

Bronchodilating effect of deep inspirations in asthma and chronic cough

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Wasilewski NV, Fisher T, Turcotte SE, Fisher JT, Loughheed MD. Bronchodilating effect of deep inspirations in asthma and chronic cough. *J Appl Physiol* 120: 1018–1028, 2016. First published March 3, 2015; doi:10.1152/jappphysiol.00737.2015.—The pathophysiologic processes distinguishing classic asthma (CA), cough-variant asthma (CVA), and methacholine (MCh)-induced cough but normal airway sensitivity (COUGH) are inadequately understood and may be a result of differences in the ability to bronchodilate following a deep inspiration (DI). The purpose of this study was to compare the bronchodilating effect of DIs in individuals with CA, CVA, and COUGH using high-dose MCh. Individuals aged 18–65 yr with CA or suspected CVA completed high-dose MCh testing to a maximum change in forced expiratory volume in 1 s (FEV₁) of 50% from baseline (MAX). Impulse oscillometry (IOS) measurements and partial and maximal-flow volume curves (used to calculate a DI index) were recorded at baseline and at each dose of MCh. Body plethysmography was performed at baseline and MAX. Twenty-eight subjects [25 women, 39.8 ± 11.9 yr (means ± SD)] were studied ($n = 11$ CA, $n = 10$ CVA, and $n = 7$ COUGH). At MAX, the percent change in FEV₁ was greater in subjects with CA compared with those with CVA ($P < 0.001$) and COUGH ($P < 0.001$), and the percent change in forced vital capacity was greater in those with CA than with COUGH ($P = 0.017$). Subjects with CA and CVA developed dynamic hyperinflation and gas trapping. In subjects with CA and CVA, all IOS parameters were significantly increased from baseline to MAX, except for central respiratory resistance (R20). In individuals with COUGH, total respiratory resistance, R20, and resonant frequency were significantly increased from baseline. At MAX, the DI index was positive in all groups, suggesting preserved bronchodilation (CA, 0.67 ± 0.97; CVA, 0.51 ± 0.73; COUGH, 0.01 ± 0.36; $P = 0.211$). We conclude that the bronchodilating effect of DIs is preserved in individuals with CA, CVA, and borderline with COUGH; however, hyperinflation and gas trapping are avoided in subjects with COUGH alone.

asthma; cough; deep inspirations; methacholine; bronchodilation

NEW & NOTEWORTHY

Our findings suggest that deep inspirations reverse the obstruction due to airway closure but not the obstruction due to large airway narrowing. This may reflect underlying differences in the airway responses of conditions that fall along a spectrum of disorders of airway hyperresponsiveness. The differential contribution of the small airways in these groups is a key finding, and could determine with which phenotype a patient presents (i.e., predominant cough symptoms or predominant symptoms of airway hyperresponsiveness).

COUGH IS A COMPLEX REFLEX that typically acts as a valuable protective airway clearance mechanism arising from irritation of the intrapulmonary or extrapulmonary airways (7, 10, 42). When

the cough reflex is activated, there is an initial inspiration phase followed by glottic closure and a prompt increase in intrathoracic pressure (3). This is followed by forced expiration and, after opening of the glottis, gas is expired at high flow rates along with the characteristic audible sound recognized as a cough (27, 45).

Classic asthma (CA) is one of the most common causes of chronic cough (18). Although it is accepted that cough can be the only or main symptom of asthma, referred to as cough-variant asthma (CVA) (1), little is understood about why some individuals with asthma either only or primarily cough, and do not develop dyspnea, chest tightness, or wheeze (75). Furthermore, a chronic cough (lasting 8 wk or more), is often accompanied by significant morbidity (39, 76), and is associated with a decreased quality of life (29). Osman et al. (61) reported that patients with asthma perceived cough to be the most troubling symptom, and rated cough and breathlessness more bothersome than wheezing, chest tightness, or sleep disturbance.

In healthy subjects, deep inspirations (DIs) produce bronchodilation in previously constricted airways, which is a favorable effect that preserves airway patency (9, 21, 69). The bronchodilating effect of DIs during inhalation-challenge testing is either absent or diminished in asthma (26, 63, 68), and depends on disease severity (70). Taking DIs before inhalation of methacholine (MCh) also has been found to bronchoprotect the airways of healthy individuals from narrowing, but not individuals with asthma (20, 41, 69). The bronchoprotective effect of DIs in individuals without asthma is potent, and prohibiting DIs before airway challenge has been shown to increase the response to MCh in healthy subjects (41, 69, 73).

In our laboratory, we identified individuals with chronic cough and suspected CVA, who cough during a MCh challenge but have normal airway sensitivity to MCh (COUGH). The pathophysiologic mechanisms that differentiate CA, CVA, and COUGH are not fully understood. It is possible that the preservation or loss of the bronchodilating effect of a DI may distinguish CA from these related conditions (48). The objectives of this research are to compare the bronchodilating effect of DIs in individuals with CA, CVA, and COUGH. We hypothesize that the bronchodilating effect of a DI falls along a spectrum and will be absent or diminished in individuals with CA, diminished in individuals with CVA, and preserved in individuals with COUGH. Preliminary results have been previously reported in the form of an abstract (80).

MATERIALS AND METHODS

Subjects

Individuals (age 18–65 yr) with asthma or chronic cough and suspected CVA were invited to participate. The subjects were recruited from tertiary care asthma and cough clinics, where they were managed according to national and international guidelines (39, 47, 54). Subjects with asthma were eligible to participate if they had a

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compatible clinical history, evidence of reversible airflow obstruction, or airway hyperresponsiveness (AHR) to MCh [defined as a provocative concentration eliciting a 20% decline in forced expiratory volume in 1 s (FEV_1) from baseline (PC_{20}) less than or equal to 16 mg/ml] (19, 47). Subjects with chronic cough were eligible to participate if after thorough investigation and management of proven or suspected causes of cough the specialist suspected CVA was the primary or contributing diagnosis. Subjects were excluded if they had any contraindication to MCh challenge testing according to American Thoracic Society (ATS) guidelines (19). Additional exclusion criteria included current smoking or a smoking history in excess of 10 pack-yr, and no coughing during MCh challenge (for subjects who displayed normal airway sensitivity to MCh). This clinical trial was registered with www.clinicaltrials.gov (NCT01659476), approved by the Queen's University Health Sciences Research Ethics Board, and a Letter of No Objection was acquired from Health Canada's Therapeutic Products Directorate.

Study Design

After participants gave their informed consent, a detailed medical history was taken and subjects were screened for exclusion criteria. This was followed by the completion of the Mini Asthma Quality of Life Questionnaire (Mini-AQLQ) (40) and the Leicester Cough Questionnaire (LCQ) (5). Subjects then completed baseline pulmonary function testing including spirometry, body plethysmography, maximal and partial expiratory flow volume curves (MEFV, PEFV, respectively), and impulse oscillometry (IOS) measurements (Fig. 1). These tests were followed by a high-dose MCh challenge. Subjects were asked to withhold bronchodilators as recommended by the ATS (19).

Methacholine Challenge Testing

MCh challenge testing was completed using methacholine chloride (Provocholine; Methapharm, Brantford, ON, Canada) as per ATS guidelines for assessing sensitivity (19), and a standardized high-dose tidal breathing procedure (77). Subjects inhaled doses of MCh from a Wright RX 160 nebulizer (Roxon Medi-Tech, Montreal, QC, Canada) for 2 min. The first dose, given as a control, was isotonic saline (0.9%), and subsequent doses ranged from 0.03 to 256 mg/ml. The nebulizer was calibrated to deliver 3.5-ml aliquots of MCh solution at a rate of 0.13 ml/min using compressed oxygen, and doses were administered within about 5 min of each other (19). Immediately after

the dose of MCh, IOS measurements were obtained, followed by PEFV/MEFV maneuvers according to a published method (77).

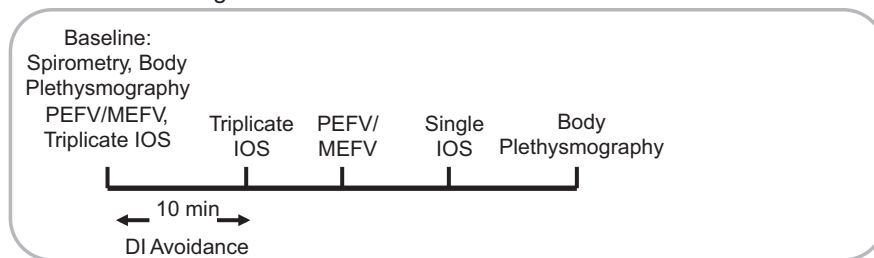
Testing was stopped if 1) FEV_1 decreased by 50% from baseline (as measured from the MEFV); 2) the subject reached a plateau (less than a 5% change in FEV_1 over two or more dose steps after an initial decrease of >10%) (82); 3) the subject could not tolerate the symptoms and desired to stop; or 4) the final dose (256 mg/ml) had been administered (77). The maximal decrease in FEV_1 from baseline was defined as MAX. Bronchoconstriction was reversed after data collection with inhaled salbutamol (200 µg every 10 min using a metered-dose inhaler and spacer) until FEV_1 was within 10% of the baseline value. PC_{20} was interpolated from dose-response curves ($\log_{10}$). Coughs were defined as audible expiratory maneuvers against a closed glottis (15, 53). Any coughs occurring during MCh bronchoprovocation were manually recorded, and coughs occurring in response to MCh (not including coughs in response to saline) were summed for analysis.

Measurements

Pulmonary function testing. Spirometry [FEV_1 ; forced vital capacity (FVC); peak expiratory flow rate; forced expiratory flow, midexpiratory phase ($FEF_{25-75\%}$); and forced expiratory flow after exhalation of 50% FVC ($FEF_{50\%}$)], performed according to ATS and European Respiratory Society Task Force standards (52), was completed using a Vmax V62J Autobox (CareFusion, Yorba Linda, CA). Body plethysmography was used to evaluate lung volumes and specific airway resistance (sRaw). To diminish the risk of frequency-dependent overestimation of thoracic gas volume during induced bronchoconstriction, the panting frequency was standardized at 1 Hz (67). Predicted values used for spirometry, lung volumes, and airway resistance followed those described by Morris et al. (55), Goldman and Becklake (34), and Briscoe and Dubois (8), respectively.

Impulse oscillometry. IOS measurements were carried out as previously described using a Masterscreen IOS (Erich Jaeger, Hoechst, Germany) (6, 60). With nose clips in place, subjects tidally breathed on the IOS device while it emitted wave impulses. At least 30 s of testing was completed from which the software calculated mean values of reactance (X) and resistance (R) at frequencies from 5 to 20 Hz, specifically X5, R5, and R20 (44). Additional measurements were the resonant frequency (f_{res}) and the reactance area. The trial was saved when the coherence (a measure of testing reliability) was acceptable (>0.8) (35, 49), and there was no evidence of artifact

A Baseline Testing



B Bronchodilating Effect

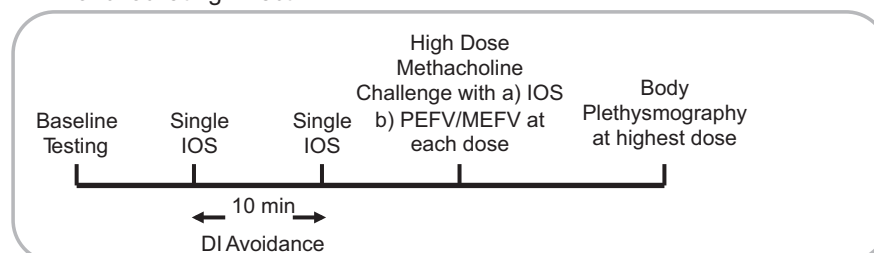


Fig. 1. Detailed outline of sequence of testing. A: baseline testing. B: assessment of the bronchodilating effect of deep inspirations (DIs) using partial expiratory flow volume (PEFV), maximal expiratory flow volume (MEFV) and impulse oscillometry (IOS) during high-dose methacholine (MCh).

including coughing, swallowing, vocalization, hyperventilation, or breath holding. The subjects' efforts were supervised and evaluated by a registered respiratory therapist. When measurements were in triplicate, the values from all three efforts for each IOS parameter were averaged for final results (2).

Bronchodilating effect. The bronchodilating effect of DIs was examined in the three groups using responses to high-dose MCh challenge testing and comparing the flow difference between the PEFV and MEFV at an isovolume of 40% of the control vital capacity (PEF₄₀ and MEF₄₀, respectively) at MAX with that recorded at baseline (59). Differences between groups were determined by ANOVA with Bonferroni correction. Specifically, the bronchodilating effect of DIs on MCh-induced bronchoconstriction was examined using 1) a bronchodilating index (DI index), which is the ratio of (MEF₄₀ – PEF₄₀)/PEF₄₀, (31, 58) (others have used this index as a measure of the bronchodilatory ability of the DI performed during an MEFV maneuver, when following a PEFV maneuver performed under induced bronchoconstriction) and 2) two closing indices, which were calculated using the following equations: *a*) the closing index by Hardaker et al. (37) [$\% \Delta FVC - \% \Delta (FEV_1/FVC) / \% \Delta FEV_1$]. (A negative value indicates predominant airway narrowing, whereas a positive closing index indicates predominant airway closure. A closing index of zero indicates that both airway narrowing and closure are equally prominent); and *b*) the closing index by Chapman et al. (14), $\% \Delta FVC / \% \Delta FEV_1$. (This closing index specifies which proportion of the change in FEV₁ is a consequence of airway closure. When the closing index is greater than 0.5, airway closure is predominant. In contrast, a value less than 0.5 indicates that airway narrowing predominates, and a value of 0.5 indicates that airway narrowing and closure are balanced.)

Statistical Analysis

Continuous variables were expressed as means $\pm$ SD unless otherwise stated. For continuous variables (such as spirometry, lung volume, IOS, AQLQ scores, and LCQ scores), within-group comparisons were made using paired *t*-tests and between-group comparisons were made using ANOVA with Bonferroni correction for multiple

comparisons. For noncontinuous variables (such as gender, medication use, and presence of plateau), between-group comparisons were carried out using Kruskal-Wallis tests with post hoc Mann-Whitney *U*-tests. Statistical analyses were performed using SPSS version 22.0 (IBM; Chicago, IL) using a conventional level of statistical significance ($P < 0.05$).

Sample size. A sample size of 36 (12 per group) was required to detect a significant difference in PEF₄₀ and MEF₄₀ at MAX, and for the planned subanalyses (such as evaluation of peripheral airway function using IOS data). To examine whether responses to high-dose MCh challenges represented a continuum between groups, ANOVA with Bonferroni correction for multiple comparisons was used to detect significant differences in the change in FEV₁, FVC, inspiratory capacity (IC), and residual volume (RV) between groups from baseline to MAX.

RESULTS

Subjects. Forty-five subjects were enrolled, of whom 28 were included in the study (Fig. 2). Characteristics of all 28 subjects are summarized in Table 1. Medication use did not differ between the three groups ($P > 0.05$). Fifteen subjects were taking inhaled corticosteroids (ICSs), nine of whom were also taking a long-acting β_2 -agonist (LABA). LCQ responses are presented in Table 2. Mini-AQLQ scores did not differ significantly between groups in any domain, or overall (Table 3). Nine subjects with chronic cough and suspected CVA were excluded from the study because they had normal airway sensitivity and did not cough in response to MCh (Fig. 2). However, otherwise they were not different from the other groups with regards to gender (66.7% female; $P = 0.934$), age (45.0 ± 17.9 yr; $P = 0.128$), height (171.5 ± 12.0 cm; $P = 0.537$), weight (84.2 ± 23.0 kg; $P = 0.323$), or body mass index (BMI) (28.3 ± 5.38 kg/m²; $P = 0.207$). Medication use in the nine excluded subjects was also not different from the other groups [66.7% were taking a short-acting β_2 -agonist

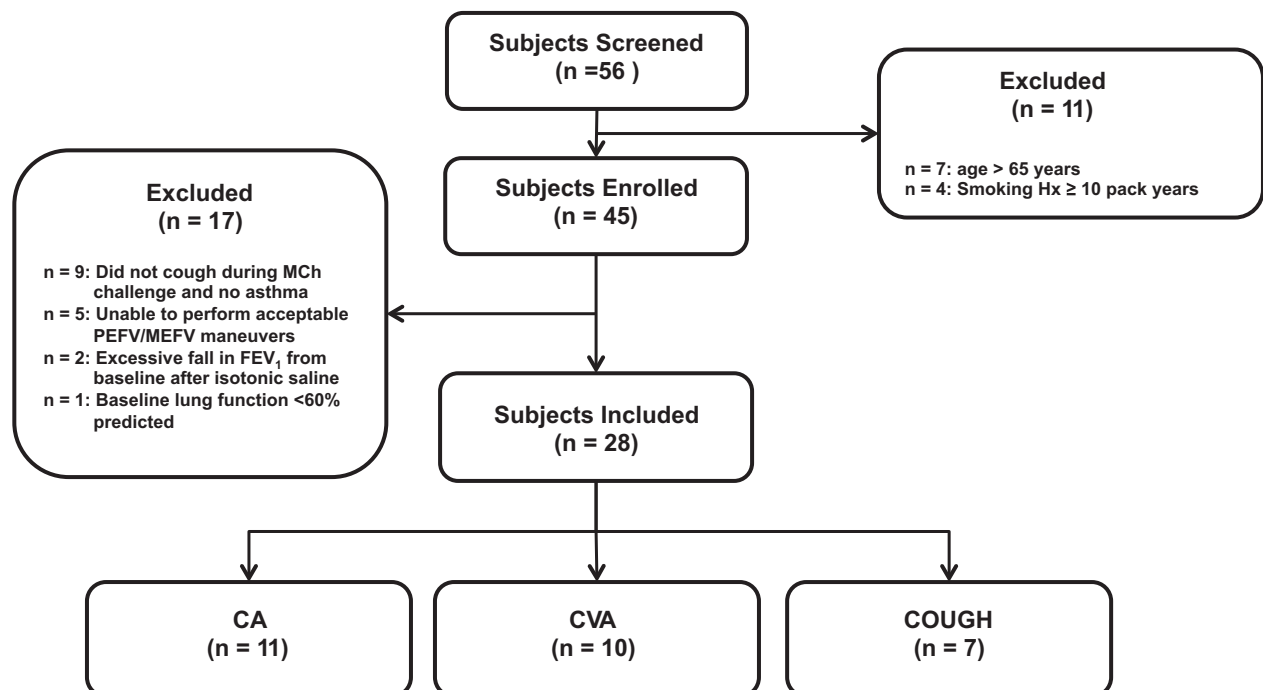


Fig. 2. Flow diagram of subject recruitment. CA, classic asthma; COUGH, methacholine (MCh)-induced cough but normal airway sensitivity; CVA, cough-variant asthma; FEV₁, forced expiratory volume in 1 s; Hx, history.

Table 1. Subject characteristics of stratified groups

	CA, n = 11	CVA, n = 10	COUGH, n = 7	P
Age, yr	39.8 ± 11.9	53.0 ± 9.9 ^a	40.9 ± 11.7	0.027
Gender, % female	72.7	80.0	71.4	0.903
Height, cm	164.0 ± 14.2	167.4 ± 7.4	168.9 ± 11.1	0.641
Weight, kg	88.4 ± 19.1	75.7 ± 16.1	74.8 ± 13.5	0.152
Body mass index, kg/m ²	32.8 ± 6.3	28.2 ± 7.0	26.7 ± 7.5	0.149
Smoking history, pack-yr	5.0 ± 3.5 ^c	1.2 ^d	5.7 ± 6.1 ^c	0.490
PC ₂₀ MCh, mg/ml	2.19 ± 2.32	5.59 ± 4.09	49.3 ± 17.0 ^{a,b,e}	0.000
MAX dose MCh, mg/ml	7.36 ± 5.97	50.0 ± 45.7	137.1 ± 86.1 ^{a,b}	0.000
Plateau, %	45.5	40.0	71.4	0.425
Allergies, %	63.6	72.7	42.9	0.403
Eczema, %	9.0	20.0	14.3	0.328
Rhinitis/sinusitis, %	45.5	36.4	57.1	0.788
SABA use, %	90.1	70.0	71.4	0.453
ICS use, %	72.7	40.0	42.9	0.519
Combination ICS/LABA use, %	45.5	20.0	28.6	0.460

All values are means ± SD. Values in bold indicate significant differences. CA, classic asthma; COUGH, methacholine-induced cough but normal airway sensitivity; CVA, cough-variant asthma; ICS, inhaled corticosteroid; LABA, long-acting β_2 -agonist; MCh, methacholine; PC₂₀, provocative concentration eliciting a 20% decline in FEV₁ from baseline; SABA, short-acting β_2 -agonist. ^aSignificantly different from CA group using ANOVA and post hoc Bonferroni correction. ^bSignificantly different from CVA group using ANOVA and post hoc Bonferroni correction. ^cn = 2. ^dn = 1. ^en = 4.

($P = 0.578$), 77.8% were taking ICSs ($P = 0.330$), and 44.4% were using combined ICS/LABA therapy ($P = 0.585$). Only one of these nine excluded subjects had a smoking history (0.5 pack-yr) ($P = 0.743$).

Baseline pulmonary function. There were no significant differences in spirometry, lung volumes, or airway resistance between groups at baseline, except for FEF_{75%} (l/s), which was greater in individuals with COUGH compared with those with CVA ($P < 0.05$) and FEF_{75%} (% predicted), which was significantly greater in individuals with COUGH compared with those with CA ($P < 0.05$).

Impulse oscillometry. The protocol at baseline (Fig. 1A) included multiple IOS measurements (i.e., before DI avoidance, after DI avoidance, after PEFV/MEFV, and after plethysmography). None of the IOS variables changed significantly when comparisons were made before and after these various maneuvers in any of the three groups (data not shown). IOS values at baseline and MAX are presented in Table 4.

IOS values also did not differ between groups at baseline (Table 4) except for f_{res} (Hz), which was greater in individuals with CA compared with those with COUGH ($P < 0.05$). During the high-dose MCh challenge, in those with CA and CVA, all IOS parameters were significantly increased from baseline to MAX, except for R20 ($P = 0.295$ and $P = 0.962$, respectively). In individuals with COUGH, R5, R20, and f_{res} increased significantly from baseline, and the change in R20 was significantly greater in those with COUGH compared with those with CA ($P < 0.05$). At MAX, there were no differences between groups in IOS parameters except f_{res} (Hz), which was

significantly greater in those with CA compared with those with COUGH ($P < 0.05$).

Spirometry and lung volume responses to high-dose MCh. Group responses to high-dose MCh are shown in Table 5. The percent change in FEV₁ at MAX was significantly greater in individuals with CA compared with those with CVA and COUGH ($P < 0.001$ and $P < 0.001$, respectively), and the percent change in FVC at MAX was greater in those with CA compared with those with COUGH ($P = 0.017$). All groups had significant changes in the mid-to-late flows (% predicted) from baseline (Fig. 3), and these were not different between groups [FEF_{50%} ($P = 0.077$), FEF_{25–75%} ($P = 0.587$), FEF_{75%} ($P = 0.536$)]. Subjects with CA and CVA developed significant dynamic hyperinflation and gas trapping (Fig. 4). Individuals with CA had a greater increase in RV compared with individuals with COUGH [$P = 0.001$ (L), $P = 0.001$ (% predicted)], and a greater increase in RV/total lung capacity (TLC) (% predicted) compared with those with CVA and COUGH ($P = 0.021$ and $P = 0.001$, respectively). IC decreased significantly from baseline in all three groups, and the change was not different between the three groups [$P = 0.203$ (L), $P = 0.193$ (% predicted)]. The percent change in FEV₁ at MAX was not significantly different between the three groups, whereas the percent change in FVC was significantly greater in those with CA than those with COUGH. Mid-to-late expiratory flows decreased comparably in all groups (Fig. 3).

Both closing indices were significantly different between the CA and COUGH groups at MAX (Table 5), indicating that the closing profile of those with CA is predominantly a result of airway closure, whereas in those with COUGH there is pre-

Table 2. Leicester Cough Questionnaire scores

	CA, n = 11	CVA, n = 10	COUGH, n = 7	P
Physical domain	6.2 ± 0.7	4.84 ± 1.2 ^a	5.1 ± 1.5	0.023
Psychological domain	6.5 ± 0.6	4.5 ± 1.7 ^b	5.0 ± 1.3	0.004
Social domain	6.5 ± 0.6	4.6 ± 1.4 ^b	4.7 ± 1.8 ^a	0.003
Total score	19.2 ± 0.9	13.9 ± 4.0 ^b	14.8 ± 4.4 ^a	0.004

All values are means ± SD. Values in bold indicate significant differences. ^a $P < 0.05$ compared with CA using ANOVA with post hoc Bonferroni comparison. ^b $P < 0.01$ compared with CA using ANOVA with post hoc Bonferroni comparison.

Table 3. Mini-Asthma Quality of Life Questionnaire scores

	CA, n = 11	CVA, n = 9	COUGH, n = 7	P ^a
Symptoms	5.6 ± 0.9	4.8 ± 1.2	4.9 ± 1.6	0.260
Activity limitation	6.3 ± 0.8	6.0 ± 1.2	5.6 ± 1.4	0.490
Emotional function	5.7 ± 1.3	5.0 ± 1.9	5.4 ± 1.0	0.605
Environmental stimuli	5.4 ± 1.6	4.5 ± 1.8	5.0 ± 1.3	0.517
Overall score	5.8 ± 1.0	5.1 ± 1.3	5.2 ± 1.1	0.348

All values are means ± SD. ^aFrom ANOVA with Bonferroni correction.

Table 4. *Impulse oscillometry values at baseline and MAX*

	CA, <i>n</i> = 11	CVA, <i>n</i> = 10	COUGH, <i>n</i> = 7	<i>P</i> ^a
Baseline				
R5, cmH ₂ O·l ⁻¹ ·s ⁻¹	5.44 ± 1.56	4.34 ± 1.21	4.07 ± 1.95	0.144
R20, cmH ₂ O·l ⁻¹ ·s ⁻¹	4.16 ± 1.34	3.48 ± 0.978	3.36 ± 1.39	0.324
R5-R20, cmH ₂ O·l ⁻¹ ·s ⁻¹	1.28 ± 0.523	0.861 ± 0.387	0.673 ± 0.78	0.072
X5, cmH ₂ O·l ⁻¹ ·s ⁻¹	-1.90 ± 0.855	-1.34 ± 0.262	-1.37 ± 1.26	0.251
AX, cmH ₂ O/l	10.43 ± 6.39	5.35 ± 2.86	5.54 ± 9.75	0.159
<i>f</i> _{res} , Hz	17.2 ± 3.27	14.2 ± 2.10	11.3 ± 6.18 ^b	0.015
MAX				
R5, cmH ₂ O·l ⁻¹ ·s ⁻¹	8.11 ± 2.06	6.66 ± 2.22	6.55 ± 3.59	0.337
R20, cmH ₂ O·l ⁻¹ ·s ⁻¹	3.93 ± 0.912	3.46 ± 0.666	4.14 ± 1.42	0.354
R5-R20, cmH ₂ O·l ⁻¹ ·s ⁻¹	4.18 ± 1.41	3.20 ± 1.64	2.37 ± 2.82	0.161
X5, cmH ₂ O·l ⁻¹ ·s ⁻¹	-6.20 ± 3.11	-4.85 ± 2.77	-2.89 ± 3.59	0.110
AX, cmH ₂ O/l	50.9 ± 28.31	39.1 ± 26.9	21.8 ± 34.7	0.147
<i>f</i> _{res} , Hz	27.9 ± 7.00	23.4 ± 4.99	18.6 ± 9.03 ^b	0.034
Change				
ΔR5, cmH ₂ O·l ⁻¹ ·s ⁻¹	2.67 ± 1.20 ^c	2.33 ± 1.70 ^c	2.48 ± 2.03 ^d	0.889
ΔR20, cmH ₂ O·l ⁻¹ ·s ⁻¹	-0.226 ± 0.679	-0.014 ± 0.903	0.771 ± 0.814 ^{b, d}	0.046
ΔR5-R20, cmH ₂ O·l ⁻¹ ·s ⁻¹	2.89 ± 1.41 ^c	2.34 ± 1.59 ^c	1.70 ± 2.10	0.107
ΔX5, cmH ₂ O·l ⁻¹ ·s ⁻¹	-4.30 ± 2.71 ^c	-3.52 ± 2.69 ^c	-1.52 ± 2.35	0.308
ΔAX, cmH ₂ O/l	40.5 ± 26.4 ^c	33.8 ± 26.8 ^c	16.3 ± 25.1	0.179
Δ <i>f</i> _{res} , Hz	10.7 ± 5.65 ^c	11.2 ± 5.49 ^c	7.33 ± 4.38 ^c	0.343

Values represent means ± SD. Values in bold indicate significant differences. AX, reactance area; Δ, change from baseline to MAX; *f*_{res}, resonant frequency; R5, total respiratory resistance; R5-R20, peripheral respiratory resistance; R20, central respiratory resistance; X5, peripheral reactance. ^aFrom ANOVA with Bonferroni correction. ^b*P* < 0.05 compared with CA. ^c*P* < 0.01 compared with baseline using paired *t*-test. ^d*P* < 0.05 compared with baseline using paired *t*-test.

dominantly airway narrowing. Subjects with CA had significantly greater increases in sRaw compared with those in the COUGH group (Table 5).

Cough during high-dose MCh challenge. The total number of coughs in response to high-dose MCh were 1.7 ± 2.9 (*n* = 9), 33.0 ± 24.0 (*n* = 9), and 32.0 ± 27.1 (*n* = 7) in CA, CVA, and COUGH, respectively. The total number of coughs was significantly different between the groups (ANOVA *P* = 0.005). Post hoc analysis using Bonferroni correction indicated significant differences in the number of coughs between CA

and the other two groups (CA vs. CVA, *P* = 0.010; CA vs. COUGH, *P* = 0.022; CVA vs. COUGH, *P* = 1.000).

Bronchodilating index. At MAX, the DI index values were greater than zero in all three groups (0.67 ± 0.97, 0.51 ± 0.73, and 0.01 ± 0.36 in CA, CVA, and COUGH, respectively). The DI indices were not significantly different between the three groups at baseline [means ± SD: -0.01 ± 0.27, -0.20 ± 0.27, and -0.29 ± 0.16, respectively (ANOVA *P* = 0.069 or MAX ANOVA *P* = 0.211)]. The change in the DI index from baseline to MAX, presented in Figure 5, was significant in the

Table 5. *Spirometry, resistance, and closing indices to high-dose MCh challenge at MAX*

	CA, <i>n</i> = 11	CVA, <i>n</i> = 10	COUGH, <i>n</i> = 7	<i>P</i>
ΔFEV ₁ , liter	-1.09 ± 0.58 ^a	-0.91 ± 0.29 ^a	-0.70 ± 0.50 ^b	0.247
%ΔFEV ₁	-37.0 ± 9.5	-34.1 ± 9.2 ^c	-21.6 ± 15.1 ^c	0.000
ΔFEV ₁ , % predicted	-33.3 ± 13.9 ^a	-28.4 ± 9.0 ^a	-20.9 ± 14.1 ^d	0.141
ΔFVC, liter	-1.12 ± 0.72 ^a	-0.77 ± 0.29 ^a	-0.49 ± 0.56	0.075
%ΔFVC	-27.9 ± 9.6	-21.8 ± 8.9	-12.2 ± 14.4 ^c	0.021
ΔFVC, % predicted	-28.5 ± 13.9 ^a	-20.7 ± 8.0 ^a	-12.5 ± 14.3	0.040
ΔFEV ₁ /FVC	-0.10 ± 0.05 ^a	-0.12 ± 0.03 ^a	-0.09 ± 0.09 ^b	0.501
ΔPEF ₄₀ , l/s	-1.43 ± 0.56 ^a	-2.02 ± 1.07 ^a	-1.72 ± 0.67 ^a	0.262
ΔMEF ₄₀ , l/s	-1.06 ± 0.52 ^a	-1.30 ± 1.00 ^d	-0.90 ± 0.43 ^d	0.517
Resistance				
ΔRaw, cmH ₂ O·l ⁻¹ ·s ⁻¹	3.75 ± 1.88 ^a	2.31 ± 2.21 ^d	2.83 ± 4.14	0.474
ΔRaw, % predicted	242.0 ± 119.4 ^a	159.2 ± 153.6 ^b	201.9 ± 307.2	0.620
ΔsRaw, cmH ₂ O·l ⁻¹ ·s ⁻¹	16.13 ± 9.47 ^a	14.01 ± 10.34 ^d	4.26 ± 4.95 ^c	0.031
ΔsRaw, % predicted	407.6 ± 248.0 ^a	355.2 ± 269.2 ^d	105.5 ± 128.3 ^c	0.036
Closing indices				
%ΔFVC/%ΔFEV ₁	0.75 ± 0.14	0.63 ± 0.15	0.36 ± 0.48 ^c	0.021
%ΔFVC - %Δ $\left(\frac{\text{FEV}_1}{\text{FVC}}\right)$				
% FEV ₁	0.38 ± 0.30	0.13 ± 0.26	-0.31 ± 0.97 ^c	0.045

All values are means ± SD. Values in bold indicate significant differences. FEV₁ = forced expiratory volume in one second; FVC, forced vital capacity; FEV₁/FVC, forced expiratory volume in 1 s divided by forced vital capacity; FRC, functional residual capacity; IC, inspiratory capacity; MCh, methacholine; MEF₄₀, expiratory flow at 40% of the forced vital capacity from the full forced vital capacity maneuver; PEF₄₀, expiratory flow at 40% of the forced vital capacity from the partial curve; Raw, airway resistance; RV, residual volume; RV/TLC, residual volume divided by total lung capacity; sRaw, specific airway resistance; TLC, total lung capacity. *P* values are from ANOVA with Bonferroni correction. ^a*P* < 0.001 compared with baseline using paired *t*-test. ^b*P* < 0.05 compared with baseline using paired *t*-test. ^c*P* < 0.05 compared with CA. ^d*P* < 0.01 compared with baseline using paired *t*-test.

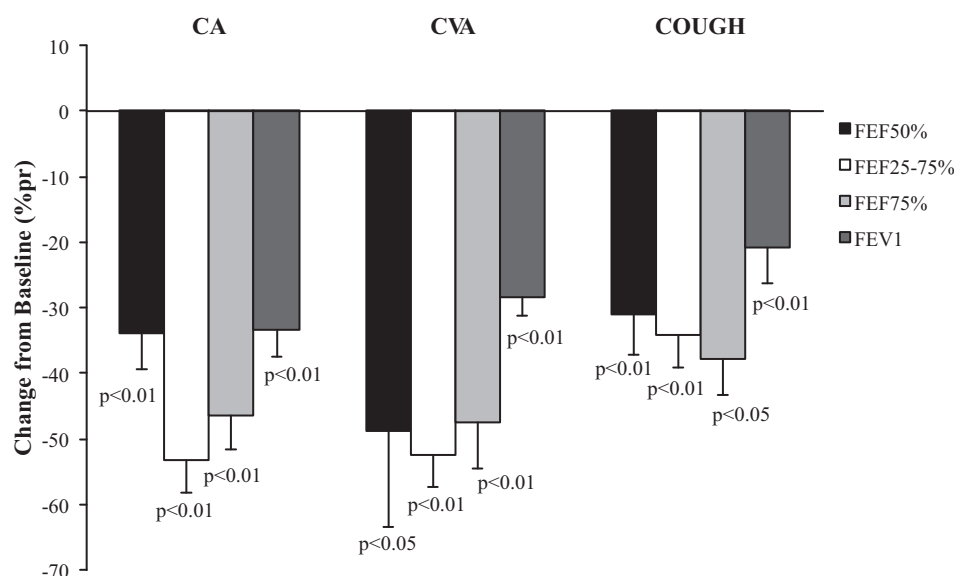


Fig. 3. FEV₁ and mid-to-late flow responses to MCh challenge at maximum change in FEV₁ of 50% from baseline (MAX) in individuals with CA ($n = 11$), CVA ($n = 10$), and COUGH ($n = 7$). Error bars represent SE. FEF_{50%}, forced expiratory flow at 50% of forced vital capacity; FEF_{25-75%}, mean forced expiratory flow during the middle half of the forced vital capacity; FEF_{75%}, forced expiratory flow at 75% of forced vital capacity. P values indicate the significance of variable changes from baseline.

CA ($P = 0.040$), CVA ($P = 0.013$), and COUGH ($P = 0.022$) groups. There was no correlation between the percent change in FEV₁ at MAX and the DI index (correlation coefficients: CA = -0.26 , CVA = 0.32 , COUGH = 0.42).

DISCUSSION

To our knowledge, this is the first study to compare the bronchodilating effect of DIs in individuals with mild CA, CVA, and COUGH. Our findings suggest that the bronchodilating effect of DIs is preserved in all three groups, although intriguingly, the bronchodilating effect was marginal in individuals with COUGH. Spirometry and lung volume responses to high-dose MCh bronchoprovocation revealed greater gas trapping as indicated by the percent change in FVC in CA compared with COUGH and greater change in RV (liters and % predicted), RV/TLC (% and % predicted) in individuals with CA compared with individuals in both the CVA and COUGH groups. Airway closure was significantly less in those in the COUGH group compared with those in the CA group. Impulse oscillometry values were not significantly different between the three groups at MAX. In individuals with CA and CVA, increases in total resistance were primarily attributable to increases in peripheral resistance (R5–R20), because central resistance (R20) did not increase significantly from baseline in either group, whereas in individuals with COUGH, the opposite pattern of changes was observed. Individuals with CA

coughed less in response to high-dose MCh than individuals with CVA and COUGH. Our results suggest that CVA and COUGH are distinct phenotypes from CA, which may reflect underlying differences in the airway responses of conditions that fall along a spectrum of disorders of AHR (51). Other contributing factors may include differences in the peripheral airway activation or central nervous system integration of afferent feedback (24, 51). Indeed, differences in the central processing of afferent feedback is only one area that deserves further attention with respect to physiologic differences between the groups, which could lead to novel therapeutic strategies.

General findings. During high-dose MCh challenge, FEV₁ and FVC declined significantly compared with baseline in individuals with CA (37% and 28%, respectively) and CVA (34% and 22%, respectively). Individuals with COUGH had FEV₁ and FVC declines of ~22% and 12% from baseline, respectively. All groups had comparable significant declines in the mid-to-late flows from baseline to MAX. The mean number of coughs in response to high-dose MCh in individuals with COUGH and CVA was greater than in those with CA. Ohkura et al. (59) also found that compared with healthy individuals, subjects with moderate or severe asthma have greater AHR and cough less in response to high-dose MCh. Others have also demonstrated that individuals with CVA cough more frequently in response to MCh challenge compared with subjects

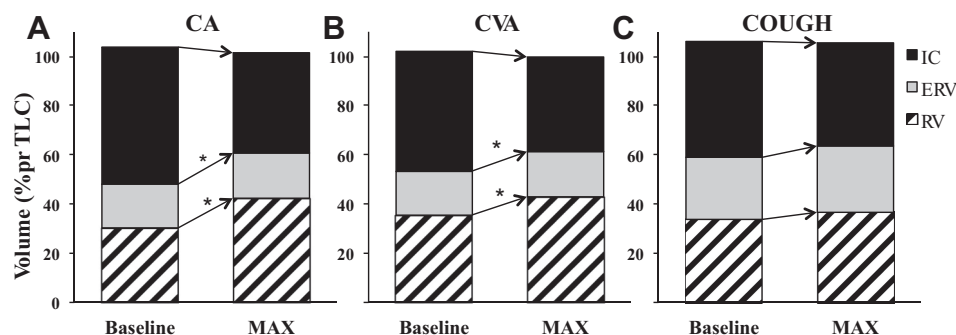


Fig. 4. Lung volume responses to MCh challenge at MAX in individuals with CA ($n = 11$) (A), CVA ($n = 10$) (B), and COUGH ($n = 7$) (C). ERV, expiratory reserve volume; IC, inspiratory capacity; RV, residual volume; TLC, total lung capacity. * $P < 0.05$ compared with baseline using paired t -test.

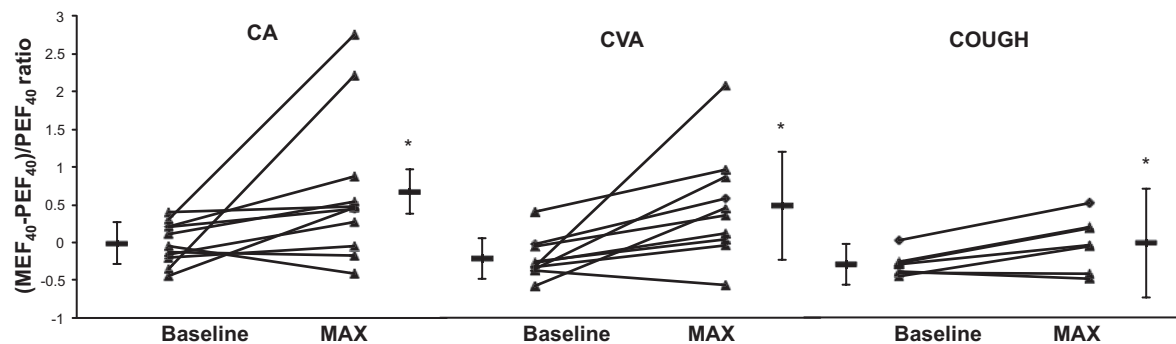


Fig. 5. Bronchodilating index at baseline and MAX in individuals with CA ($n = 11$), CVA ($n = 10$), and COUGH ($n = 7$). Bars represent means $\pm$ SD. MEF_{40} , expiratory flow at 40% of the forced vital capacity from the full forced vital capacity maneuver; PEF_{40} , expiratory flow at 40% of the forced vital capacity from the partial curve. * $P < 0.05$ compared with baseline using paired t -test.

with CA (50). In healthy individuals who do not have asthma, MCh-induced cough is associated with the bronchodilating effect of a DI (58). The coughing observed in response to MCh by those with COUGH and CVA may bronchodilate the airways and reduce the MCh-induced bronchoconstriction, because a DI usually precedes a cough (64). At MAX, IOS values were not different between the three groups (except for f_{res} , which was greater in individuals with CA compared with those with COUGH). At this unique frequency, f_{res} , the total impedance to airflow (resistive and reactive) is entirely attributable to resistance, and f_{res} is considered normal when it is approximately 6–12 Hz (36, 83). Obstructive defects of the distal respiratory tract cause f_{res} to be increased above normal (11, 16). Because f_{res} was greater than 12 in individuals with CA and CVA at baseline, but not in those with COUGH, we suggest that pathological processes in the distal airways differentiate CA and CVA from COUGH.

Although R5 increased significantly in all three groups compared with baseline, in CA and CVA this was primarily attributable to increases in the peripheral resistance (R5–R20) because R20 did not increase significantly from baseline in either group. In contrast, the significant increase in R5 in the COUGH group was primarily attributable to increases in central resistance (R20) because R5–R20 did not increase significantly from baseline. However, it is important to note that the specific level of the tracheo-bronchial tree cannot be definitively established using R20, thus the distinction between peripheral and central airways is somewhat arbitrary (72). Nevertheless, these findings support our hypothesis that a defect in the peripheral airways is a distinguishing feature of these related conditions.

It is interesting that despite not having AHR, those with COUGH had similar decreases in the mid-to-late flows when given a strong enough MCh stimulus. We believe that patients with COUGH represent a distinct phenotype, the clinical significance of which remains to be elucidated. We excluded nine subjects with chronic cough and suspected CVA who neither coughed in response to MCh nor demonstrated AHR to MCh. Despite having similar clinical presentation, demographics, medication use, and smoking history, these subjects did not have the same tussive response to MCh as those we included in our study in the COUGH group. Perhaps the individuals we excluded are yet another group that fall along the theoretical continuum of disorders of airway responsiveness (48).

Bronchodilation. The DI index used by Ohkura et al. (58) provides a method to quantify the bronchodilating effect of DIs. In our study, the value of the DI index was greater than zero at MAX in all three groups, which suggests that the bronchodilating effect of a DI was preserved. The DI index was not different between the three groups at baseline or at MAX. In all three groups, the DI index increased significantly from baseline to MAX, indicating an increase in the bronchodilatory ability at MAX. It is important to note that the response to DIs is related to the magnitude of “spontaneous” obstruction (46), and individuals with obstruction at baseline can bronchodilate (62). There were no differences between the three groups at baseline [except $FEF_{75\%}$ (liters and % predicted)], and nothing to suggest that there was baseline obstruction in any group, thus it is not surprising that the DI index did not differ at baseline, and was less than zero.

Among those with asthma, there is variability in the ability to bronchodilate after DIs, which appears to be dependent on the severity of disease (70), and loss of bronchoprotection, which may relate to airway distensibility and gas trapping (66). Using AHR to MCh to quantify disease severity, the majority of the subjects with CA in our study (7/11) had mild-to-moderate asthma (1 had borderline AHR), and all subjects with CVA had borderline-to-mild asthma. If the sample contained more individuals with severe asthma, it is possible that the bronchodilating effect would not have been preserved in CA. Lung-volume history (i.e., repeated DIs) inherent to a multiple-dose MCh challenge may influence the ability of an individual to bronchodilate the airways. Because the bronchodilating effect of a DI lasts approximately 1–2 min (56), and the MEFV/PEFV maneuvers were performed every 4–5 min after the previous maneuver, it is not likely that any bronchodilation from a DI during the MEFV maneuver would affect a subject’s ability to subsequently bronchodilate. However, the bronchoprotective effect of DIs may have had an effect on our findings. Five DIs before the inhalation of a single dose of MCh bronchoprotects the airways of healthy individuals but not individuals with asthma (20, 41, 69). This effect was found to be transient, and abolished when measured 10 min after MCh inhalation even in healthy subjects (20). Because the doses of MCh were separated by about 5 min, it is possible that the MEFV maneuver, which requires a DI, could have bronchoprotected the airways, and affected any subsequent bronchodilation. However, Chapman et al. (12) have shown that an

exhalation to RV abolishes the bronchoprotective effect. Because the MEFV maneuver used in the protocol requires an exhalation to RV, any bronchoprotection from the DI likely was abolished.

Airway closure. At MAX of the high-dose MCh challenge, individuals with COUGH had significantly less airway closure than those with CA. This may be because individuals with COUGH did not bronchoconstrict enough to induce airway closure; however, those individuals demonstrated significant declines in their mid-to-late flows at MAX, which was comparable in magnitude to those with CA and CVA. Thus we surmise that the obstruction induced by MCh in the COUGH group was predominantly due to airway narrowing. This was likely a result of narrowing of the large airways because R20 was significantly increased in the COUGH group (with no change observed in R5–R20). In those with CA, the obstruction induced by MCh was predominantly the result of airway closure, since in response to MCh, RV and RV/TLC increased more, and functional residual capacity decreased more in the CA group compared with that of the COUGH group. Furthermore, both closing indices and R5–R20 suggest that there was more airway closure in those with CA compared with COUGH. The significant increase in R5–R20 (without a significant increase in R20) localizes the MCh response to the small airways and further supports our conclusion of airway closure in CA. Thus it is possible that there is some functional difference in the peripheral airways (perhaps a consequence of airway remodeling), which permits airways to close more readily in individuals with CA. Additionally, because those with CA demonstrated the ability to bronchodilate, which was at most borderline in those with COUGH (DI index = 0.01), DI appears to reverse the obstruction due to closure but not the obstruction due to large airway narrowing. Although the relationship between cough and induced airway trapping is unknown, there is speculation that the pathophysiology of cough in CA and CVA may relate to small airway obstruction, gas trapping, and intrinsic positive-end expiratory pressure (48). It has been suggested that surfactant might play a role in the ability of DIs to protect against airway closure (12, 13). Perhaps the increased airway closure in CA is due to the inhibitory effects of inflammation on surfactant (38). Although most of the subjects with CA (8/11) were taking ICSs, the small airways may not be adequately treated because not all available formulations of ICSs efficiently access the small airways (57), and many patients have an improper inhalation technique (43, 65).

Impulse oscillometry. A major strength of the design of our study is that we examined airway responses to high-dose MCh with IOS, which does not require DIs. Although the IOS values were not different between the three groups (except for f_{res}), it is likely that greater sample sizes will be required to definitively confirm or refute statistically significant differences. Furthermore, we have directly assessed the effects of maneuvers performed as part of our protocol on IOS values. In our subjects, we were able to demonstrate that 10 min of DI avoidance, MEFV/PEFV maneuvers, and plethysmography do not affect IOS measurements. Therefore, it is likely that changes in IOS values represent the effects of MCh on the airways and not artifact from a preceding maneuver (such as plethysmography, or DI avoidance). However, we acknowledge the potential for a type II error (28) related to small

sample size, and therefore, the need to verify these observations in a larger sample.

Medication use. Medication use was not significantly different among individuals in the three groups, and two of seven patients with COUGH were taking combined ICS/LABA therapy (compared with 5 of 11 with CA and 2 of 10 with CVA), without demonstrating AHR. This is interesting and concerning because the use of LABAs has been controversial (81). Perhaps the prescription of LABAs to those with cough represents a lack of resources in the community for objective diagnosis, or perhaps the prescription of ICS/LABA in those without AHR is a result of the difficulty of treating chronic cough (22, 23, 39).

Study limitations. The main limitation of this study is the small sample size, particularly in the COUGH group, and thus we risk a type II error (28) if we conclude that responses to DIs were comparable in our groups. Consequently, this limits interpretation of nonsignificant differences between groups.

Although medication use was not significantly different between groups, ICS therapy may have influenced our results. ICS use has been found to reduce sensitivity to MCh in individuals with CVA (30), decrease cough sensitivity to capsaicin in subjects with asthma (25), and restore the bronchoprotective effect of a DI in subjects with mild AHR (71). However, in those with mild-to-severe AHR, ICSs did not have an effect on the bronchodilating effect of a DI (71), whereas leukotriene inhibitor therapy has been shown to enhance DI-induced bronchodilation during MCh challenge (4).

Obesity can affect airway responsiveness and can lead to bronchoconstriction in response to a DI (74). Many subjects in our study were obese (BMI >30 kg/m²), which could have affected the bronchodilating effect of a DI; however, because there were no significant differences in BMI between the three groups, the effect of obesity on DIs would have influenced our findings in all three groups.

We separated our subjects into three distinct groups, which reflects our previous hypothesis that subjects can be divided along a continuum of airway responsiveness (48). However, there was overlap in asthma severity (in terms of PC₂₀) between those with CA and CVA, which could potentially influence the magnitude of the difference between the two groups. In this regard, comparing the bronchodilating effect in individuals with more severe CA with those with CVA may be informative. Furthermore, even within the COUGH group there was a variable response to MCh, which could affect the interpretation of the results. The problematic nature of defining asthma has been extensively discussed (17), and it is clear that no single test has sufficient sensitivity and specificity to reliably diagnose a syndrome with such variable manifestations (78). Although all subjects in the COUGH group, by definition, did not demonstrate AHR to MCh, it is possible that another stimulus (e.g., mannitol or cold air) could elicit AHR in these individuals (79). This deserves further attention with respect to identification of the phenotypic differences between CA, CVA, and COUGH. Although it is possible (albeit unlikely) that cough was attributable to eosinophilic bronchitis in our subjects with COUGH, 43% were taking ICSs before the beginning of the study, but still suffered from a chronic cough (7, 33).

Future directions. A larger sample size is required, including subjects with more severe asthma, to help to distinguish po-

tential differences between CA and CVA and determine whether these differences fall along a spectrum of disease severity that is related to the loss of the bronchodilating effects of DIs. Sputum induction and exhaled nitric oxide testing would help define the phenotype of the subjects. Additionally, because the bronchoprotective effect of DIs may be stronger than the bronchodilating effect (69), it would be valuable to compare the bronchoprotective effect of DIs in these three groups. Furthermore, because the FEV₁ and FVC response to high-dose MCh in those with COUGH was comparable to those previously studied without airway disease (32), it would be beneficial to examine what distinguishes individuals with COUGH from healthy individuals in terms of airway responses to high-dose MCh.

Conclusion. In conclusion, comparison of individuals with mild CA, CVA, and COUGH suggests that the bronchodilating effect of a DI is preserved in CA and CVA, and is at most borderline in COUGH. Individuals with COUGH had significantly less airway closure than those with CA, despite all groups demonstrating comparable significant declines in mid-to-late expiratory flows. In both CA and CVA, IOS data indicate primary involvement of the peripheral airways in response to high-dose MCh, whereas COUGH exhibited predominant increases in central resistance. Thus DIs are able to reverse the obstruction due to closure but not the obstruction due to large-airway narrowing. The functional difference in the peripheral airways of these groups may contribute to the different airway responsiveness phenotypes observed and merit further delineation to personalize therapies. Comparing these findings with a group of individuals with more severe asthma would be informative. Furthermore, examination of the bronchoprotective effect of DIs would help to elucidate pathophysiological differences between these related conditions.

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AUTHOR CONTRIBUTIONS

N.V.W., S.E.T., and M.D.L. conception and design of research; N.V.W. and T.F. performed experiments; N.V.W. analyzed data; N.V.W., J.T.F., and M.D.L. interpreted results of experiments; N.V.W. prepared figures; N.V.W. drafted manuscript; N.V.W., S.E.T., J.T.F., and M.D.L. edited and revised manuscript; N.V.W., T.F., S.E.T., J.T.F., and M.D.L. approved final version of manuscript.

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