Airway remodeling is a pathologic feature of asthmatic airways characterized by airway smooth muscle (ASM) thickening, subepithelial fibrosis, mucus cell hyperplasia, and airway neovascularization. Airway remodeling is most profound in severe asthma where it may underlie, at least in part, persistent airway narrowing, airway hyperresponsiveness, lung function decline, and corticosteroid resistance.

Recent research has implicated mechanical forces in the initiation of these pathologic features. We review these developments and discuss them in light of previous studies and therapeutic opportunities. It is well recognized that mechanical forces influence tissue biology. Weight-bearing exercise induces mineralization of bone, leading to decreased fracture rates; high BP leads to cardiac and blood vessel remodeling; and weight lifting leads to skeletal muscle hypertrophy. Mechanical forces caused by bronchoconstriction have been shown in animal and human in vitro and in vivo work to not only induce symptoms but also influence airway biology akin to the...
changes induced by mechanical force in other tissues.\textsuperscript{1,4,5} As the influence of mechanical force on the airways is more understood, practicing respiratory physicians may be able to take advantage of this knowledge and improve outcomes for their patients.

What Are the Mechanical Forces Within the Airway?
Forces acting on the airways are complex and varied and occur from the earliest stages of development. In utero, the lung epithelium is a secretory structure, and normal lung development depends on pressure generated by liquid secretions and resistance from the developing larynx.\textsuperscript{6} Following birth, the lung stretches to fulfill its function, expanding and contracting physiologically each breath with lung inflation and deflation. Excessive lung stretch, which may be induced by mechanical ventilation, induces lung injury, with protective ventilator strategies used as standard practice to reduce this risk.\textsuperscript{1,7} Whereas lung stretch is physiologic, forces generated in the airway by symptomatic bronchoconstriction are believed to be present only in disease.

What Are the Consequences of Mechanical Forces During Bronchoconstriction: What Is Happening Physically?
ASM contraction leads to a reduction in airway caliber, increased resistance to airflow, and the clinical syndrome of asthma.\textsuperscript{2,3,8} As the airway narrows, either individual epithelial cells must reduce in size or the internal surface of the airway must fold. Bronchial epithelial cells sit on a relatively noncompressible subepithelial membrane, and epithelial cells resist acute changes in size.\textsuperscript{1,4,5,9,10} This leads to epithelial folding during bronchoconstriction, with areas of high pressure generated as the airway folds back on itself.\textsuperscript{6,11,12} Figure 1 shows diagrammatically our current understanding of the mechanical results of bronchoconstriction.

How Does the Epithelium Respond to Mechanical Stress?
Various in vitro models have been used to address whether the bronchial epithelium responds to compressive stress resulting from bronchoconstriction. Compression of bronchial epithelial cells, either apically to mimic airway folding or laterally to mimic direct lateral stress, has shown the bronchial epithelium to be mechanoresponsive.\textsuperscript{13-15} Human bronchial epithelial cells grown at an air-liquid interface apically compressed using warmed humidified air (at pressures up to 30 cm H\textsubscript{2}O) show an increased release of endothelin 1 and endothelin 2 as well as transforming growth factor-\(\beta\) (TGF-\(\beta\)) 2 into basal medium.\textsuperscript{16} Endothelin 1 induces smooth muscle contraction\textsuperscript{17} and is implicated in airway remodeling,\textsuperscript{18} and genetic polymorphisms have been associated with asthma, although small trials of endothelin receptor antagonists in asthma have been disappointing.\textsuperscript{19} TGF-\(\beta\)2 is considered to be a fundamental molecule in the pathogenesis of airway remodeling, although attempts to modulate its actions in the airway have also been disappointing.\textsuperscript{20}

Only short periods of apical compressive stress (minimum of 1 h) were sufficient to commit the cells to induce signaling in this model. To model the airway in more detail, a coculture method was developed where epithelial cells above a porous membrane were cultured with fibroblasts below the membrane and pressure applied only to the epithelial cells.\textsuperscript{14} This model demonstrated that epithelial compression induced production of collagen I, III, and IV by fibroblasts in a time- and pressure-dependent manner. Lateral epithelial compression shows similar results, with three-dimensional gel-embedded coculture models undergoing compressive strain leading to increased production of collagen by fibroblasts just beneath the epithelium, indicating a concentration gradient from the epithelial surface.\textsuperscript{21}

Mechanical stress also alters epithelial barrier function, disrupting tight junctions and increasing the transduction of lentivirus across the epithelial surface.\textsuperscript{22} Repeated apical stress on cultured epithelial cells induces epithelial metaplasia with an increase in mucus production, even when apical stress is applied for as little as 10 min/d,\textsuperscript{23} as well as inducing the release of YKL-40, a mediator associated with asthma severity and a reduction in lung function.\textsuperscript{24}

These data suggest that the epithelium is receptive to mechanical (especially compressive) stress, inducing the release of mediators that drive extracellular matrix deposition in the airway following such stress. In addition, mechanical stress associated with bronchoconstriction increases mucus production and may induce changes in epithelial tight junctions and barrier function, all of which may be relevant in asthma pathology.

How Does the Epithelium Detect Mechanical Stress?
To react to mechanical stress, the bronchial epithelium must detect this stress, and understanding the underlying mechanism may provide opportunities for intervention. Using air-liquid interface cultures of human bronchial
epithelial cells, it has been demonstrated that apical mechanical pressure on the cells shrinks the intercellular spaces while cell volumes themselves remain constant. This loss of intercellular fluid results in matrix metalloproteinase-dependent shedding of heparin-binding epidermal growth factor from one cell, binding to the epidermal growth factor receptor (EGFR) on the adjacent cell, with the resultant EGFR phosphorylation within 5 to 20 min of compression. Extending this work from cell culture to an ex vivo whole-tissue model using isolated mouse trachea, methacholine application to isolated tissue resulted in phosphorylation of the EGFR in the airway epithelium, a signaling event known to regulate mucus cell hyperplasia. This result was only found when mechanical stress occurred, when bronchoconstriction was abrogated by preadministration of isoproterenol no EGFR phosphorylation was detected.

What Is the Role of the ASM?
The effects of bronchoconstriction on the airway may not only be on the epithelium. Recent work has shown that contraction of human ASM cells in vitro induces activation of TGF-β by the ASM cells. The mechanism of this, which involves αvβ5-integrin signaling, was shown to involve reorganization of the cytoskeleton.

In addition, several mechanosensitive transcription factors have been described in ASM, including serum response factor, myocardin, and myocardin-related transcription factor. The function of these transcription factors appears directly regulated by the actin cytoskeleton and the master regulator of smooth muscle contraction RhoA. It is well established that these transcription factors are activated by TGF-β in ASM, and the activation of muscarinic receptors that regulate bronchoconstriction enhance the functional effects of both passive mechanical stress and growth factors such as TGF-β. For example, muscarinic receptor agonism promotes the expression of myosin light chain kinase (MLCK) in concert with mechanical stretch. MLCK is the key to force production in smooth muscle. Furthermore, muscarinic receptor agonism acts synergistically in combination with TGF-β in the regulation of mRNA-to-protein translation of contractile markers (eg, smooth muscle α-actin) in ASM and induces TGF-β release and the expression of contractile proteins (eg, smooth muscle myosin) in guinea pig lung slices.

Does the Asthmatic Airway Respond Differently to Mechanical Stress?
The epithelium and smooth muscle are both known to be abnormal in asthma. The epithelium produces increased mucus, is of higher permeability, has a greater sensitivity to oxidants, and has a deficient innate immune response to virus compared with the normal epithelium. The ASM in asthma is thickened due to both cellular hyperplasia and hypertrophy, and increased airway reactivity to spasmogens is a typical feature of asthma. The findings discussed previously of epithelial and smooth muscle responses to bronchoconstriction could be normal physiologic responses of tissues to an abnormal mechanical force, and indeed, many of the epithelial experiments were performed on normal cells, suggesting an inherent response to mechanical stress. It could, however, also be that the abnormal epithelium and
smooth muscle present in asthma respond differently to mechanical stress compared with normal tissue and, indeed, this appears to be the case. Experimental ASM constriction leads to more active TGF-β release from asthmatic than from healthy ASM, and asthmatic ASM has increased MLCK compared with normal ASM. The epithelium from patients with asthma also shows an augmented response to mechanical stress, releasing increased TGF-β and granulocyte-monocyte colony-stimulating factor compared with normal cells. These findings suggest that there is a multistep abnormality in asthma, with abnormal physical stress (bronchoconstriction) leading to amplified downstream signaling and pathologic airway responses.

What Is the Real-Life Relevance?

Multiple experimental systems examining the effect of mechanical forces on the respiratory epithelium in vitro are of interest; however, relevance in human disease is debatable without in vivo evidence. We have shown that the responses found in vitro are indeed mimicked by human airway responses. To investigate the effects of bronchoconstriction on airway responses, volunteers with mild allergic asthma who were only taking, as required, short-acting β-agonists as bronchodilator therapy were recruited and exposed to one of four repeated inhaled challenges: allergen (inducing both bronchoconstriction and eosinophilic inflammation), methacholine (inducing bronchoconstriction but no increase in eosinophilic inflammation), and two control conditions (saline inhalation and methacholine challenge preceded by inhaled albuterol to control for any direct chemical effect of methacholine). The allergen and methacholine groups were matched for an initial drop in FEV1, Bronchial biopsy specimens were obtained before and after the repeated challenges and the respiratory epithelium examined by immunohistochemistry. There was a similar degree of epithelial activation (increased TGF-β immunoeexpression in the epithelium), airway metaplasia to a more mucus-producing phenotype (increased epithelial mucus staining, possibly as a result of EGFR activation), and airway remodeling (subepithelial collagen deposition) in the methacholine and allergen groups, with no increases in the saline and albuterol/methacholine groups. This finding suggests that bronchoconstriction induced both epithelial activation and airway remodeling. Other researchers have found similar results, where blocking bronchoconstriction with long-acting β-agonists (LABAs) prevents remodeling as measured by myofibroblast numbers following allergen challenge.

Consequently, these findings suggest that (better adherence to) bronchodilator drugs may improve patient outcomes and help to prevent clinical manifestations related to remodeling, such as persistent airway narrowing, lung function decline, and corticosteroid resistance.

How Are the Effects of Bronchoconstriction Related to Inflammation?

If bronchoconstriction can cause airway structural changes, what exactly is the role of inflammation? Histopathology of severe asthma in childhood suggests that inflammation and remodeling can be dissociated, and additional studies indicate that the severity of remodeling in asthma is unrelated to the severity of inflammation. Multiple in vitro studies have demonstrated that inflammation and remodeling can be dissociated and studied separately in models of disease. Tschumperlin et al showed that EGFR activation in response to bronchoconstriction can be achieved in naïve animals not previously exposed to allergen; similarly, using naïve guinea pig precision cut lung slices, Onema et al demonstrated that bronchoconstriction induced by methacholine and histamine was sufficient to enhance the expression of smooth muscle-specific marker proteins in the large and small airways. This involved a mechanism similar to that reported by Tatler et al, including the release of bioactive TGF-β.

Although the exact role of inflammation in the remodeling process is not yet completely clear, a role for the inflammatory response in creating a local airway microenvironment more susceptible to bronchoconstriction is plausible. This would explain both the aforementioned findings and the number of studies reporting effects of antiinflammatory therapies on remodeling in animal models of asthma. Intriguingly and in support of this hypothesis, knockout of the muscarinic M1 receptor that regulates bronchoconstriction reduces and prevents various aspects of remodeling but with no effect on airway inflammation. Future studies are needed to address this question in more detail.

Can We Reinterpret Previous Studies in Light of the Evidence Regarding the Influence of Mechanical Forces in the Airways?

With the accumulation of data suggesting that bronchoconstriction may induce airway remodeling through the release of mediators from the epithelium and smooth muscle, a reexamination of previous studies with these data now available is warranted. Attempts to reduce airway remodeling in vivo by increasing antiinflammatory medication have shown conflicting results.
potentially because corticosteroids reduce exacerbation and, therefore, bronchoconstriction frequency. Airway remodeling itself is associated with corticosteroid resistance in asthma. Many studies have demonstrated, however, that LABAs in combination with inhaled corticosteroids (ICSs) improve lung function over and above the improvement from ICSs alone. These data have been interpreted either as a direct effect of bronchodilation in combination with the antiinflammatory effects of the ICS or as a result of an additional antiinflammatory effect of the LABAs when used in combination with ICS. These conclusions have been reached because of evidence that LABAs have antiinflammatory effects in vitro, although there is good evidence that LABAs do not have any clinically relevant antiinflammatory effect when given alone, neither does formoterol reduce airway inflammation following allergen challenge. Kelly et al examined the effects of budesonide/formoterol or budesonide alone on allergen-induced airway responsiveness, inflammation, and airway remodeling. Budesonide alone reduced airway inflammation but had no effect on myofibroblast accumulation or ASM mass (used as markers of remodeling). In contrast, the combination of budesonide and formoterol, which only slightly increased the antiinflammatory effect compared with budesonide alone, completely prevented the remodeling induced by the allergen challenges. Possibly, these effects of the combination therapy are not due to an enhanced antiinflammatory effect of the formoterol/budesonide combination but may be due to the introduction of a bronchodilator in the protocol. Unfortunately, formoterol monotherapy was not studied in a separate group of patients possibly because this is not used clinically due to concerns related to increased mortality from LABA monotherapy.

Kariyawasam et al showed that inflammation and remodeling changes can be isolated from each other in time, with remodeling persisting and inflammation being transient following allergen challenge. Interestingly, several markers of airway remodeling continued to increase up to 7 days following a single-allergen challenge. However, in light of the role of bronchoconstriction in the induction of remodeling, the study protocol actually included three methacholine challenges, two occurring after the allergen challenge. The continuing remodeling, which was attributed to a delayed response to the inflammation at the time of the allergen challenge, should now be interpreted in light of the repeated methacholine challenges, which were not controlled for and have been shown to induce airway remodeling.

Conclusions
Recent in vitro and in vivo findings have dramatically reshaped our view on the role of bronchoconstriction in asthma pathophysiology. From merely being a symptom of disease, it is now clear that bronchoconstriction may be at the root of the disease and the worsening of symptoms. Although airway inflammation may provide a local airway microenvironment that facilitates bronchoconstriction, a direct role for the inflammatory response in regulating remodeling may be questioned. Consequently, when considering (novel) therapies aimed at reducing airway remodeling in asthma, their bronchodilatory capacities should be taken into consideration. In addition, the potential role of bronchoconstriction in airway remodeling should be considered in the design of clinical trials, particularly those where tests of airway hyperreactivity are planned as outcome measures.

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References

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