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Mechanisms of Airway Hyperresponsiveness in Asthma: The Past, Present and Yet to Come

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Abstract

Airway hyperresponsiveness (AHR) has long been considered a cardinal feature of asthma. The development of the measurement of AHR forty years ago initiated many important contributions to our understanding of asthma and other airway diseases. However, our understanding of AHR in asthma remains complicated by the multitude of potential underlying mechanisms which in reality are likely to have different contributions amongst individual patients. Therefore the present review will discuss the current state of understanding of the major mechanisms proposed to contribute to AHR and highlight the way in which AHR testing is beginning to highlight distinct abnormalities associated with clinically relevant patient populations. In doing so we aim to provide a foundation by which future research can begin to ascribe certain mechanisms to specific patterns of bronchoconstriction and subsequently match phenotypes of bronchoconstriction with clinical phenotypes. We believe that this approach is not only within our grasp but will lead to improved mechanistic understanding of asthma phenotypes and hopefully better inform the development of phenotype-targeted therapy.

1. Introduction

Airway hyperresponsiveness (AHR) is defined as the predisposition of the airways of patients to narrow excessively in response to stimuli that would produce little or no effect in healthy subjects (Figure 1). Cockcroft et al (1) are largely credited with popularising the non-specific test of AHR almost forty years ago; however, the abnormal responses of asthmatics to non-specific stimuli were first described by Tiffeneau and Beauvallet in 1945 (2) and later developed during the 1960s in both Europe (3) and the United States (4). AHR has long been considered a cardinal feature of asthma and its measurement has provided profound insights into the underlying pathophysiology of the disease. Our view of AHR has greatly matured, and because of recent findings it is important to re-assess the current knowledge of AHR particularly in our understanding of the underlying mechanisms. While we did not embark upon a systematical evaluation of all literature regarding mechanisms of

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AHR in asthma we have endeavoured to provide an extensive update on the most recent findings. We will first introduce a few key clinical studies to provide a foundation for discussing the way in which future research of the mechanisms of AHR may contribute significantly to clinical practice.

But let's start with a pair of case studies. Both authors have at one time responded positively to bronchial challenge. One (DC) had AHR to methacholine despite being concurrently negative to mannitol challenge. Following treatment, he is now in "AHR remission". The other (CI) responded positively in the past to exercise, methacholine and histamine when inhaled but negatively during systemic administration. Currently he does not respond to methacholine even up to high doses. Despite this small sample size, a few key concepts emerge. Firstly, AHR is a moving target, in that its severity and presence are dependent upon many factors, including the modality of agonist chosen and level of treatment. Secondly, AHR is likely due to a plethora of underlying mechanisms that will have greater or lesser contributions in individual patients. This heterogeneity is not always well appreciated. While the clinical utility of AHR has been extensively reviewed elsewhere (5–7) we believe that understanding the heterogeneity of the mechanisms underlying AHR is an often over-looked yet important piece of the puzzle. Therefore we propose that future research into the mechanisms of AHR should aim to can move our understanding from a "one size fits all" approach to ascribing specific mechanisms of AHR to distinct patient populations. In this way we believe that it will provide much needed insight into the ever-developing recognition of distinct clinical phenotypes of asthma.

2. Clinical Importance

Airway hyperresponsiveness is used as a tool in the diagnosis, classification of severity (8) and management (9, 10) of asthma. AHR is useful in those who report symptoms (9), particularly in those with normal baseline lung function as measured by spirometry (11). The presence of AHR is associated with increased decline in lung function (12), even in those with asymptomatic AHR (13), increased risk for the development of asthma (12) and increased likelihood of the persistence of wheeze from childhood to adulthood (14). Furthermore, the severity of AHR is associated with an increased risk of exacerbation (15), increased asthma severity as measured by symptoms (16) and an increased level of treatment required to control symptoms (1). While the clinical implications relating specifically to the loss of the maximal response plateau in asthma are unclear, an increased or absent plateau represents uninhibited airway narrowing or closure that has the potential for life-threatening exacerbations (17). Understanding the factors contributing to the presence and severity of AHR therefore provides an important component for improving asthma control and reducing disease progression.

Although AHR is considered a hallmark of asthma, it is important to recognise that the severity, and even presence, of AHR is not stable. AHR to non-specific stimuli, such as histamine and methacholine, is increased in some, but not all, subjects following allergen challenge (18). This increase in AHR occurs most frequently in those subjects with a late asthmatic response and its persistence can be short-lived or remain for up to several months from exposure (19). It is not surprising, then, that seasonal allergen exposure alters the

severity of AHR (20). In addition, anti-inflammatory therapy profoundly improves AHR and since its widespread introduction many patients on appropriate treatment regimens may not respond positively to bronchial challenge in the range associated with asthma. For example, in a population of poorly controlled, chronically undertreated asthmatics, Reddel *et al* (21) reported that 16 weeks of high dose inhaled corticosteroid (ICS) followed by dose titration led to a 4.0 doubling dose increase in $PD_{20}FEV_1$ (reduction in AHR). After 72 weeks, 40% of subjects had responses to methacholine challenge within the normal range. Consistent with this, in asthmatic subjects on regular controller therapy, the sensitivity of a positive methacholine challenge for the diagnosis of asthma is only 77% (22). This sensitivity is further reduced in Caucasian and non-atopic patients. Furthermore, the measurement of AHR is confounded by its moderate repeatability, with estimates of within-subject repeatability ranging from 1–3 doubling doses (reviewed in (23)). Variability of AHR is further increased in those with non-atopic disease and those over 50 years of age (24). It is important to acknowledge that the variability of AHR is not only due to variability in the underlying mechanisms but also due to the imprecision of the measurement itself. Although it is clear that a negative challenge does not exclude the presence of asthma, interpretation of a negative bronchial challenge must consider the presence or absence of current symptoms. In a patient with current symptoms a negative challenge may suggest that diagnoses other than asthma should be considered. However, a negative challenge in a period without symptoms does not preclude asthma and in consideration of history it may be more appropriate to label such a patient as "*currently* negative AHR".

3. Measurement of AHR: To what do we respond and how do we measure it?

Traditionally measurements of airway responsiveness have been presented using two different, yet qualitatively similarly, calculations. The provocative concentration (or dose) causing a 20% fall in FEV_1 ($PC_{20}FEV_1$) is calculated by interpolation from the dose causing ≥20% fall in FEV_1 and the penultimate dose on a semi-log scale. It is maybe not surprising that determining the actual dose delivered (PD_{20}) appears to be a more robust measure than simply using the concentration of agonist (PC_{20}) (25). The dose response slope (or DRS), also referred to as the response dose ratio (RDR), is calculated as the slope of the dose response curve plotted with a linear dose axis. The advantage of the DRS is that it provides a continuous measure of airway responsiveness allowing inclusion of subjects who do not reach a 20% fall in FEV_1. In a more philosophical sense, the DRS more accurately reflects that airway responsiveness is a continuous variable in which AHR merely describes those people at one extreme. As both calculations provide similar information studies providing either calculation will be subsequently mentioned without differentiation.

a. Methacholine vs Mannitol

There are two groups of stimuli utilised in the measurement of AHR; those which allegedly act directly on the airway smooth muscle to induce bronchoconstriction, such as methacholine and histamine, and those which indirectly cause bronchoconstriction through the release of upstream mediators. Indirect challenge tests include exercise, eucapnic voluntary hypernea (EVH), hypertonic saline, mannitol, adenosine 5'monophosphate (AMP)

and various allergens. Although extensive review of the clinical application (6) and mechanisms (5) of these various stimuli have been provided elsewhere, the increasing interest in mannitol is worth considering as an example of an indirect challenge. Airway eosinophilia, measured by both sputum eosinophils and exhaled NO (eNO), is more strongly associated with AHR to mannitol than to methacholine (26, 27). Although this suggests that mannitol may provide a marginally better reflection of airway eosinophilia, the clinical utility of this finding is unclear. In addition, mannitol is more variable than methacholine which probably reflects variability in underlying inflammation. Therefore mannitol might be useful in predicting those patients who respond best to anti-IL-13 or anti-IL-5 monoclonal antibody treatment given the projected high cost of these treatments. Compared to treatment guidelines based upon symptoms and lung function, treatment strategies targeting reductions in AHR to mannitol (STAMINA trial) (28) or methacholine (AMPUL trial) (9) both lead to improvements in the number of mild exacerbations. Mannitol appears more specific in detecting a diagnosis of asthma although it is less sensitive than methacholine (27, 29, 30). Interestingly, the biggest distinction may be in the effect of allergen exposure with increased responsiveness to methacholine but reduced responsiveness to mannitol three hours after allergen challenge (31). This highlights the distinct underlying mechanisms between the two tests and suggests that comparison of the two methods may be useful in identifying phenotypes of AHR, and the underlying mechanisms, in subpopulations of patients. Indeed, one recent study reported 15% of asthmatics in primary care had AHR only to methacholine while another 15% had AHR only to mannitol (32). Similar variability in response has been reported when comparing exercise, EVH and methacholine challenge (33). Further research is required to determine the clinical importance of these distinct phenotypes of AHR.

b. What lung function measurement to use?

Traditionally AHR has been measured as reductions in spirometric parameters, most particularly FEV_1. However, spirometry is highly effort dependent and therefore requires considerable subject co-operation which is impossible for children under five and difficult for the elderly and those with increased disease severity. In contrast, the forced oscillation technique (FOT) is a measure of the mechanics of the respiratory system which can be acquired without special breathing manoeuvers. The FOT imposes oscillations over tidal breathing with the subsequent changes in pressure and flow analysed to provide measures of respiratory system resistance (Rrs) or its inverse conductance (Grs), as measures of airway calibre, and reactance (Xrs), as a measure of elastance. Recent advancements have allowed the FOT to cross the divide into clinical practice. FOT is capable of detecting patients with AHR, as assessed by spirometry, during methacholine (34), mannitol (35) and carbachol challenges (36). Furthermore, the repeatability of Grs and Xrs is not different to that of FEV_1 (2.0 and 1.95 vs 1.67 doubling doses, respectively, (35)). However, it should be noted that the aforementioned comparative studies included deep inspirations (inherent in spirometry) which provide beneficial effects in non-asthmatic but not moderate to severe asthmatic subjects (37). More importantly, removing deep inspirations during FOT measurement may also alter responsiveness in asthmatics with mild AHR since reduced responses are observed in mild disease when challenge inhalation is performed with the deep inspiration method compared to the tidal breathing method (38). Therefore, measuring responses to bronchial challenge during only tidal breathing with FOT may reduce the

ability to discriminate borderline AHR from normal responsiveness. Furthermore, decades of research indicates the clinical utility of AHR measured by spirometry, leaving us with a scenario where the use of FOT during bronchial challenge may best be suited as an adjunct to spirometry. Comparison of spirometry and FOT responses to bronchial challenge, as well as comparisons between FOT variables, may provide important clues as to the underlying pathophysiology. Lastly, FOT measurements may be capable of detecting differences in the pattern of response between stimuli (39) which may further aid in assigning phenotypes of AHR to distinct clinical populations.

4. Mechanisms of Airway Hyperresponsiveness

Despite decades of research, there is still little consensus as to the mechanisms underlying AHR in asthma. This is most likely due to the numerous pathophysiological abnormalities associated with asthma and the likely reality that different mechanisms or a combination of these gives rise to AHR in different patient populations. The definition of asthma as an inflammatory airways disease characterised by exaggerated airway narrowing immediately brings attention to the role of airway inflammation and the airway smooth muscle (ASM) in the manifestation of AHR. In addition, the structural remodelling reported in many patients with asthma is also likely to contribute in some, but presently unclear, way to the severity of AHR. Lastly, there is currently renewed interest in airway closure as a cause of AHR rather than merely a consequence.

a. Genetics

The role of familial inheritance in asthma was formally acknowledged by Coca and Cooke in 1923 (40) and the heritability of AHR has since been reported to be approximately 30% (41). However, the mechanisms linking genetics and AHR remain to be defined. Levitt and Mitzner (42) showed the large genetic contribution to AHR in mice by demonstrating that airway responsiveness to acetylcholine was controlled by a single autosomal recessive gene. Genome-wide association studies have revealed a substantial number of genes associated with susceptibility to asthma (recently reviewed in (43)), with genes corresponding to inflammatory pathways, airway epithelial function and ASM function likely contributing to AHR (mechanisms discussed below). Indeed, β_2-adrenergic receptor genotype appears to partially determine the improvements in AHR to methacholine following salmeterol/ICS therapy (44). Although not substantiated, this may represent distinct genotype-dependent mechanisms of AHR rather than differences in general treatment efficacy since improvements in baseline lung function, eNO and bronchodilator responsiveness were not affected by genotype. Similarly, it has been reported that allergen exposure in mice induces epigenetic changes in the transforming growth factor-β signaling pathway which are associated with development of AHR (45). Despite considerable advancement of our understanding of genetics in asthma future research is required to determine if and how genetic/epigenetic alterations are causally linked to the development and severity of AHR in human asthma.

b. Airway Inflammation

Asthma is a disease associated with chronic inflammation and the influx of inflammatory proteins likely contributes to AHR. Although allergic asthma has long been known to be associated with increased eosinophils in the airways, recent research suggests that subsets of asthmatic patients have elevated neutrophils with or without increased eosinophils (46). In asthmatic subjects there is a positive correlation between the severity of AHR and the number of eosinophils and metachromic cells in sputum (47, 48), as well as the number of mast cells in the airways (49). Furthermore, the level of exhaled nitric oxide (eNO), considered a biomarker for eosinophilic inflammation (50), correlates with the severity of AHR to methacholine in asthmatic subjects (51, 52). Interestingly, the link between eNO and AHR appears driven by airway narrowing, but not airway closure (53). In a small study of mild asthmatics, Brusasco et al (54) found no relationship between baseline AHR and inflammatory cells in bronchoalveolar lavage. However, after allergen challenge a strong correlation was reported between the increase in AHR and increase in eosinophils, further supporting the role of eosinophils in AHR. In contrast, there is little evidence that airway neutrophilia contributes to the severity of AHR in asthma. Indeed, Porsbjerg *et al* (48) were unable to show any correlation between neutrophils or neutrophil mediators and AHR. However, sputum neutrophils were correlated with an increased contribution of airway closure to the overall level of bronchoconstriction. Taken together, these findings suggest that eosinophillic airway inflammation may contribute to the severity of AHR whereas airway neutrophilia may be associated (causally or coincidentally) with an alteration in the type of bronchoconstriction towards predominance of airway closure.

c. Airway Smooth Muscle

Bronchoconstriction is due, at least in part, to constriction of the airway smooth muscle (ASM) surrounding the airway. Therefore it is not surprising that increased contractility of the ASM has long been touted as a principal cause of AHR. Abnormal ASM function could be due to intrinsic abnormalities of the ASM itself or to the effects of the asthmatic environment in which it resides.

i. Intrinsic factors—Despite considerable research it is still unclear as to whether asthmatic ASM is intrinsically hyper-contractile, and if so, what factors are mechanistically involved. Recent gene expression profiling of ASM revealed four novel genes that not only differentiated asthmatic and non-asthmatic patients, but were related to the severity of AHR (55). Furthermore, the expression of contractile proteins α-smooth muscle actin and desmin in ASM from asthmatics correlates with the severity of AHR (56) suggesting a role of intrinsic ASM dysfunction. Some *in vitro* studies have reported increased force generation of ASM from asthmatic patients (57, 58), while others have reported no difference when compared to healthy controls (59–61). However, increased airway narrowing could be due to an increase in the shortening velocity of ASM despite normal force generation. This would theoretically occur because a muscle that shortens quickly would produce greater airway narrowing during expiration before the dilatory effect of the proceeding inspiration (62). Indeed, *in vivo* findings support an effect of ASM shortening velocity on the magnitude of ASM shortening (63).

ASM contraction involves the formation of actin-myosin cross-bridges with the rate of formation dependent upon the activity of myosin light chain kinase (MLCK) and myosin light chain phosphatase (MLCP). An increase in the activity of either MLCK or MLCP would lead to increased shortening velocity of ASM. Indeed, both an increased expression of MLCK and increased shortening velocity of ASM have been reported in asthma (64). Furthermore, the fast myosin heavy chain isoform is also increased in patients with asthma which murine models suggest would also increase cross-bridge cycling and AHR (65). Functionally, this may not be important as increased shortening velocity in asthma is not a consistent finding (61). Asthmatic ASM is also more sensitive to oxidative stress with the extent of oxidative damage within the ASM bundle correlated with the severity of AHR (66). This relationship is in part mediated by increased NOX4 expression since siRNA knockdown of NOX4 attenuates *in vitro* ASM contractility. In contrast to dysregulation of the molecular pathways controlling ASM contraction, subcellular structure of the ASM appears similar between asthmatic and non-asthmatic subjects (67).

ii. Extrinsic factors—The asthmatic airway resides in a pro-inflammatory environment which likely contributes to ASM dysfunction independent of any intrinsic abnormalities. Pro-inflammatory cytokines such as IL-4, IL-13 and tumour necrosis factor-α (TNFα) increase ASM responsiveness *in vitro*, possibly via effects on calcium signaling (68). Proteases, such as matrix metalloproteinase-1 (MMP-1), are increased within ASM bundles of asthmatics and also regulate *in vitro* ASM contractility (69) and structural integrity. Additionally, the number of mast cells within the ASM correlates with the severity of AHR in asthma (70). Although the mechanisms are not yet clear, mast cell mediators such as histamine, leukotriene D_4 (71) and prostaglandin D_2 (72) may contribute to increased basal ASM tone. Bossé and colleagues (73) reported that ovine tracheal ASM adapts to increased basal tone so that subsequent ASM shortening is synergistically amplified. Computational modelling suggested that this synergistic effect on ASM shortening, termed force adaptation, would translate to an increase in airway narrowing as high as 48% and increase in airflow resistance up to 274% for a prototypic ninth generation airway (74). Although force adaptation has recently been demonstrated in mice *in vivo* (75), it is unknown whether force adaptation occurs in humans *in vivo* and to what extent, if any, that would contribute to AHR measured by spirometry.

Alternatively, the inflammatory milieu may induce the transition of asthmatic ASM from a contractile to a “synthetic” phenotype (reviewed in (76)). This synthetic ASM phenotype is characterised by reduced contractile-associated proteins but increased proliferation and chemokine secretion. *In vitro* stimulation of human ASM with TNFα or IL-1β induces the secretion of chemokines such as regulated on activation, normal T cells expressed and secreted (RANTES), interleukin-6 (77, 78) and IL-8 (79, 80). This proliferative/secretory phenotype is associated with reduced expression of contractile proteins such smooth muscle myosin heavy chain, smooth muscle α-actin, myosin light chain kinase (81). It is presently unclear whether the transition of ASM to the synthetic phenotype confers protection against, or further contributes to, AHR.

Damage to the airway epithelium, which provides an initial barrier for inhaled spasmogens, also likely contributes to AHR. Disruption of the airway epithelium would increase the

amount of stimulus interacting with the ASM and thus potentiate bronchoconstriction. In addition, epithelial damage or dysregulation likely reduces the ability of the epithelium to maintain relaxation of ASM via release of epithelial-derived relaxing factor(s) (82). For example, intratracheal administration of cationic proteins reduces both the barrier effect and control of ASM relaxation by the airway epithelium, and results in AHR in animal models (83). Additionally, damage to the epithelium may also directly contribute to airway narrowing. Recent murine *in vitro* findings suggest that rupture of small airway epithelial cells induce intracellular [Ca^{2+}] waves and subsequent contraction in neighboring ASM (84). The contribution of damage of the airway epithelium is most likely to be highly relevant to AHR following exposure of noxious inhalants, such as in occupational asthma.

d. Structural airway remodelling

The lung of the asthmatic exhibits a gamut of structural pathologies that are collectively termed airway remodelling. Remodelling here merely means that the structure is no longer normal with the implication that the change is permanent. These changes include subepitheial fibrosis (85), ASM hypertrophy/hyperplasia (86), angiogensis (87) and changes in extracellular matrix composition (88). AHR correlates with airway wall thickening (89, 90) , reticular basement membrane thickness (91) and components of the extracellular matrix (56, 92), although others have been unable to replicate these findings (70).

Thickening of the airway wall could contribute to excessive bronchoconstriction in two ways. Airway resistance is inversely related to airway radius such that an increase in the submucosal area would amplify the reduction in airway calibre for any given degree of ASM shortening. Although an attractively simple explanation, it remains unclear whether reduced airway calibre is causally associated with increased severity of AHR. On one hand, several studies report a correlation between baseline airway calibre and AHR, measured by FEV_1/FVC (53, 93) and $FEF_{25–75}$/FVC (94, 95). On the other hand, *improvements* in airway calibre appear dissociated from improvements in AHR. Salome et al (96) administered fenoterol prior to histamine challenge and reported that although baseline FEV_1 and AHR both improved, AHR returned to control levels much more rapidly than FEV_1. This disconnect was later strengthened by Britton et al (97) who reported that ipratropium did not alter AHR despite increasing baseline FEV_1 and sGaw. However, determining whether *reductions* in airway calibre are similarly disassociated from AHR is confounded by an inability to reduce airway calibre without confounding effects, such as those related to transpulmonary pressure or ASM tone.

An increase in the thickness of the adventitial layer has the potential to uncouple the ASM layer from the surrounding parenchyma. Under this condition, the ASM is essentially untethered from the lung parenchyma, reducing the load against which the ASM shortens. This would allow for increased ASM shortening. Increased ASM mass, due to either hypertrophy or hyperplasia, is thought to increase the total force generated by ASM and thus exaggerate airway narrowing without any alteration in ASM contractile function (98). Indeed, increased ASM area in explanted bronchial segments from asthmatics correlates with increased *in vitro* airway narrowing (99). However, airway remodelling may protect against AHR. Should the remodelling processes increase airway wall stiffness, it would in

fact oppose, and therefore limit, airway narrowing during bronchoconstriction (100). On the other hand, some features of airway remodeling may be a consequence, rather than a cause, of AHR since bronchoconstriction itself is sufficient to induce subepithelial fibrosis and mucous metaplasia without affecting AHR (101).

e. Airway Closure

It is not well recognized that bronchoconstriction is also associated with increased airway closure. Moreover, it is important to determine whether increased airway closure is merely the consequence of exaggerated airway narrowing (102) or whether asthma is associated with a predisposition to airway closure. Increased airway closure assessed by bronchial challenge is associated with increased disease severity (103), oral steroid use (104) and a history of exacerbations requiring intubation (103). Irvin and Bates (105) reviewed the literature that supports the notion that bronchoncostriction in asthma is not due to central airway narrow as commonly assumed, but rather due to peripheral airway closure. Consistent with this hypothesis, AHR in allergically sensitised mice can be fully attributed to an increased susceptibility to small airway closure (106, 107). The importance of airway closure to human asthma was validated by the finding that the extent of airway closure during methacholine challenge was a significant determinant of the severity of AHR, independent of airway narrowing (53). However, unlike in the allergically sensitised mouse, there is great variability in the contribution of airway closure to bronchoconstriction in human asthma. As shown in Figure 2A and B, asthmatic patients can respond to bronchial challenge through predominantly airway narrowing or airway closure. It is important to highlight that these examples are the extremes of a continuum with the majority of subjects falling in between. Hence, the severity of AHR in specific phenotypes of asthmatic patients is likely due in large part to airway closure. However, the clinical features or underlying mechanisms of patients who respond predominantly due to airway closure require further investigation.

There are several mechanisms by which asthma pathophysiology may lead to increased airway closure during bronchoconstriction. Firstly, mucous plugging would obviously induce airway closure so it is not surprising that pharmacologically blocking the release of mucous protects against AHR in allergically inflamed mice (108). Secondly, increased airway closure may be due to surfactant dysfunction caused by inflammation (109, 110). Similarly, fibrin is known to inactivate surfactant (111), accumulate in the airways of asthmatics and is associated with AHR (107). A role of surfactant dysfunction in AHR is consistent with the protective effect of inhaled surfactant against allergen-induced bronchoconstriction (112). On the other hand, recent computational and physiological evidence suggests that increased baseline ventilation heterogeneity may promote increased airway closure during bronchoconstriction. Venegas et al (113) developed a highly advanced lung model that takes account of the effects of the parenchymal tethering forces, the intra- and extra-luminal pressures and ASM forces. The model predicted that uniform ASM contraction with the addition of small, random heterogeneities in airway calibre would lead to the abrupt development of airway closure when ASM contraction reached a critical level of instability. The validity of the model predictions have been strengthened by subsequent findings that the severity of AHR in asthma strongly correlates with the degree of baseline

ventilation heterogeneity (52, 114, 115). This has recently been extended by the report that baseline ventilation heterogeneity correlates with the increase in airway closure during methacholine challenge (116). Importantly, the association between baseline ventilation heterogeneity and AHR remained following three months of ICS treatment suggesting that it is independent of (steroid-responsive) airway inflammation (52). Further research is needed to ascertain the causes of the baseline ventilation heterogeneity and whether they can be targeted to treat AHR, which may provide more effective treatment strategies for asthma.

5. Can AHR contribute to our understanding of asthma phenotypes?

Asthma is not a single disease but a combination of many pathophysiological features culminating in the clinical presentation of asthma symptoms. This underlies the importance of personalised medicine, in which the foundation has been built on improved phenotyping of asthmatic patients utilising a variety of clinical, inflammatory and physiological features (117, 118). However, these approaches are yet to include charateristics of AHR, such as differences between modalities or the pattern of bronchostriction. Below we highlight three phenotypes associated with worse asthma control and discuss the current understanding of AHR in each group (Table 1).

a. Asthma in the elderly

Asthma control worsens with age (119) with one recent study reporting that 25% of asthma patients over 65 years experienced at least one severe exacerbation in the preceding year (120). Hardaker et al (114) recently compared the physiological determinants of AHR in young and elderly asthmatics. In those below 55 years, the severity of AHR was predicted by increased eosinophilic airway inflammation (exhaled NO) and baseline ventilation heterogeneity in conducting airways. In contrast, AHR in the elderly was associated with baseline gas trapping and ventilation heterogeneity in acinar airways. This suggests that AHR in the elderly is associated with more peripheral disease. This is consistent with previous reports that AHR in the elderly is associated with increased airway closure during methacholine challenge (121). This may be due to the increase in neutrophilia with age, since increased airway closure during bronchoconstriction in elderly asthmatics correlates with sputum neutrophil levels (48). Alternatively the distinct AHR of the elderly may be due to ural change of the loss of elastic recoil due to emphysema-like changes associated with aging (122). Bronchial challenge with AMP has been suggested to induce a more peripheral response than that due to methacholine (123) and therefore age may have greater effects on AHR to AMP than to methacholine challenge. However, it is unclear whether the more peripheral disease is due to the additive effects of age on asthma or a synergistic effect of disease duration.

b. Asthma in the obese

Cross-sectional studies report an increased prevalence of asthma in the obese (124), while obesity and weight gain appear to precede the development of asthma (125). Obesity appears to worsen asthma control (126) while weight loss leads to an improvement in asthma symptoms (127). There is growing recognition that obese asthmatics comprise two distinct clinical populations; those with high IgE and early-onset asthma (allergic), and those

with late-onset disease and low serum IgE (non-allergic). Following weight loss, only the non-allergic obese asthmatics had an improvement in methacholine responsiveness suggesting that obesity negatively impacts AHR only in those with non-allergic disease (128). This effect of obesity on AHR is due to increased collapsibility of peripheral airways that predisposes to increased airway closure during methacholine challenge (129, 130). This is illustrated in Figure 2C, in which the extent of airway closure, adjusted for the level of airway narrowing, is substantially greater in non-allergic obese asthmatics compared to non-obese asthmatics (ie significantly steeper slope). Interestingly, following weight loss, the response to methacholine appears identical to that of non-asthmatic obese subjects (ie position and slope of regression). These data suggest that allergic obese asthmatics may have asthma that is complicated by obesity, whereas non-allergic obese asthmatics have asthma secondary to obesity. Since the severity of AMP appears closely associated with atopy and IgE levels (131, 132), it is possible that AMP challenge may be better able to differentiate these two phenotypes of obese asthma.

c. Asthma in smokers

Asthmatics who smoke report worse asthma control (133) and smoking history is associated with the severity of AHR (134). Airway closure during AMP challenge is increased in asthmatic smokers compared to non-smokers, but not during methacholine challenge (135). This is consistent with a more peripheral response during AMP than methacholine challenge (123). Interestingly, improvements in AHR to AMP following smoking cessation occur earlier than improvements in AHR to methacholine (136). This may reflect a greater sensitivity of AMP to smoking-related pathophysiology. On the other hand, methacholine challenge may better reflect underlying structural changes than AMP (131) such that differences between the two stimuli may reflect distinct mechanisms underlying the severity of AHR in smoking asthmatics. Further research will determine whether these differences can be used to detect early smoking-related disease or determine those smokers who may respond to asthma guideline therapy and those unlikely to benefit.

6. Looking through the crystal ball: the future of AHR testing

We must ensure that our view of the measurement of AHR does not remain as a “one size fits all” approach. On one hand we have many different stimuli for bronchial challenge testing and on the other, an extensive list of potential mechanisms underlying AHR. Currently we do not completely understand whether specific mechanisms play a greater role in AHR assessed by one challenge test over another. Similarly, we do not know whether differences between challenge modalities provide a better assessment of the various clinical asthma phenotypes. Physiologists have long known that FEV_1 is a polyvalent measure of lung function that provides little information about the precise pattern of bronchoconstriction. Understanding the pattern of bronchoconstriction in an individual patient, whether through comparison of spirometric variables or from measurements such as the FOT and inert gas washout, may allow us to ascribe certain phenotypes of bronchoconstriction to specific clinical phenotypes. This, too, would help elucidate the underlying mechanisms and may contribute to more targeted therapies. As discussed above, a combination of these two approaches has already been applied to asthma in the obese,

elderly and those who smoke. While only in its infancy, the evidence to date suggests that phenotyping AHR may help to uncover the pathophysiology contributing to poor asthma control in numerous distinct subsets of patients with asthma.

7. Conclusion

The development of the measurement of AHR forty years ago sparked many important contributions to our understanding of asthma and other airway diseases. However, it is time to re-evaluate our assumptions of AHR in light of the current population of asthmatic patients. We must look towards the future, embracing the technological advancements which provide potentially complimentary techniques to measure the response to bronchial challenge. These complementary measurements may lead us to better partition global bronchoconstriction into its components of airway narrowing and airway closure as well as proximal and distal airway effects. This enhancement has to the potential of allowing us to assign certain mechanisms to specific patterns of bronchoconstriction, opening the door for matching phenotypes of bronchoconstriction with clinical phenotypes. In doing so we are likely to gain improved mechanistic understanding of asthma phenotypes, and help better focus as well as better assess the development of phenotype-targeted therapy.

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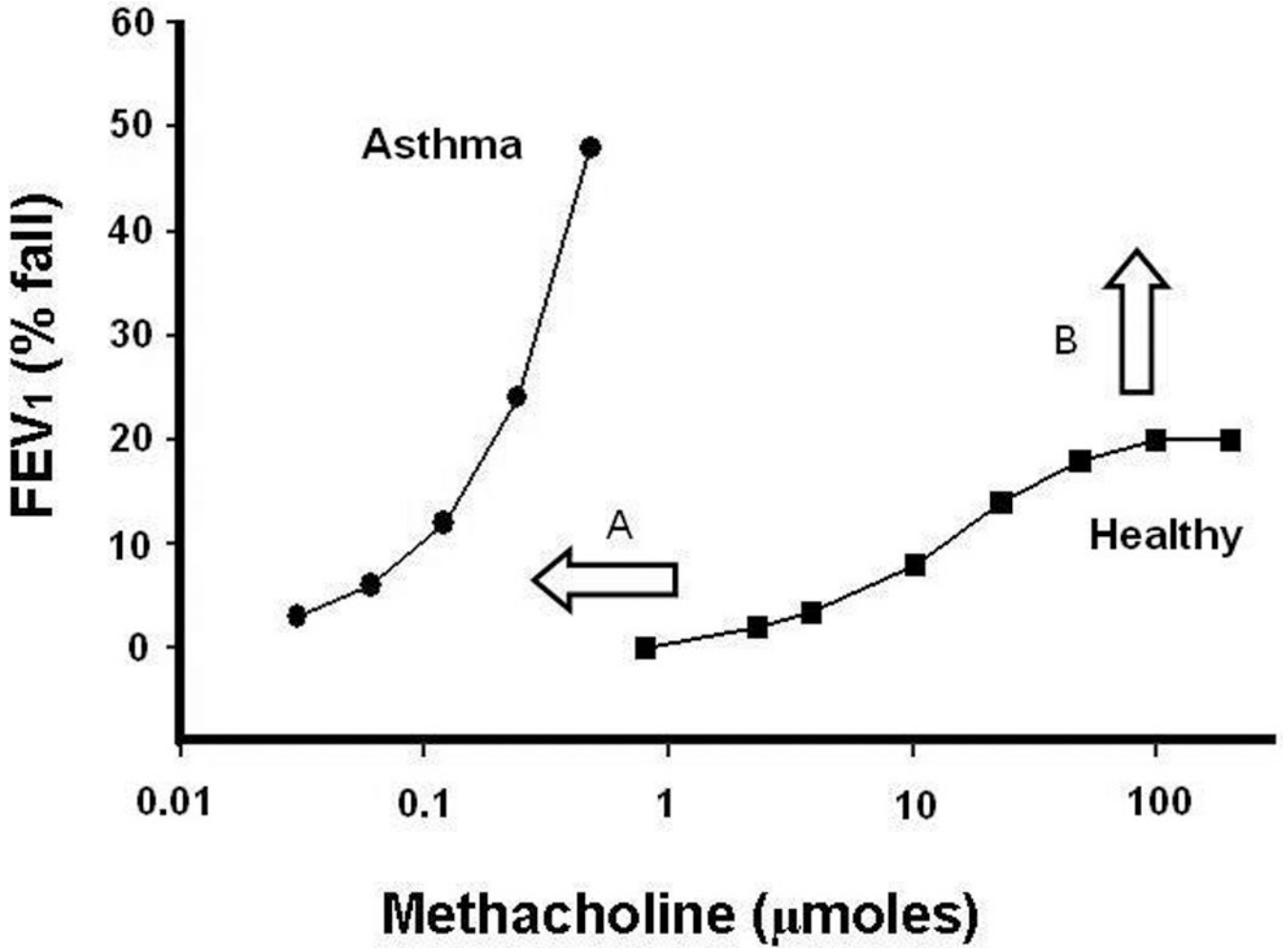

Figure 1. Representative dose response curves (DRC) to methacholine in a healthy and a severely asthmatic subject

Airway hyperresponsiveness is characterised by both an increased sensitivity, seen as the leftward shift in the DRC of the asthmatic patient (A), and excessive bronchoconstriction, resulting in the loss/increase in the maximal response plateau (B).

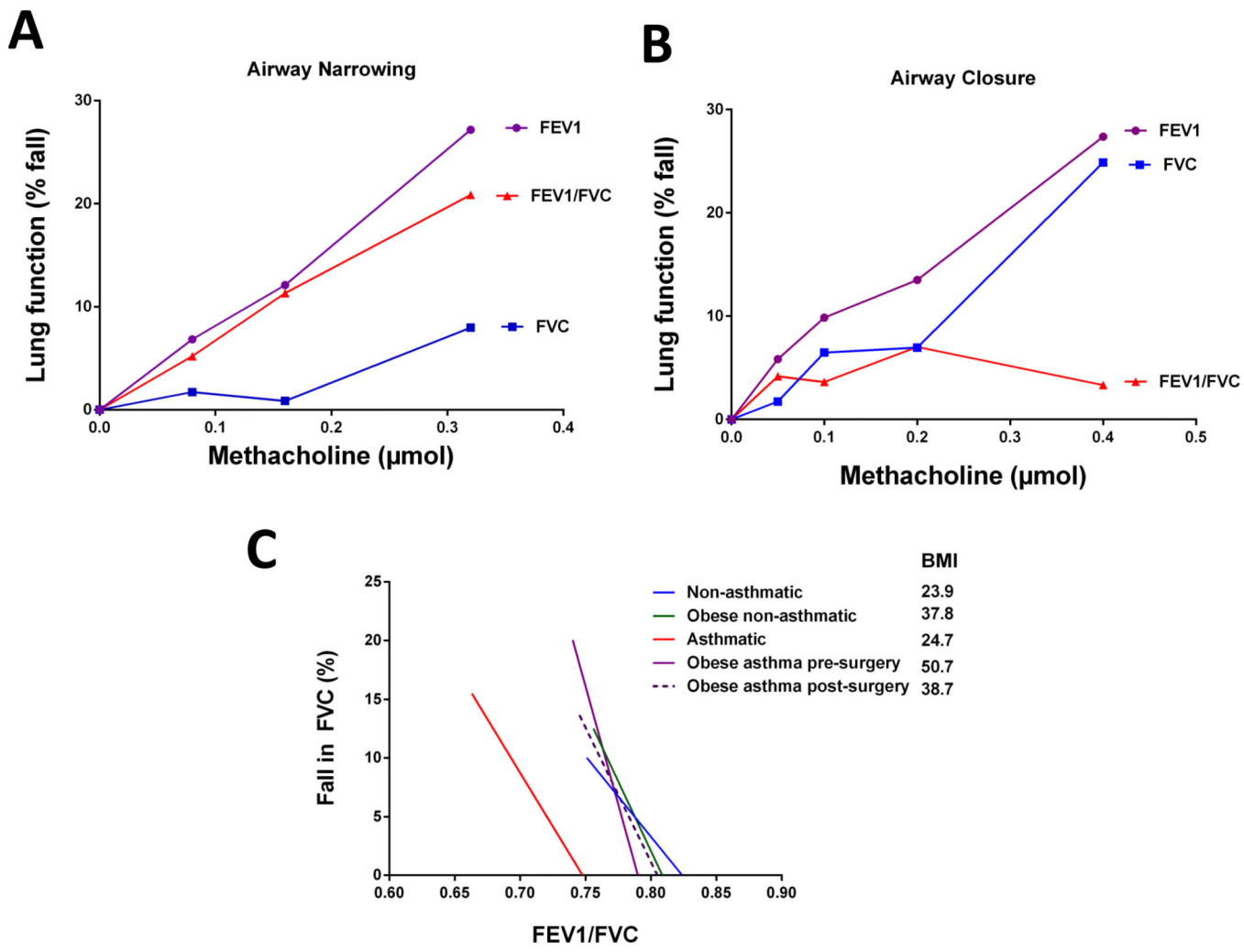

Figure 2. The contribution of airway narrowing and airway closure to the fall in FEV_1 during bronchial challenge

FEV_1 is reduced by airway narrowing because a narrowed airway loses some capacity to transmit flow. However, FEV_1 is also determined by the number of parallel airways contributing to flow and is thus reduced by functional airway closure (both true airway closure and severe airway narrowing). By contrast, FVC is determined by the volume of air in communication with the mouth and is reduced by functional airway closure but not by airway narrowing. Air narrowing, per se, is thus reflected in the ratio FEV_1/FVC. There is substantial variation in the contribution of airway narrowing and airway closure to the fall in FEV_1 amongst patients with asthma. Shown are dose response curves for an asthmatic subject with predominantly airway narrowing (A: 24 years old, baseline FEV_1 122%pred, $PC_{20}FEV_1$ 0.23µmol) and one with predominantly airway closure (B: 24 years old, baseline FEV_1 78%pred, $PC_{20}FEV_1$ 0.28µmol). These examples represent extremes of a continuum of responses, with the majority of subjects falling in between. The extent of airway narrowing is expected to contribute to airway closure so to determine excessive airway closure we have analysed the relationship between %fall FVC and FEV_1/FVC (C). A steeper slope represents greater airway closure for a given level of airway narrowing. Absolute

FEV_1/FVC, rather than % fall FEV_1/FVC, maintains the contribution of baseline airway calibre. Representative regression lines were calculated from the mean baseline FEV_1/FVC, mean fall in FEV_1/FVC and mean % fall FVC for lean non-asthmatics (blue), lean asthmatics (red), obese non-asthmatics (green), non-allergic obese asthmatics prior to bariatric surgery (purple) and the same subjects 12 months following bariatric surgery (dashed purple). Data were adapted from two of our previous studies (53, 130). Important to note is the increased slope in asthmatics compared to non-asthmatics, and in all obese groups compared to the two lean groups. Following weight loss, the slope of the obese non-allergic asthmatics decreased suggesting reduced predisposition to airway closure. Interestingly, the position and slope of obese non-allergic asthmatics post-surgery is almost identical to obese non-asthmatics suggesting that the effect of obesity on airway closure is dependent upon the level of adiposity in non-allergic subjects.

Table 1

Current understanding of AHR in asthma phenotypes associated with worse control

Asthma Phenotype	Severity of AHR	Pattern of AHR (Closure vs narrowing)	Modality	Associated pathophysiology
Elderly	Increased	Closure	Methacholine	↑ Neutrophils ↓ Elastic recoil
Obese				
• non-allergic	Increased	Closure	Methacholine	↓ FRC volume ↑ airway compliance ↓ surfactant (?)
• allergic	Unaltered	Closure	Methacholine	↓ FRC volume
Smoking	Increased	Closure	AMP	Inflammation/ Acutely reversible
	Increased	~ Equal	Methacholine	Structural

FRC = functional residual capacity