. 3(*a*), the relengthening increased with time, from 2.7±0.7% at the 11 min time point to 7.1±1.4% at the 60 min time point. The relengthening at each time point was subtracted from the length data obtained with interventions from the same trachea to obtain the net effect. The net effect of salbutamol alone, H1152 alone, or FO alone was not significantly different from that of the time control at each time point (Fig. 3(*a*), $n = 5$, $p = 0.295$, 0.522, 0.288, respectively), indicating that the interventions, if applied individually, did not cause significant relengthening.

**3.5 Synergy Was Found in All Combination Treatments.** At the 60 min time point, relengthening by (H1152 + salbutamol), (FO + salbutamol), (FO + H1152), and (H1152 + salbutamol + FO) reached 28±5%, 34±6%, 30±6%, and 55±6% MS, respectively. Each combination treatment (time control subtracted) showed statistically significant difference from time control at all four time points (Figs. 3(*b*)–3(*e*), $n = 5$, $p = 0.014$,

**Table 1 Active stress generation and maximal isotonic shortening to ACh $3 \times 10^{-5}$ M of all groups before any treatment was applied**

| Group | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | One way RM ANOVA ($p$) |
|---|---|---|---|---|---|---|---|---|---|
| Active stress (kPa) | 172.3 ± 20.9 | 181.1 ± 18.6 | 205.7 ± 18.1 | 188.1 ± 16.4 | 178.6 ± 21.6 | 189.1 ± 38.0 | 195.9 ± 17.2 | 191.8 ± 20.6 | 0.96 |
| Maximal shortening ($L_{ref}$) | 0.46 ± 0.02 | 0.46 ± 0.03 | 0.44 ± 0.01 | 0.41 ± 0.01 | 0.45 ± 0.02 | 0.41 ± 0.02 | 0.44 ± 0.02 | 0.41 ± 0.01 | 0.43 |
| Treatment | TC | Sal | H1152 | Sal + H1152 | FO | FO + Sal | FO + H1152 | FO + Sal + H1152 | |

Note: Data are mean ± SE ($n = 5$).

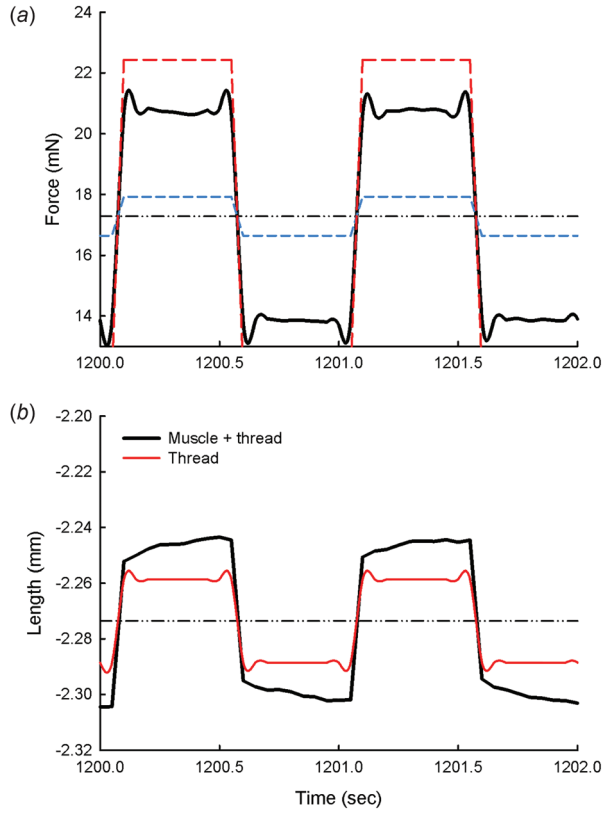


**Fig. 2 An example of force and length tracings obtained during FO. (*a*) Force traces: black solid line, set amplitude of 6% $F_{max}$ was 3.57 mN. The peak to peak amplitude was 7.1 mN. Black dotted horizontal line, set isotonic load (30% $F_{max}$), also the midline of oscillation. Red dashed line, predicted oscillation due to deep inspirations. Blue dashed line, predicted oscillation due to tidal breathing. (*b*) Length traces: black solid line, length oscillation of muscle + thread. Black dotted horizontal line, midline of oscillation. Red solid line, measured thread response alone to the same set parameters of force oscillation indicating thread compliance.**

0.006, 0.02, $< 0.001$, respectively). Synergistic effect on the relengthening was determined after the comparison of relengthening due to combination treatments and the sum of relengthening caused by individual treatments separately. For example, Fig. 3(*b*) shows time course of H1152 ($10^{-7}$ M) alone, salbutamol ($10^{-7}$ M) alone, their mathematical sum, and when both H1152 and salbutamol were added to the tissue bath at the same time. The difference between both drugs added at the same time and the mathematical sum of the response to each drug alone was significant at all four time points ($p = 0.034$). This comparison indicates a synergistic effect between $\beta_2$ agonist and ROCK inhibitor. Similarly, as shown in Figs. 3(*c*)–3(*e*), synergy exists when FO was applied in combination with salbutamol ($p = 0.008$), H1152 ($p = 0.007$), and both salbutamol and H1152 ($p = 0.007$). Time control is included in each panel to illustrate the lack of difference between time control and single interventions.

**3.6 Muscle Stiffness Was Reduced by Force Oscillation in Combination With H1152.** The stiffness of the shortened muscle was measured at the same arbitrary time points (11, 21, 40, and 60 min). It was calculated by dividing the change in force by the change in length. The change in length was corrected for thread compliance. As shown in Fig. 4, the corrected stiffness in the time control group increased with time. Muscle stiffness at all four time points was significantly greater than that at time 0 ($p < 0.01$). The stiffness at 40 and 60 min was not different from each other but was different compared to 11 and 21 min time points ($p < 0.01$). Interestingly, when a drug (salbutamol or H1152) was applied alone or in combination, the stiffness was not reduced compared with time control. In fact they appear to increase the stiffness (Fig. 5(*a*)) although the increase was not significant ($p > 0.3$). However, when FO was applied (Fig. 5(*b*)), with or without the drugs, with one or both drugs, the mean stiffness at each time points appeared lower than time control. No statistically significant difference was found when time control was compared to FO ($p = 0.118$) or to FO + salbutamol ($p = 0.11$). However, statistically significant difference was found when time control was compared to FO + H1152 (treatment $p = 0.021$, time $p = 0.018$), or to FO + salbutamol + H1152 (treatment $p = 0.027$, time $p < 0.001$).

## 4 Discussion

In this study, we selected three interventions to relax isotonically shortened ASM: $\beta_2$ agonist salbutamol, ROCK inhibitor H1152, both at a low concentration ($10^{-7}$ M), and a force oscillation that delivers pressures lower than those induced by DIs. We chose 30% $F_{max}$, which gives a relatively large amount of shortening and minimal relengthening in time control to maximize the resolution for the response due to interventions. When these interventions were applied alone, they each had no effect on muscle relengthening just like the time control. However, when used in combination of any two interventions, they reversed ~30% of the shortening. When all three interventions were combined at the same time, they reversed 50–60% of the shortening. At all of the time points throughout the observed 60 min we found statistically significant synergy. Because airway resistance is inversely related to the fourth power of the airway diameter, 30–50% relengthening of ASM could translate into 65–80% reduction in airway resistance.

Our data showing synergistic effects of multiple interventions suggest that $\beta_2$ agonists at low concentrations will be able to reverse airway resistance substantially if used in combination with ROCK inhibitor (H1152), also at very low concentration, or in combination with FO. Clinically $\beta_2$ agonists have been used regularly to abolish ASM tone which is often the culprit of asthma exacerbation. However, it is known that regular use of long acting $\beta_2$-agonists could lead to tolerance [15], making them ineffective or less effective in treating asthma attacks [16]. Safety concerns for $\beta_2$ agonists include cardiovascular side effects and death [17–19]. Desensitization of the $\beta_2$-adrenoceptors and activation of phosphodiesterases, which break down cAMP, has been suggested as an underlying mechanism for the loss of effectiveness of $\beta_2$-agonists in long-term asthma treatment [20,21]. It was our intention to keep the doses of the drugs and amplitude of FO low so that when used in vivo would help to reduce side effects and avoid the development of tolerance to the treatment normally associated with higher dose of drugs.

One of the possible mechanisms for the synergistic effects observed in our combined multiple-intervention experiments is that the interventions collectively or individually lead to deactivation and softening of ASM, which would allow FO to more effectively induce strain in ASM, resulting in further relaxation of the muscle. $\beta_2$ agonists are well known for their effects of reducing active force in ASM, which also leads to reduction in muscle stiffness due to decreased myosin cross-bridge attachment to actin filaments; ROCK inhibitors are known for their similar effects on active force as $\beta_2$ agonists, but with additional effects in reducing noncrossbridge related muscle stiffness (i.e., cytoskeletal stiffness) [9,10]. Therefore, one would expect that the combined effect of salbutamol and H1152 would be muscle relaxation and stiffness reduction. However, in the absence of FO, salbutamol and H1152 interventions, collectively or individually, resulted in no reduction in muscle stiffness (Fig. 5(*a*)). These surprising results could be due to the low concentrations of the drugs used. The synergistic effect of salbutamol + H1152 in causing muscle relaxation

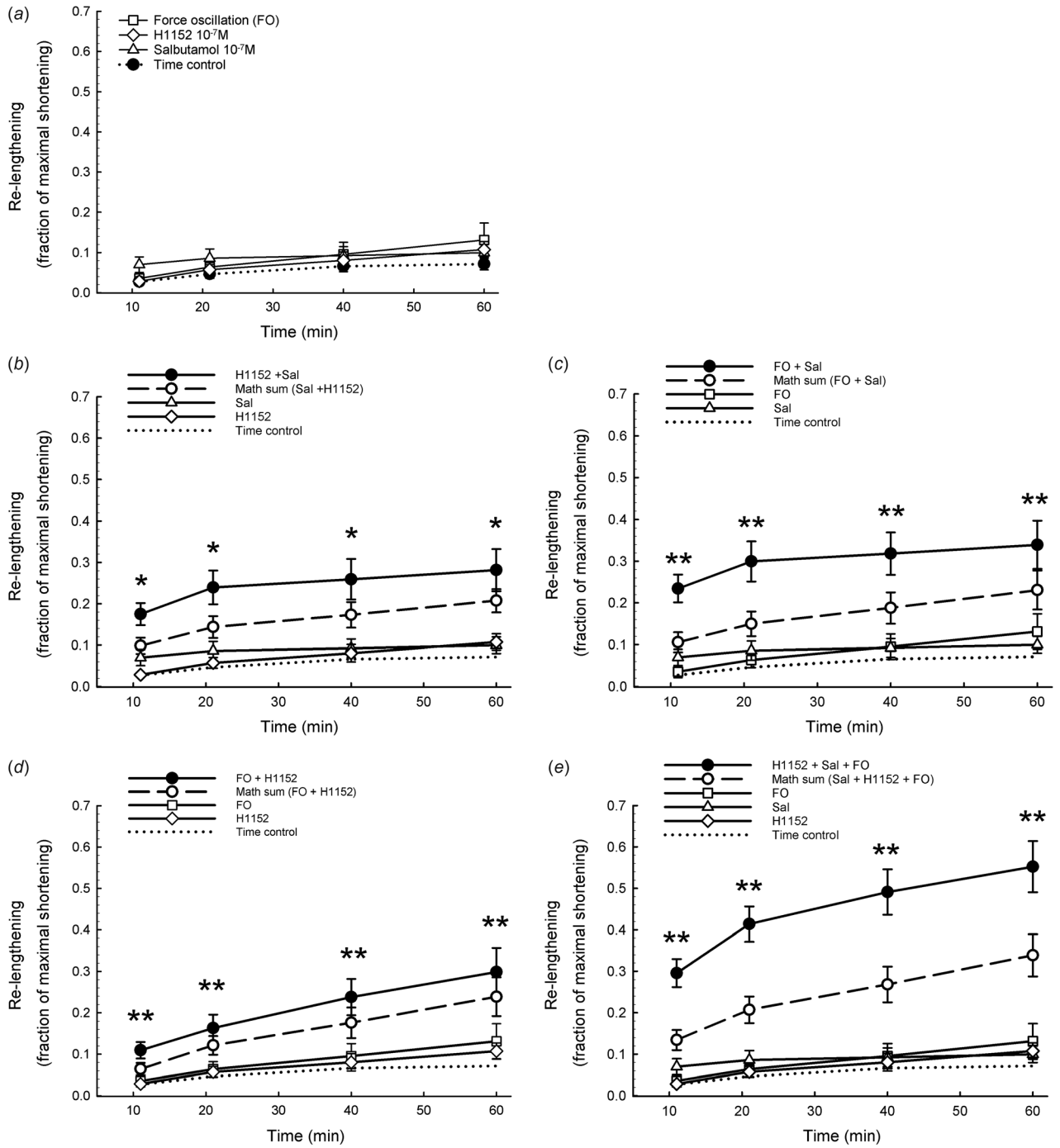


**Fig. 3 Relengthening measured from individual and combined interventions as well as the mathematical sums: (*a*) time control and three individual interventions, (*b*) H1152 and salbutamol (Sal), both $10^{-7}$ M, (*c*) FO and Sal, (*d*) FO and H1152, and (*e*) H1152, Sal, and FO. Data are mean ± SE. *$p<0.05$, **$p<0.01$, combined intervention compared to mathematical (math) sum of individual interventions, two-way repeated measure ANOVA ($n=5$). Time control has already been subtracted from all interventions.**

observed in our experiments, therefore, cannot be explained by mechanisms related to alteration of ASM stiffness.

$\beta_2$ agonist had been suggested to reduce airway wall stiffness. Ansell et al. [22] proposed that there is a synergistic effect in airway segments between $\beta_2$ agonist isoprenaline and breathing maneuvers simulated by fixed transmural pressure oscillations in dilating the airways. The authors suggested that the bronchodilator reduced the stiffness of the airway wall which then allowed the oscillation to induce greater relaxation. Our study first of all showed a trend for an increase in stiffness over time, which is not surprising. In chronically shortened ASM, we observed an increase in the muscle's ability to generate force, which we coined force adaptation [23]. The increase in stiffness with time may be related to this phenomenon. Second, compared to time control at equivalent time points, salbutamol at $10^{-7}$ M did not reduce muscle stiffness. This finding does not contradict that of Ansell et al. because we did not test the same bronchodilator nor at the same concentration. They used doses from $10^{-7}$ to $10^{-4}$ M isoprenaline, and it was only at higher end of the concentration spectrum that isoprenaline enhanced the effect of oscillation. In fact, they showed that the $\beta_2$ agonist at $10^{-7}$ M did not increase ASM strain during pressure oscillation.

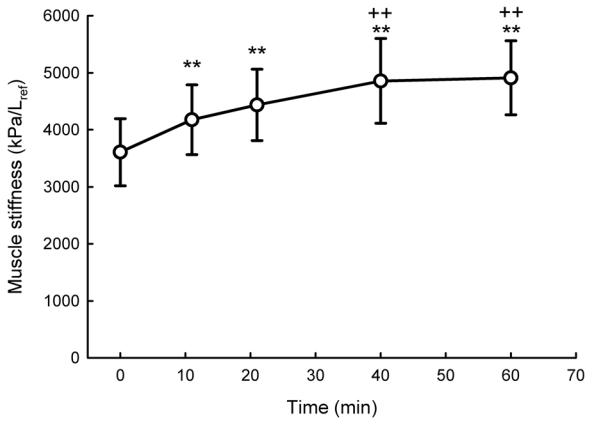


**Fig. 4 Changes in muscle stiffness after an isotonic shortening reached its plateau. No intervention was applied; the change in stiffness, therefore, represents a baseline stiffness with which changes in muscle stiffness due to experimental interventions are compared (see next figure). Muscle stiffness was corrected for the thread compliance (see text). Data are mean ± SE. **$p<0.01$, compared to time 0, ++$p<0.01$, compared to 11 and 21 min time points. One way repeated measure ANOVA ($n=5$).**

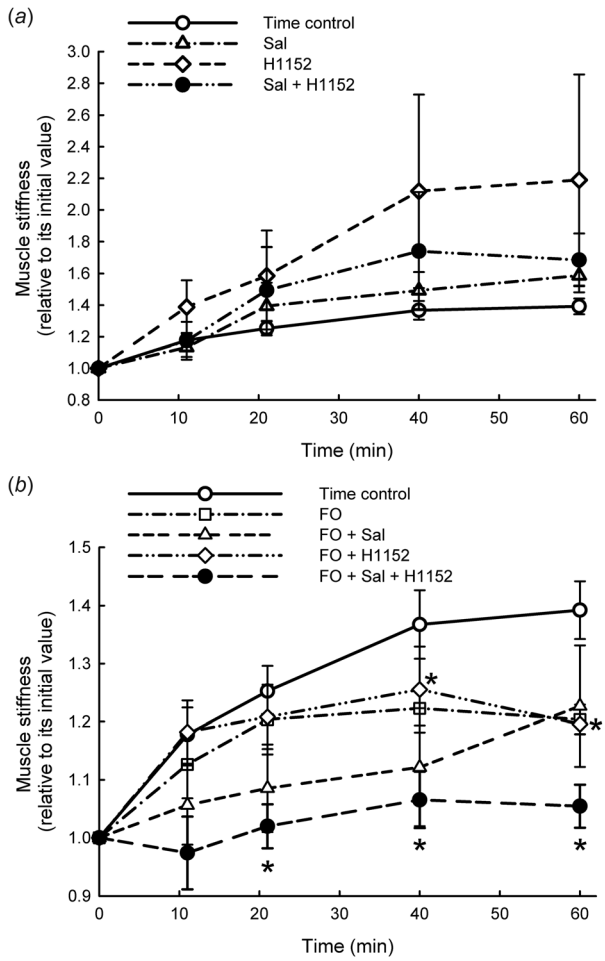


**Fig. 5 Muscle stiffness relative to its zero-time value in the absence (time control) and presence of various interventions. Time zero represents the time point when the isotonic shortening just reached its plateau: (*a*) interventions without FO and (*b*) interventions with FO. Data are mean ± SE. *$p<0.05$, compared to time control, two-way repeated measure ANOVA ($n=5$).**

Even with FO, salbutamol at the low concentration of $10^{-7}$ M did not cause a reduction in muscle stiffness (Fig. 5(*b*)). However, H1152 with FO, with or without salbutamol, resulted in a significant reduction in muscle stiffness (Fig. 5(*b*)). This suggests that the synergistic effects observed in combined interventions that involved H1152 and FO could stem from alterations in cytoskeletal stiffness. It is not clear why H1152 and FO alone did not change muscle stiffness, but the combination did. It could be that the low intensity of each of the intervention alone could not overcome the threshold for stiffness change, but the combination could.

Findings from this study suggest that we can use small amplitude of pressure oscillation in combination with drugs to more effectively relax or soften ASM and maintain airway patency without excessive pressure oscillation. These results could lead to the development of a new type of therapy for asthma using combinations of drugs in low doses and also a nonmedicinal component—pressure oscillation which would result in softening of ASM. While patients maintain their normal tidal breathing at ~0.2 Hz, a high frequency pressure oscillation (1 Hz) would be superimposed on top of the tidal breathing through this device. A notable advantage of this type of therapy is that the low drug doses will likely reduce side-effects and the development of tolerance. To deliver the appropriate pressure oscillation to the lung, a device similar to the CPAP machine could be developed. The disease mechanisms are likely very different for obstructive sleep apnea and asthma, and the positive airway pressure used for treatment of sleep apnea [12] may not be necessary for treating asthma. However, based on our findings, a similar device that provides a pressure oscillation riding on top of tidal breathing may work as an asthma therapy.

An important objective of this report is to provide preliminary data on synergistic mechanopharmacological effects on ASM and justification for developing devices that could alleviate asthma symptoms while minimizing medication doses to avoid side-effects. On a parallel note, the experimental design developed here could also be tested in different experimental paradigms where ASM contractility and passive stiffness maybe increased such as animal models of asthma and/or airway inflammation/remodeling in order to gain further insights into disease mechanisms and to suggest new combination therapies.

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