# Spirometric Response to Bronchodilator and Eucapnic Voluntary Hyperpnea in Adults With Asthma

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**BACKGROUND: The spirometric response to fast-acting bronchodilator is used clinically to diagnose asthma and in clinical research to verify its presence. However, bronchodilator responsiveness does not correlate with airway hyper-responsiveness measured with the direct-acting stimulus of methacholine, demonstrating that bronchodilator responsiveness is a problematic method for diagnosing asthma. The relationship between bronchodilator responsiveness and airway hyper-responsiveness assessed with indirect-acting stimuli is not known. METHODS: Retrospectively, the spirometric responses to inhaled bronchodilator and a eucapnic voluntary hyperpnea challenge (EVH) were compared in 39 non-smoking adult subjects with asthma (26 male, 13 female; mean ± SD age 26.9 ± 7.8 y; mean ± SD body mass index 26.3 ± 4.7 kg/m$^2$). All subjects met one or both of 2 criteria: ≥ 12% and 200 mL increase in $FEV_1$ after inhaled bronchodilator, and ≥ 10% decrease in $FEV_1$ after an EVH challenge. RESULTS: Overall, $FEV_1$ increased by 9.9 ± 7.9% after bronchodilator (3.93 ± 0.97 to 4.28 ± 0.91 L, $P < .001$) and decreased by 23.9 ± 15.0% after the EVH challenge (3.89 ± 0.89 to 2.96 ± 0.88 L, $P < .001$). However, the change in $FEV_1$ after bronchodilator did not correlate with the change after EVH challenge (r = 0.062, $P = .71$). Significant bronchodilator responsiveness predicted a positive response to EVH challenge in 9 of 33 subjects (sensitivity 27%). Following EVH, the change in $FEV_1$ strongly correlated with the change in FVC ($FEV_1$ percent change vs FVC percent change, r = 0.831, $P < .001$; $FEV_1$ ΔL vs FVC ΔL, r = 0.799, $P < .001$). CONCLUSIONS: These results extend previous findings that demonstrate a lack of association between bronchodilator responsiveness and methacholine responsiveness. Given the poor concordance between the spirometric response to fast-acting bronchodilator and the EVH challenge, these findings suggest that the airway response to inhaled $\beta_2$-agonist must be interpreted with caution and in the context of its determinants and limitations.** *Key words: airway hyperresponsiveness; bronchoconstriction; bronchoprovocation challenge; bronchodilator responsiveness; spirometry.* [Respir Care 2021;66(8):1282–1290. © 2021 Daedalus Enterprises]

## Introduction

Asthma is characterized by variable episodes of airway narrowing that result in dyspnea, chest tightness, wheeze, and cough. The airway narrowing is consequent to an underlying airway inflammation and airway hyper-responsiveness (AHR), which is increased sensitivity and responsiveness of the airway smooth muscle to a variety of stimuli. The spirometric response to inhaled fast-acting $\beta_2$-agonist is used clinically to diagnose asthma and in clinical research to verify its presence. However, a recent review highlights the poor diagnostic utility of bronchodilator responsiveness and the subsequent costs of misdiagnoses on the individual and health care system.[1] Indeed, bronchodilator responsiveness does not correlate with AHR measured with the direct-acting stimulus, methacholine,[2,3] which indicates that bronchodilator responsiveness is a problematic method for diagnosing asthma. However, the association between the spirometric response to inhaled fast-acting $\beta_2$-agonist and indirect assessment of AHR is not known. Compared with direct-acting bronchoprovocation tests, the indirect tests of AHR more accurately reflect the biological mechanisms of airway narrowing in asthma.[4] Eucapnic voluntary hyperpnea (EVH) is a well-established indirect bronchoprovocation challenge that causes airway narrowing through the actions of biological mediators released from resident and nonresident airway cells.[5,6]

We compared the spirometric responses to inhaled fast-acting $\beta_2$-agonist and EVH in 39 adult males and females who demonstrated either significant bronchodilator responsiveness or significant AHR in response to an EVH challenge, or both. Given the wide inter-individual variation in asthma pathophysiology and phenotype, and the temporal variability in airway function within individual patients with asthma,[7-9] we hypothesized that the spirometric responses to the inhaled $\beta_2$-agonist and EVH challenge would not correlate with each other.

## Methods

All subjects in this retrospective analysis were participants in research protocols that took place between August 2008 and July 2019 in the Exercise Physiology Lab at Northern Vermont University-Johnson. Subjects were recruited by a combination of advertisement in local print or online publications (eg, neighborhood forums, recreational clubs), flyers posted on campus and in the surrounding community, and by word of mouth. All subjects provided written consent to participate in the studies, and all research was approved by the Northern Vermont University institutional review board for research involving human subjects. All subjects were identified with asthma by meeting at least 1 of 2 criteria: $\geq$ 12% and 200 mL increase in $FEV_1$ after inhalation of a fast-acting $\beta_2$-agonist, or $\geq$ 10% decrease in $FEV_1$ after EVH challenge.

In all subjects, bronchodilator responsiveness was conducted as a preliminary screening study prior to participation in one of several research projects. The EVH challenge was conducted either as a preliminary screening study or as an experimental intervention in previous research.[10,11] In all cases, bronchodilator responsiveness was assessed during each subject's first visit to the lab. The EVH challenge was completed between a second and sixth visit to the lab, depending on the research study protocol being completed by the subject. In all subjects, at least 24 h separated the bronchodilator responsiveness and EVH challenge visit.

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### QUICK LOOK

**Current knowledge**

The airway response to an inhaled fast-acting bronchodilator is routinely used to diagnose asthma and to verify its presence in clinical research. However, studies indicate that bronchodilator responsiveness does not correlate with airways hyper-responsiveness (AHR) assessed via methacholine challenge.

**What this paper contributes to our knowledge**

In a group of 39 adults with asthma, the spirometric response to inhaled fast-acting bronchodilator did not associate with the response to a eucapnic voluntary hyperpnea challenge (EVH). In the subjects in whom EVH caused significant airway narrowing ($n = 33$), a minority (9 of 33) demonstrated significant bronchodilator responsiveness. In response to EVH, the spirometric response was characterized by reductions in both $FEV_1$ and FVC, demonstrating involvement of the peripheral airways in response to the indirect bronchoprovocation challenge.

All subjects were non-smokers between the ages of 18 and 45 y, had a negative history for cardiovascular disease and other chronic illness (excepting asthma), and had an absence of respiratory infection during the 4 weeks prior to participation. Many of the subjects used an inhaled fast-acting $\beta_2$-agonist on an as-needed basis; these subjects were instructed to refrain from such use for 8 h prior to each study. Subjects were instructed to avoid caffeinated food and beverages for 4 h prior to each study, and to avoid strenuous exercise for 8 h prior to each study. None of the subjects were using inhaled corticosteroids at the time of their participation.

An automated pulmonary function system was used to collect all spirometry data reported here (MGC Diagnostics, St Paul, Minnesota). Maximum forced exhalations were completed in the seated, upright position according to recommendations by the American Thoracic Society and European Respiratory Society.[12] During each measurement, subjects completed maximum volitional forced exhalations to determine FVC, $FEV_1$, forced expiratory flow during the middle half of the FVC maneuver ($FEF_{25-75\%}$), and peak expiratory flow (PEF). Predicted values for FVC and $FEV_1$ are from Quanjer et al.[13] Predicted values for $FEF_{25-75\%}$ and PEF are from Hankinson et al.[14]

Following baseline spirometry, subjects inhaled 4 actuations of a fast-acting $\beta_2$-agonist (4 $\times$ 90 $\mu$g albuterol sulfate). During each inhalation, subjects exhaled to residual volume, depressed the actuator, inhaled slowly through a holding chamber (Aerochamber, AbbVie, North Chicago,

Table 1. Demographic Characteristics and Baseline Pulmonary Function

| Variable | All Subjects ($N = 39$) | Group 1 ($n = 6$) | Group 2 ($n = 9$) | Group 3 ($n = 24$) |
|---|---|---|---|---|
| Male/Female | 26/13 | 3/3 | 5/4 | 18/6 |
| Age, y | 26.9 ± 7.8 | 29.3 ± 8.2 | 29.9 ± 9.2 | 25.2 ± 6.9 |
| Weight, kg | 78.6 ± 19.3 | 64.0 ± 8.9 | 8.8 ± 19.5 | 81.4 ± 20.0 |
| Height, m | 1.72 ± 0.10 | 1.68 ± 0.06 | 1.73 ± 0.11 | 1.73 ± 0.10 |
| Body mass index, kg/m$^2$ | 26.3 ± 4.7 | 22.6 ± 2.3 | 26.9 ± 4.4 | 27.0 ± 4.9 |
| FVC, L | | | | |
| $\beta_2$-agonist | 5.27 ± 1.18* (110 ± 16) | 4.56 ± 0.8 (103 ± 13) | 4.91 ± 0.91 (109 ± 18) | 5.58 ± 1.25* (112 ± 15) |
| EVH | 5.27 ± 1.09* | 4.61 ± 0.90 | 4.93 ± 1.00 | 5.60 ± 1.09* |
| $FEV_1$, L | | | | |
| $\beta_2$-agonist | 3.92 ± 0.98 (96 ± 18) | 3.20 ± 0.45* (87 ± 13) | 3.20 ± 0.79* (84 ± 16) | 4.38 ± 0.87 (104 ± 15) |
| EVH | 3.89 ± 0.89 | 3.4 ± 0.38 | 3.29 ± 0.9 | 4.24 ± 0.81 |
| $FEV_1$/FVC | | | | |
| $\beta_2$-agonist | 0.75 ± 0.09 | 0.71 ± 0.07 | 0.65 ± 0.08† | 0.79 ± 0.06 |
| EVH | 0.75 ± 0.10 | 0.75 ± 0.11 | 0.66 ± 0.09 | 0.79 ± 0.08 |
| $FEF_{25-75\%}$, L/s | | | | |
| $\beta_2$-agonist | 3.35 ± 1.34* (76.8 ± 26.1) | 2.37 ± 0.48* (61.0 ± 12.2) | 2.14 ± 0.90*† (50.2 ± 15.2)† | 4.05 ± 1.15* (90.6 ± 21.6) |
| EVH | 3.29 ± 1.23* | 2.89 ± 0.89 | 2.26 ± 1.14* | 3.78 ± 1.08* |
| PEF, L/s | | | | |
| $\beta_2$-agonist | 8.48 ± 1.86* (93 ± 16) | 7.63 ± 1.14 (92 ± 9) | 7.58 ± 2.09* (86 ± 15) | 9.03 ± 1.75 (98 ± 16) |
| EVH | 8.34 ± 1.77* | 08.02 ± 0.74 | 7.24 ± 2.45* | 8.83 ± 1.49 |

Values are presented as mean ± SD. Groups are based on the $FEV_1$ responses to inhaled fast-acting $\beta_2$-agonist and eucapnic voluntary hyperpnea. Percent of predicted in parentheses.
* $P < .05$ vs predicted values.
† $P < .05$ vs Group 3.
$FEF_{25-75\%}$ = forced expiratory flow during the middle half of the FVC maneuver
PEF = peak expiratory flow

Illinois) to total lung capacity, and held their breath for 5 s prior to exhaling. Spirometry was assessed 5 min after $\beta_2$-agonist and up to, but never after, 30 min after drug inhalation. In all cases, the post-bronchodilator maneuver with the highest $FEV_1$ was selected for analysis. The largest FVC and PEF were selected from the same measurement time as the highest $FEV_1$; however, the highest values did not necessarily occur during the single maneuver with the highest $FEV_1$. On average, the highest postbronchodilator $FEV_1$ occurred 15.4 ± 6.6 min after inhalation (range 5–30 min). The highest $FEV_1$ and highest FVC were used to calculate $FEV_1$/FVC. An increase in $FEV_1$ of > 12% and 200 mL from baseline was considered a significant response.[15]

Following baseline spirometry, subjects completed an EVH challenge according to previously published methods.[16] Briefly, subjects ventilated dry gas from a tank of compressed gas (21% $O_2$, 5% $CO_2$, balance nitrogen) for 6 min at a target ventilation equal to $FEV_1 \times 30$. Spirometry was assessed serially (at 5, 10, 15, 20, and 30 min) following the EVH challenge. The lowest post-hyperpnea $FEV_1$ measured after the challenge was selected for analysis. The FVC and PEF were selected from the same post-EVH time point with the lowest $FEV_1$; however, they were not necessarily from the same maneuver. A ≥ 10% decrease in $FEV_1$ was considered a positive EVH response.[16]

All tabular data are reported as mean ± SD. Subjects were partitioned into 3 groups (Groups 1–3) on the basis of their responses to inhaled fast-acting $\beta_2$-agonist and the EVH challenge. Due to the resulting unequal group sizes, a nonparametric test (ie, Kruskal-Wallis) was used to compare demographic and spirometry results among the 3 groups. Pairwise comparisons using the Mann-Whitney $U$ test, including Bonferroni-corrected $P$ values, were used to determine significant group differences. Within-group observations were analyzed using dependent $t$ tests. Pearson correlation coefficients were used to assess associations between variables. Statistical significance was set at $\alpha < 0.05$. The statistical software SYSTAT 12 (IBM, Armonk, New York) was used for all analyses.

## Results

Subject characteristics and results for baseline spirometry are shown in Table 1. The table contains results for all subjects and for the 3 subgroups based on subject responses to the 2 interventions. On average, subjects were 26.9 ± 7.8 y old and had a mean body mass index of 26.3 ± 4.7 kg/m$^2$. When all subjects were analyzed collectively, FVC was larger than predicted values ($P < .05$), whereas $FEV_1$ was not different from the predicted values. On average,

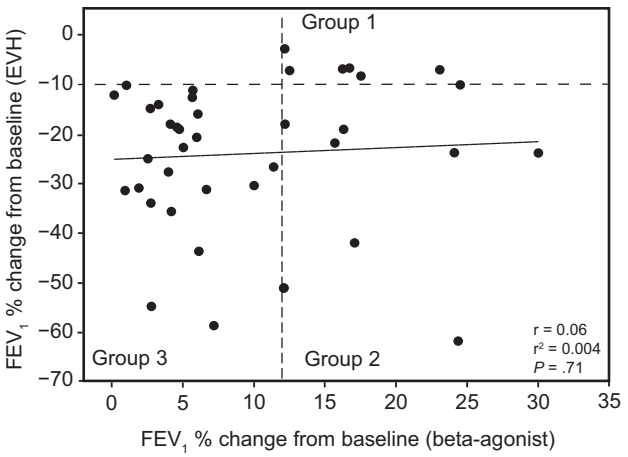


Fig. 1. Correlation between the change in $FEV_1$ after inhalation of fast-acting $\beta_2$-agonist and after eucapnic voluntary hyperpnea (EVH) challenge in 39 adults with asthma. As described in the text, subjects were partitioned into 3 separate groups on the basis of their responses to inhaled $\beta_2$-agonist and the EVH challenge. There was no association between the $FEV_1$ response to inhaled $\beta_2$-agonist and EVH. The horizontal and vertical dashed lines indicate significant responses to the EVH challenge and inhaled $\beta_2$-agonist, respectively.

PEF was 93% of predicted values (8.48 ± 1.86 vs 9.04 ± 1.62 L/s for measured vs predicted, $P = .02$), $FEF_{25-75\%}$ was 76.8% of predicted values (3.35 ± 1.34 vs 4.30 ± 0.61 L for measured vs predicted, $P = .002$), and $FEV_1$/FVC was 0.75. Collectively, these spirometry results suggest an overall mild airway obstruction.

Figure 1 shows the correlation between the $FEV_1$ response to inhaled fast-acting $\beta_2$-agonist and the EVH challenge. There was no association between the response to $\beta_2$-agonist and EVH ($r = 0.06$, $P = .71$). A positive bronchodilator responsiveness predicted a positive response to EVH challenge in 9 of 33 subjects (sensitivity 27%). Thus, in the 33 subjects with a significant decrease in $FEV_1$ after EVH, 73% (24 of 33) did not have significant bronchodilator responsiveness.

As shown in Figure 1, the subjects were divided into 3 groups on the basis of their $FEV_1$ responses to the 2 tests: Group 1 had a positive response to fast-acting $\beta_2$-agonist and negative response to EVH; Group 2 had a positive response to both challenges; and Group 3 had a negative response to $\beta_2$-agonist and positive response to EVH. Subject characteristics and baseline spirometry in the 3 groups are shown in Table 1. In Group 1 and Group 2, $FEV_1$ was lower than predicted values ($P = .045$ and .02, respectively), whereas it was not different from predicted values in Group 3. $FEF_{25-75\%}$ was significantly lower than predicted values in all 3 groups. $FEF_{25-75\%}$ was significantly lower in Group 2 than in Group 3 ($P = .008$), and there was a nonsignificant trend for it to be lower in Group 1 than in Group 3 (Bonferroni-corrected $P = .08$). In Group 2, PEF was lower than predicted values ($P = .03$), whereas it was not different from predicted values in Group 1 or Group 3. $FEV_1$/FVC was lower in Group 2 compared with Group 3 (0.65 vs 0.79, $P = .008$). In Group 1, $FEV_1$/FVC was 0.71; however, this was not statistically different from the other 2 groups. In summary, the spirometry results demonstrate mild-to-moderate airway obstruction in Group 1 and Group 2, whereas airway caliber was largely normal in Group 3.

Figure 2 presents correlations between $FEV_1$ and FVC following fast-acting $\beta_2$-agonist (A, C) and the EVH challenge (B, D) in all subjects. The responses are shown both as percentage changes from baseline and as the absolute differences in liters following the 2 interventions. With inhaled $\beta_2$-agonist, the change in $FEV_1$ was significantly correlated with the change in FVC ($FEV_1$ ΔL vs FVC ΔL, $r = 0.63$, $P < .001$). However, 95% of the subjects had a larger absolute increase in $FEV_1$ than FVC (37 of 39 subjects). With $\beta_2$-agonist, $FEV_1$ increased by an average of 9.86%, whereas FVC increased by 1.18% (+346 ± 236 vs +61 ± 175 mL for $FEV_1$ vs FVC). Following the EVH challenge, the change in $FEV_1$ was strongly and significantly correlated with the change in FVC (FVC percentage change vs $FEV_1$ percentage change, $r = 0.83$, $P < .001$) (Fig. 2B). $FEV_1$ decreased by a greater amount than FVC in 82% (32 of 39) of subjects (–932 ± 614 vs –603 ± 653 mL for $FEV_1$ vs FVC). In the 7 subjects in whom FVC decreased by a greater amount than $FEV_1$, the decrease in FVC was 214 ± 181 mL greater than the decrease in $FEV_1$ (–1.26 ± 0.87 vs –1.05 ± 0.74 L for FVC vs $FEV_1$).

Results from correlations between baseline spirometry ($FEV_1$ percent of predicted; FVC percent of predicted; $FEV_1$/FVC) and the $FEV_1$ response to inhaled $\beta_2$-agonist and the EVH challenge are shown in Figure 3. Baseline $FEV_1$ and $FEV_1$/FVC were negatively correlated with the response to $\beta_2$-agonist ($P < .001$), but they did not correlate with the response to the EVH challenge. Baseline FVC was not significantly associated with the responses to either $\beta_2$-agonist or EVH.

Figure 4 depicts the group mean maximum changes in $FEV_1$ and FVC after inhaled fast-acting $\beta_2$-agonist and EVH in Groups 1–3. Following $\beta_2$-agonist, the increase in $FEV_1$ in Group 3 was less than that for Group 2 ($P < .05$). The increase in $FEV_1$ after $\beta_2$-agonist was the same in Group 1 and Group 2. After the EVH challenge, the decreases in $FEV_1$ and FVC were greater in both Group 2 and Group 3 than Group 1 ($P < .05$); however, the decreases in $FEV_1$ and FVC were not different between Group 2 and Group 3. Figure 5 shows individual subject and group mean values for $FEV_1$ at baseline and after inhaled $\beta_2$-agonist in Groups 1–3. There was a significant main-effect for the differences in baseline $FEV_1$ among the groups ($P = .007$); however, after $\beta_2$-agonist, $FEV_1$ was

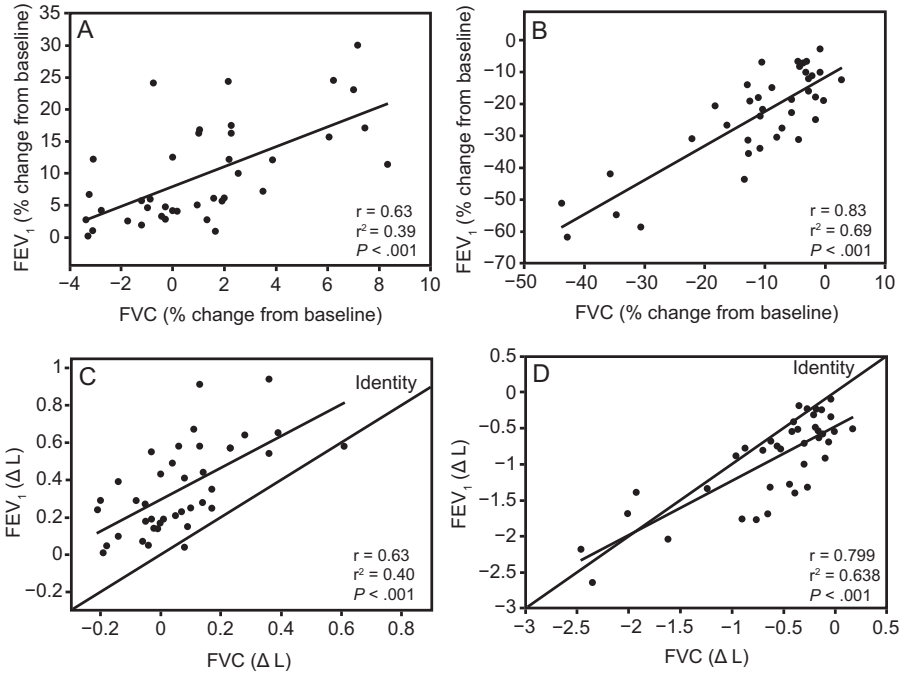


Fig. 2. Correlations between the change in $FEV_1$ and FVC after inhaled $\beta_2$-agonist (A, C) and after a eucapnic voluntary hyperpnea challenge (B, D). Results are shown as a percentage change from baseline (A, B) and as the absolute change (C, D). Identity lines are shown on the 2 graphs depicting the changes in L.

not different among the 3 groups. Following $\beta_2$-agonist, $FEV_1$ in all 3 groups was at or above 100% of predicted.

## Discussion

We compared the spirometric response to inhaled fast-acting $\beta_2$-agonist with the response to EVH challenge in 39 adults with asthma. The most important new finding in this study is that the spirometric response to inhaled $\beta_2$-agonist did not correlate with the response to the EVH challenge (Fig. 1). Second, in many of the subjects with significant airway narrowing after the EVH challenge, FVC also decreased substantially (Fig. 2). This finding indicates dysfunction of the peripheral airways in response to the EVH challenge. Finally, baseline $FEV_1$ and $FEV_1$/FVC correlated with the response to inhaled $\beta_2$-agonist but not with the response to the EVH challenge (Fig. 3). Given the poor concordance between the spirometric response to inhaled $\beta_2$-agonist and the EVH challenge, these findings support the conclusion that the airway response to inhaled $\beta_2$-agonist must be interpreted with caution and in the context of its determinants and limitations.

With regard to the principal new finding in this study (ie, that the airway response to inhaled fast-acting $\beta_2$-agonist and EVH challenge did not correlate in a group of adults with asthma), previous work has shown weak concordance between the airway responses to inhaled $\beta_2$-agonist and methacholine responsiveness.[2,17,18] However, whereas methacholine is a direct-acting smooth muscle agonist that acts via binding to muscarinic receptors on airway smooth muscle, EVH causes airway narrowing indirectly through the actions of pro-inflammatory mediators released by resident and nonresident airway cells.[19] Similarly, the amount of bronchoconstriction following exercise (another indirect bronchoprovocation test) did not correlate with bronchodilator responsiveness in a group of 21 adults with asthma in whom both methacholine responsiveness and degree of airway inflammation demonstrated a range of severity.[20]

The poor concordance between bronchodilator responsiveness and EVH is not altogether surprising upon consideration of the factors that mediate the airway responses to the 2 tests. First, bronchodilator responsiveness is very much dependent on baseline airway caliber; in general, responsiveness increases as a function of reduced baseline caliber.[2] Our findings also demonstrate a strong dependence of bronchodilator responsiveness on initial airway caliber, as baseline $FEV_1$ and $FEV_1$/FVC were significantly associated with the change in $FEV_1$ after fast-acting $\beta_2$-agonist inhalation (Fig. 3). Because daily airway caliber varies in patients with asthma, bronchodilator responsiveness should also be variable. In support of this, Silkoff et al[9] reported that within-subject bronchodilator responsiveness was highly variable across 5 visits over 12 months in patients with asthma. Moreover, temporal variability in bronchodilator responsiveness was seen in subjects with both moderate and severe disease, all of whom were prescribed low-moderate and high-dose inhaled

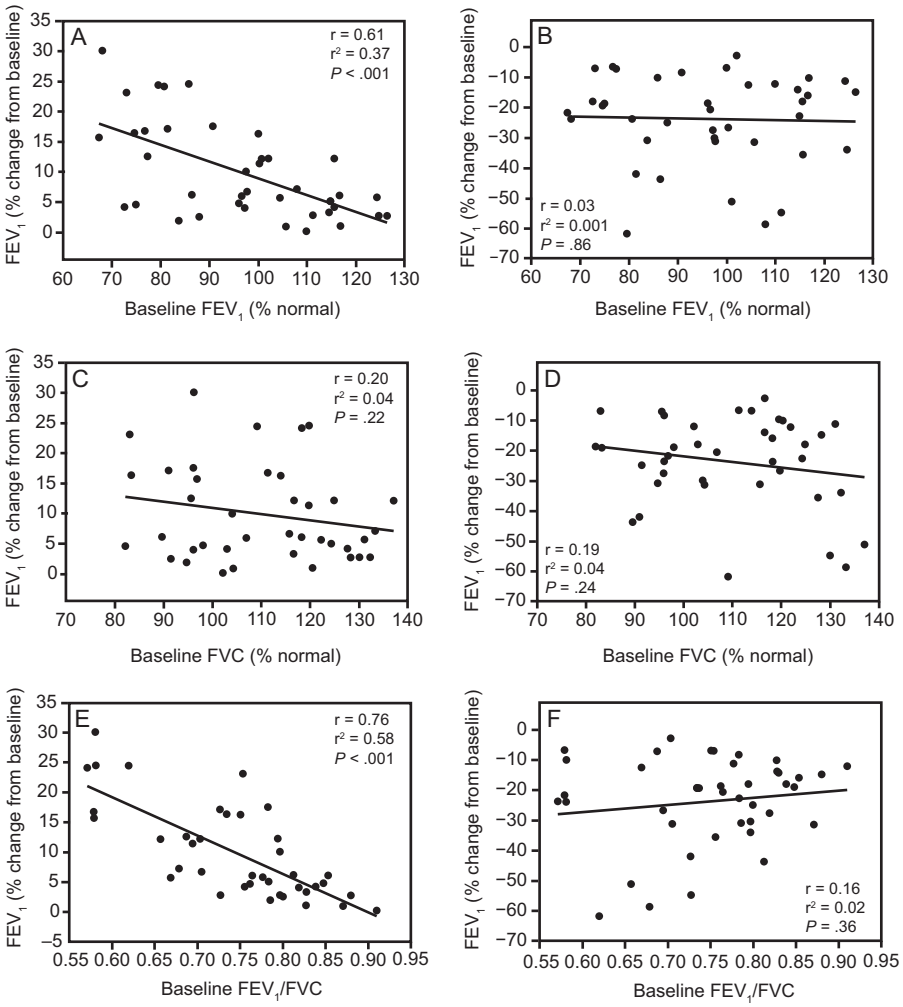


Fig. 3. Correlations between baseline $FEV_1$, FVC, and $FEV_1$/FVC and the percent change in $FEV_1$ after inhaled fast-acting $\beta_2$-agonist (A, C, E) and after eucapnic voluntary hyperpnea challenge (B, D, F) in 39 adults with asthma. Baseline $FEV_1$ and $FEV_1$/FVC were significantly associated with the $FEV_1$ response to inhaled $\beta_2$-agonist but not with the response to the eucapnic voluntary hyperpnea challenge.

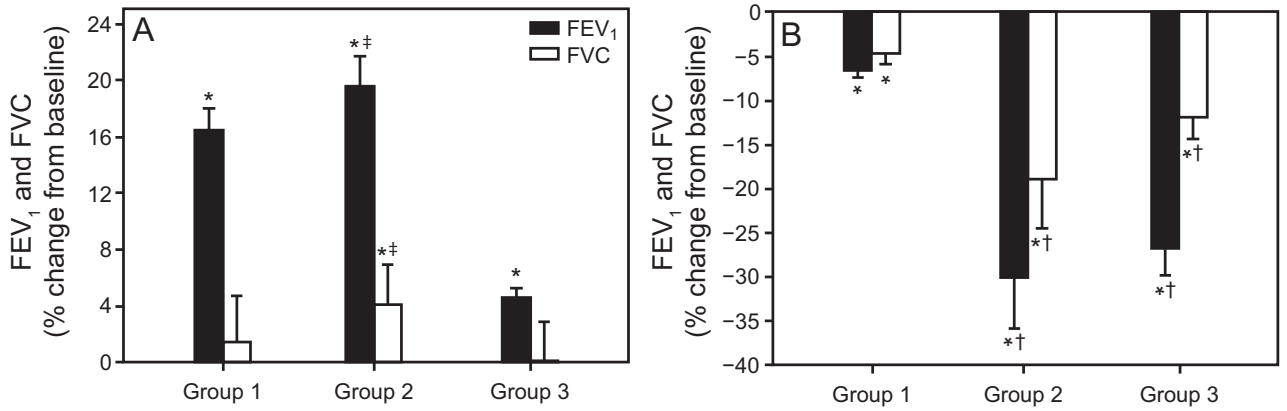


Fig. 4. Changes in $FEV_1$ and FVC after inhaled fast-acting $\beta_2$-agonist (A) and after eucapnic voluntary hyperpnea (EVH) challenge (B) by group. $FEV_1$ and FVC are expressed as percent change from baseline. These findings demonstrate the discordant responses between inhaled $\beta_2$-agonist and EVH in Group 1 and Group 3, whereas subjects in Group 2 responded significantly to both inhaled $\beta_2$-agonist and EVH. *$P < .05$ vs baseline; †$P < .05$ vs Group 1; ‡$P < .05$ vs Group 3.

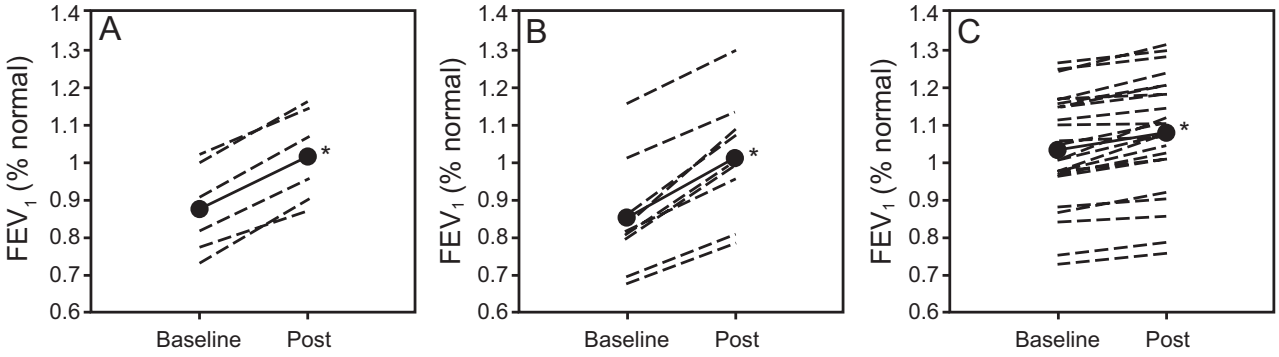


Fig. 5. Individual subject and group mean values for $FEV_1$ measured at baseline and after inhaled fast-acting $\beta_2$-agonist (Post) in 3 subject groups. Note that Post $FEV_1$ values met or exceeded the predicted normal values in all 3 subject groups. *$P < .05$ vs baseline value.

corticosteroids, respectively.[9] Clinicians should therefore be mindful of the fact that the bronchodilator responsiveness will vary over time in adults with asthma, which appears to span both disease severity and phenotype.[9] Conversely, previous work showed that baseline airway caliber did not correlate with the spirometric response to EVH.[21,22] Similarly, in our subjects, baseline $FEV_1$ did not correlate with the response to the EVH challenge (Fig. 3B, D, F).

Second, bronchodilator responsiveness is affected by chronic maladaptations to airway structure, such that maximum bronchodilation will be limited by increases in airway wall thickness or altered interdependence between the airways and surrounding lung parenchyma. Whereas treating airway inflammation will improve airway function by resolving the mutable inflammatory sequelae such as excessive bronchiolar smooth muscle contraction and airway wall edema,[23,24] its influence on features of airway remodeling are not clear.[25] In contrast, analyses suggest that structural maladaptations to the airway walls will, if anything, increase the airway response to bronchoprovocative challenges by causing exaggerated narrowing in response to a given amount of airway smooth muscle shortening.[26-28] We note, however, that remodeled airways may be stiffer and thus not only more resistant to collapse but also would provide an increased load on airway smooth muscle.[29]

Finally, one can argue that bronchodilator responsiveness and EVH assess 2 fundamentally different phenomena. On the one hand, the bronchodilator responsiveness measures the acute increase in airway caliber due principally to airway smooth muscle relaxation. On the other hand, EVH assesses airway narrowing in response to an indirect stimulus. Airway narrowing requires some combination of the presence of inflammatory cells in the airway wall, hyperresponsive airway smooth muscle, and altered airway wall structure.[4] Thus, the stimulus (ie, exogenous drug vs endogenous mediators), response (bronchodilation vs bronchoconstriction), and physiology of the response are different.

In this study, subjects were post priori placed into 1 of 3 groups depending on their spirometric responses to the 2 interventions. Several implications arise from comparisons among the 3 groups. While baseline spirometry did associate with bronchodilator responsiveness in our subjects, it did not correlate with their response to EVH; AHR was seen in our subjects both with (Group 2) and without (Group 3) significant bronchodilator responsiveness. Furthermore, while baseline spirometry was compromised in subjects in Group 2, it was largely normal in subjects in Group 3. This indicates that the lack of bronchodilator responsiveness in Group 3 was not due to remodeling of the airways and an inability to dilate. The baseline airway obstruction in subjects in both Group 1 and Group 2 was largely reversed with $\beta_2$-agonist inhalation (Fig. 5). Collectively, the differences in baseline airway function, bronchodilator responsiveness, and AHR among the three groups reflects the important, ongoing challenge to unravel the phenotypic expressions of asthma. The group comparisons also highlight a critical point that baseline spirometry should not be used as a proxy for AHR.

In our subjects with significant AHR, the decrease in $FEV_1$ was significantly associated with a decrease in FVC following the challenge (Fig. 2). The decrease in FVC suggests that peripheral airway dysfunction with premature small airway closure is an important component of the airway narrowing induced by voluntary hyperpnea and, ostensibly, other indirect stimuli (eg, exercise, inhaled mannitol). Given findings that peripheral airway dysfunction is associated with asthma severity and control,[30,31] our results provide additional evidence that characterization of a small airway phenotype might benefit clinical care of patients with asthma.

### Limitations

In all subjects, bronchodilator responsiveness was completed first and the EVH challenge second; the order of the 2 interventions was not randomized. However, the fact that baseline spirometry was the same on the 2 experimental days (Table 1) suggests that the results were not affected by visit number. The number of days separating the 2 visits

varied among the subjects. In individuals with asthma, temporal variability in airway function is inconsistent and unpredictable, and thus in most cases will not display any consistent time-dependent behavior.[9] Additionally, although we made every effort to study each subject at the same time of the day, some subjects were not able to complete both visits at the same time of day. Finally, given the unequal group sizes, these data must be interpreted with caution. We intend to continue to add subjects to this data set as they are studied.

## Conclusions

In this study, bronchodilator responsiveness did not correlate with the spirometric response to EVH challenge in a group of adults with asthma. Thus, our data demonstrate that a significant proportion of patients with asthma (ie, demonstrated AHR to one or more stimulus) will not routinely exhibit a significant bronchodilator responsiveness. This finding complements previous work that indicated no correlation between bronchodilator responsiveness and methacholine responsiveness in adults with asthma. While bronchodilator responsiveness should be included in the assessment and ongoing care of patients with asthma, our results should be interpreted with caution and with an understanding of the complexity of functional and structural features determining the degree of responsiveness.

### REFERENCES


1. Saglani S, Menzie-Gow AN. Approaches to asthma diagnosis in children and adults. Front Pediatr 2019;7:148.
2. Louis R, Bougard N, Guissard F, Paulus V, Henket M, Schleich F. Bronchodilation test with inhaled salbutamol versus bronchial methacholine challenge to make an asthma diagnosis: do they provide the same information? J Allergy Clin Immunol Pract 2020;8(2):618-625.
3. Hunter CJ, Brightling CE, Woltmann G, Wardlaw AJ, Pavord ID. A comparison of the validity of different diagnostic tests in adults with asthma. Chest 2002;121(4):1051-1057.
4. Hallstrand TS, Leuppi JD, Joos G, Hall GL, Carlsen KH, Kaminsky DA, et al. ERS technical standard on bronchial challenge testing: pathophysiology and methodology of indirect airway challenge testing. Eur Respir J 2018;52(5):1801033.
5. Kippelen P, Larsson J, Anderson SD, Brannan JD, Dahlén B, Dahlén SE. Effect of sodium cromoglycate on mast cell mediators during hyperpnea in athletes. Med Sci Sports Exerc 2010;42(10):1853-1860.
6. Bood JR, Sundblad BM, Delin I, Sjödin M, Larsson K, Anderson SD, et al. Urinary excretion of lipid mediators in response to repeated eucapnic voluntary hyperpnea in asthmatic subjects. J Appl Physiol (1985) 2015;119(3):272-279.
7. Choi IS, Ki WJ, Kim TO, Han ER, Seo IK. Seasonal factors influencing exercise-induced asthma. Allergy Asthma Immunol Res 2012;4(4):192-198.
8. Hargreave FE, Nair P. The definition and diagnosis of asthma. Clin Exp Allergy 2009;39(11):1652-1658.
9. Silkoff PE, Laviolette M, Singh D, FitzGerald JM, Kelsen S, Backer V, et al. Longitudinal stability of asthma characteristics and biomarkers from the Airways Disease Endotyping for Personalized Therapeutics (ADEPT) study. Respir Res 2016;17(1):43.
10. Rossman MJ, Nader S, Berry D, Orsini F, Klansky A, Haverkamp HC. Effects of altered airway function on exercise ventilation in asthmatic adults. Med Sci Sports Exerc 2014;46(6):1104-1113.
11. Klansky A, Irvin C, Morrison-Taylor A, Ahlstrand S, Labrie D, Haverkamp HC. No effect of elevated operating lung volumes on airway function during variable workrate exercise in asthmatic humans. J Appl Physiol (1985) 2016;121(1):89-100.
12. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardization of Spirometry. Eur Respir J 2005;26(2):319-338.
13. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations. Eur Respir J 2012;40(6):1324-1343.
14. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. Am J Respir Crit Care Med 1999;159(1):179-187.
15. Standardization of Spirometry, 1994 Update. American Thoracic Society. Am J Respir Crit Care Med 1995;152(3):1107-1136.
16. Anderson SD, Brannan JD. Methods for "indirect" challenge tests including exercise, eucapnic voluntary hyperpnea, and hypertonic aerosols. Clin Rev Allergy Immunol 2003;24(1):27-54.
17. Fruchter O, Hardak E, Yigla M. The response to bronchodilators in adults is not predictive of bronchial-hyperreactivity. J Asthma 2009;46(5):455-459.
18. Petanjek BB, Grle SP, Pelicaric D, Vrankovic D. Bronchodilator response in patients with persistent allergic asthma could not predict airway hyperresponsiveness. Allergy Asthma Clin Immunol 2007;3(4):123-127.
19. Brannan JD, Turton JA. The inflammatory basis of exercise-induced bronchoconstriction. Phys Sportsmed 2010;38(4):67-73.
20. Haverkamp HC, Dempsey JA, Miller JD, Romer LM, Pegelow DF, Rodman JR, et al. Gas exchange during exercise in habitually active asthmatic subjects. J Appl Physiol 2005;99(5):1938-1950.
21. Aronsson D, Tufvesson E, Bjermer L. Comparison of central and peripheral airway involvement before and during methacholine, mannitol and eucapnic hyperventilation challenges in mild asthmatics. Clin Respir J 2011;5(1):10-18.
22. Rundell KW, Evans TM, Baumann JM, Kertesz MF. Lung function measured by impulse oscillometry and spirometry following eucapnic voluntary hyperventilation. Can Respir J 2005;12(5):257-263.
23. Laitinen LA, Laitinen A, Haahtela T. A comparative study of the effects of an inhaled corticosteroid, budesonide, and a beta 2-agonist, terbutaline, on airway inflammation in newly diagnosed asthma: a randomized, double-blind, parallel-group controlled trial. J Allergy Clin Immunol 1992;90(1):32-42.
24. Booth H, Richmond I, Ward C, Gardiner PV, Harkawat R, Walters EH. Effect of high dose inhaled fluticasone propionate on airway inflammation in asthma. Am J Respir Crit Care Med 1995;152(1):45-52.
25. Durrani SR, Viswanathan RK, Busse WW. What effect does asthma treatment have on airway remodeling? Current perspectives. J Allergy Clin Immunol 2011;128(3):439-448.
26. Bramley AM, Thomson RJ, Roberts CR, Schellenberg RR. Hypothesis: excessive bronchoconstriction in asthma is due to decreased airway elastance. Eur Respir J 1994;7(2):337-341.
27. Macklem PT. A theoretical analysis of the effect of airway smooth muscle load on airway narrowing. Am J Respir Crit Care Med 1996;153(1):83-89.

28. Affonce DA, Lutchen KR. New perspectives on the mechanical basis for airway hyperreactivity and airway hypersensitivity in asthma. J Appl Physiol (1985) 2006;101(6):1710-1719.
29. Brackel HJ, Pedersen OF, Mulder PG, Overbeek SE, Kerrebijn KF, Bogaard JM. Central airways behave more stiffly during forced expiration in patients with asthma. Am J Respir Crit Care Med 2000;162(3 Pt 1):896-904.
30. Farah CS, King GG, Brown NJ, Downie SR, Kermode JA, Hardaker KM, et al. The role of the small airways in the clinical expression of asthma in adults. J Allergy Clin Immunol 2012;129(2):381-387, 7.e1.
31. Postma DS, Brightling C, Baldi S, Van den Berge M, Fabbri LM, Gagnatelli A, et al. Exploring the relevance and extent of small airways dysfunction in asthma (ATLANTIS): baseline data from a prospective cohort study. Lancet Respir Med 2019;7(5):402-416.