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L-citrulline prevents Asymmetric Dimethylarginine-Mediated reductions in Nitric Oxide and Nitrosative Stress In Primary Human Airway Epithelial Cells

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Abstract

Introduction—Asthma is associated with reduced systemic levels of L-arginine and increased asymmetric dimethylarginine (ADMA). This imbalance leads to nitric oxide synthase (NOS) uncoupling with reduced nitric oxide (NO) formation and greater oxidative and nitrosative stress. Whether this imbalance also occurs in the bronchial epithelium of asthmatics is unknown.

Methods—Subjects with asthma and healthy controls underwent bronchoscopy to obtain human bronchial epithelial cells (HBECs), which were cultured and stimulated with IL-13 and IFN γ (both 10ng/ml) to determine the effects of ADMA and varying concentrations of L-arginine, on NO- metabolites (nitrites + nitrates or NOx), hydrogen peroxide and nitrotyrosine levels. HBECs were also treated with L-citrulline to prevent ADMA-mediated effects in NO bioavailability and nitrosative stress.

Results—In HBECs IL-13 and IFN γ stimulated NOS2 and increased NOx levels. The addition of ADMA reduced NOx and increased H₂O₂ levels (p<0.001). Treatment with L-citrulline (800 μ M, 1600 μ M) rescued NOx when the L-arginine media concentration was 25 μ M but failed to do so with higher concentrations (100 μ M). Under reduced L-arginine media conditions, HBECs treated with L-citrulline increased the levels of argininosuccinate, an enzyme that metabolizes L-citrulline to L-arginine. L-citrulline prevented the ADMA-mediated increase in nitrotyrosine in HBECs in cells from asthmatics and controls.

Conclusion—Increasing ADMA reduces NO formation and increases oxidative and nitrosative stress in airway epithelial cells. L-citrulline supplementation restores NO formation, while preventing nitrosative stress.

Introduction

Nitric oxide (NO) in exhaled air is a biomarker for increased Type 2 helper T cell (Th-2)-related inflammation in asthma; however, NO is also constitutively produced in airways, functioning as an endogenous bronchodilator in addition to having other physiological properties (1–4). The elevated fractional excretion of NO (FeNO) in asthma is thought to be derived from inducible nitric oxide synthase (NOS2) in bronchial epithelial cells through a

process that is largely dependent on availability of L-arginine as substrate (5). Factors that reduce the bioavailability of this amino acid, such as increased arginase activity, limit the formation of NO impairing its airway bronchodilator effects (6). Further, under conditions of substrate limitation, asymmetric dimethyl arginine (ADMA), a byproduct of protein catabolism, may lead to NOS uncoupling; this preferentially induces reactive oxygen species (ROS) formation at the expense of NO production, while at the same time generating secondary reactive oxygen and nitrogen oxides (7). These metabolic changes have significant pathophysiological and clinical implications. In experimental models ADMA reduces NO-related metabolites while increasing oxidative and nitrosative stress and enhancing lung inflammation (8, 9). In asthmatics, plasma and sputum ADMA levels are higher than in controls and plasma ADMA is inversely related to FeNO, while lower plasma L-arginine levels and increased plasma arginase activity have been associated with worse asthma severity (6, 10). Reduction in the L-arginine/ADMA balance has particular implications for obese late onset asthma. In this group of patients, increasing body mass index (BMI) categories are linked to decreased L-arginine/ADMA ratios in plasma, which are associated with lower FeNO, reduced lung function, more frequent respiratory symptoms and poorer asthma-related quality of life (11). Reduced NO bioavailability coupled with greater oxidative and nitrosative stress, which impairs normal airway function could explain the observed associations.

Therefore, therapeutic interventions resulting in increased L-arginine availability for NOS may help reverse the downstream effects of ADMA induced NOS uncoupling in asthma airways, specifically in patients with the late onset obese phenotype.

We used primary human bronchial epithelial cells (HBECs) from asthmatics and healthy controls to study our hypotheses that a) ADMA-mediated NOS uncoupling reduces epithelial production of NO and increases oxygen and nitrogen reactive species, and b) L-citrulline (as an L-arginine donor) can reverse this mechanism by recoupling NOS, restoring NO production and reducing oxidative and nitrosative stress.

Methods

The study population for the HBECs studies was derived from healthy volunteers and from asthmatics undergoing a baseline bronchoscopy for a separate study of asthma and electrophilic fatty acids (NCT01733485). Asthma was defined by having a physician diagnosis in addition to either a 12% or greater post bronchodilation increase in forced exhalation volume in one second (FEV₁) or a methacholine-induced bronchial hyperresponsiveness (≥ 20% reduction in baseline FEV₁ with a methacholine concentration dose < 16mg/dl). Participants were recruited from University of Pittsburgh Clinics and the Asthma Institute registry, signed a consent form and agreed to undergo bronchoscopy using an IRB approved conscious sedation protocol.

All study participants were nonsmokers for at least 1 year prior to recruitment and had smoked a total of < 10 pack-years. The study was IRB approved by the University of Pittsburgh and all subjects signed a consent form prior to starting in the study.

Cell Culture

HBEC samples were obtained during bronchoscopies using the Severe Asthma Research Protocol (SARP), as previously described (12). Briefly, participants with asthma and healthy controls underwent bronchoscopy using an IRB approved conscious sedation protocol after signing informed consent. A total of 10 cytology brushings were obtained from 3rd–4th airway generation branches from different subsegments. Epithelial cells were placed directly into 10 mL of ice-cold PBS, centrifuged, washed, and resuspended in 1 mL of serum-free, hormonally supplemented bronchial epithelial growth medium (Clonetics, San Diego, Calif) containing 50 µg/mL gentamicin and 50 µg/mL amphotericin. A total of 4×10^5 cells were seeded into 60-mm tissue-culture dishes coated with rat-tail type I collagen (BD Discovery Labware, Bedford, Mass). Cells were cultured at 37°C in a 5% CO₂ environment. When the epithelial cells reached 70% to 80% confluence, they were dissociated with trypsin-EDTA and passed onto collagen-coated polyester trans well inserts of 12 mm in diameter (pore size, 0.4 µm) at 4×10^4 cells/cm². After a week of being in immersed culture, epithelial cells reached 100% confluence and were shifted to an air liquid interface (ALI) condition by removing all but 50 µL of the apical medium. Cell media was changed every other day. At day 6, cells were stimulated with IL-13 + IFNγ (each 10 ng/ml). As shown previously by Chibana et al, IL-13 stimulation increases iNOS expression and the concentration of NO metabolites (NOx) (12) and IFNγ was also added to simulate the Th1 immune polarization described in obese asthmatics (13, 14). After 8 days in ALI state, cells are treated with increasing concentrations of ADMA 48 hours prior to harvest. To determine the effect of L-citrulline on NOx production, exogenous L-citrulline within a concentration range used in other *in vitro* studies (15, 16), was added at day 9 in the presence of 100µM ADMA (7). After 10 days, cells are harvested and media collected for analysis.

To determine whether physiologic (high) or sub-physiologic (low) concentrations of L-arginine modify the effects of ADMA to reduce (presumably due to uncoupling NOS) or of L-citrulline to restore NO-related metabolites, the experiments were respectively performed with 100 and 25 µM of L-arginine containing media.

NOx (Nitrites + Nitrates) determination

Lower supernatant nitrite and nitrate concentrations were quantified using a colorimetric assay based on the Griess reaction (Parameter Total Nitric Oxide and Nitrite/Nitrate Assay; R&D Systems, MN). Briefly, nitrite was quantified and nitrate was converted to nitrite using nitrate reductase, followed by the addition of Griess reagent to produce an azo dye compound. The absorbance was measured at 540 nm. NOx concentration was calculated from a standard curve prepared from serial nitrite dilution.

Cytotoxicity

It was performed using Pierce LDH Cytotoxicity assay kit according to the manufacture. Briefly the assay was made by transferring cell culture media from treated and untreated cells into a microplate and adding the kit reagents. After incubation at room temperature for 30 minutes, reactions were stopped and LDH activity was determined by spectrophotometric absorbance at 490nm.

Protein Assay

Protein concentration was estimated with bicinchoninic acid following the procedure described by Smith et al, 1985 et al(17). Bovine serum albumin fraction V was used as the standard protein.

Dot-blot analysis for 3-nitrotyrosine

Total proteins prepared (20 µg) from HBECs with and without ADMA treatment were blotted onto nitrocellulose membranes using a Bio Dot apparatus (Bio-Rad, Hercules, CA). The membrane were rinsed in 20 ml TBST and blocked with 20 ml 5% nonfat milk in TBST for 1 h, followed by an incubation with a 3-nitrotyrosine (3-NT) antibody (1:1000, Calbiochem, San Diego, CA) at 4°C overnight. After three washes with TBST, the membranes were incubated with goat anti-mouse IgG horseradish peroxidase-conjugated secondary antibody (1:2000, Pierce, Rockford, IL) for 1 h at room temperature. After washing again, the dots were visualized with chemiluminescence using a Kodak Digital Science Image Station (NEN) and analyzed using the KED-1 software. The same membranes were probed with β-actin antibody to normalize for loading.

Western blot analysis

Total proteins (20 µg/well) prepared from HBECs were separated on 4%–12% sodium dodecylsulfate (SDS) polyacrylamide gels and transferred to polyvinylidenedifluoride membranes (PVDF). Immunoblotting were performed using the appropriate antibodies in Tris-base buffered saline with 0.1% Tween 20 and 5% bovine serum albumin. After washing, the membranes were probed with horseradish peroxidase-conjugated goat antiserum to rabbit or mouse. Reactive bands were visualized using chemiluminescence (SuperSignal West Femto; Pierce) on a Kodak 440CF image station. Bands were quantified using Kodak image station software (Kodak 1D 3.6). Loading was normalized by probing the membranes with β-actin antibody.

Hydrogen peroxide was determined using the Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit, which detects hydrogen peroxide (H₂O₂) or peroxidase activity (Invitrogen). Briefly, culture medium from the upper chamber was removed on day 10, and washed twice with 200µl PBS, then 100µl of PBS was added following 3h incubation, H₂O₂ was then measured in the upper chamber supernatants using the Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit (Molecular Probes, Eugene, OR). The fluorescent signal was read at 530nm excitation, 590nm emissions, using the Infinite 200 PRO (Tecan, Männedorf, Switzerland).

Immunofluorescence

Epithelial cells were fixed in 4% paraformaldehyde/PBS for 45 minutes. The cells were then permeabilized with 0.2% Triton X-100 in PBS for 5 minutes at room temperature. Argininosuccinate synthase (ASS) expression in human bronchial epithelial cells was demonstrated using a polyclonal antibody directed against ASS (dilution 1:100) purchased from Santa Cruz Biotechnology (H-231). The cells were then incubated in fluorochrome-conjugated secondary antibody (dilution 1:400) for 1–2 hr at room temperature in the dark

followed by DAPI staining for 5 minutes at room temperature. After three washes in PBS, the coverslips were mounted in polyvinyl alcohol (Sigma-Aldrich). Fluorescently labeled cells were visualized with a Zeiss Axioplan fluorescence microscope using $\times 20$ or $\times 40$ objectives.

Sputum Induction and Processing

Sputum was induced in patients using previously validated methods for consistency and quality control (18, 19). Subjects underwent spirometry before and 10 minutes after 4 puffs of inhaled albuterol to ensure post-bronchodilator FEV₁ greater than 50% predicted. A 12-minute sputum induction was then performed. Sputum was collected and mixed with an equal volume of 10% sputolysin using a serological pipette. The sample was then placed in a 37°C shaking water bath for 15 minutes and the process was repeated 3 times. Cell differentials were calculated as the percent of cells in the whole sputum expectorate. Sputum samples with ~80% squamous epithelial cells were not included in the analysis. Sputum supernatants were collected and stored in an -80°C freezer.

Statistical analyses

Normally distributed data was summarized as mean and standard deviations. The t-test and one way ANOVA were used to compare normally distributed data between two or more groups respectively, the Wilcoxon or Kruskal Wallis test were alternatively used for non-parametric data between two or more groups. Statistical significance was considered at a $p < 0.05$. Statistical analyses were done with Stata 13 (College, TX).

Results

Demographics

HBECs were obtained from 11 subjects with asthma and 6 controls. Compared to controls, asthmatics were slightly older, more likely to be female and had a similar BMI distribution. Asthmatics also had greater FeNO, and lower FEV₁ and FEV₁/FVC ratios; however, given the sample size, these comparisons were not statistically significant. On average, the asthmatics were border line controlled with an ACT of 19 and 30% were on regular inhaled corticosteroids (ICS) (See Table 1).

ADMA effect on NOx production by HBECs is dependent on L-arginine concentration in the culture media

Stimulation of HBECs with IL-13 and IFN γ increased NOx as previously shown (12) (Figure 1). The addition of 100 μ M of ADMA to the culture supernatant significantly decreased NOx concentrations without reducing the levels of iNOS expression, suggesting that NOx reduction is primarily due to NOS inhibition by ADMA (Figure 2).

In stimulated HBECs from asthmatic and control subjects, increasing ADMA reduced NOx in a nonlinear fashion, with relatively smaller reductions at doses greater than 100 μ M (Figure 3). ADMA also decreased NOx, albeit to a lesser extent, in cells without any cytokine stimulation.

L-citrulline reduces ADMA-mediated reductions in NO in stimulated HBECs

L-citrulline (800µM and 1600 µM) was added to the cell media in addition to increasing ADMA concentrations. The ability of L-citrulline to reverse ADMA-mediated reduction in NOx was tested at lower (25 µM) and higher (100 µM) L-arginine concentrations in the media. At lower L-arginine concentrations, the addition of L-citrulline prevented the ADMA-mediated reduction in NOx and restored NOx to levels similar to control (Figure 4A). In contrast, at higher L-arginine concentrations, L-citrulline was ineffective in raising NOx levels (See Figure 4B). These results suggest that HBECs only synthesize L-arginine from L-citrulline under conditions in substrate availability is limited. To investigate this further, we determined the effect of L-citrulline supplementation on the concentration of argininosuccinate synthase (ASS) protein, a key enzyme in the recycling of L-citrulline to L-arginine. Treatment with L-citrulline significantly increased the ASS protein concentration in HBECs (Figure 5). However, this only occurred at low L-arginine concentration in the media (Figure 6A&B)

Effect of ADMA on 3-NT and H₂O₂

Increasing ADMA concentrations in the supernatant of stimulated HBECs significantly enhanced the production of H₂O₂ (Figure 7) in HBECs from asthmatics in conditions of limited L-arginine availability. The addition of L-citrulline did not significantly alter H₂O₂ concentrations (Data not shown) yet it significantly reduced 3-NT levels (Figure 8).

Effect of ADMA and citrulline on cytotoxicity

We found that neither ADMA (100µM, 500 µM) nor L-citrulline (1600 µM of) induced cell injury as determined by changes in lactic dehydrogenase levels (Figure 9).

Discussion

This study shows in human bronchial epithelial cells isolated from asthmatic and control subjects that ADMA inhibits and may uncouple NOS, thereby reducing production of NO metabolites and increasing oxidative and nitrosative stress. These effects were greater when the concentration of L-arginine in the culture media was reduced below the systemic levels seen in the normal population (81.6 ± 7.3 µM/L in young men and 113.7 ± 19.8 µM/L in elderly men, as compared with 72.4 ± 6.7 µM/L in young women and 88.0 ± 7.8 µM/L in elderly women (20, 21), yet considerably higher than the median level observed in the sputum of asthmatics 2.5µM [Q₁–Q₃: 4 – 10.3]) (10). L-citrulline at concentrations of 800µM and 1600µM was effective in preventing ADMA-induced reductions in NOx, and also lessened the degree of nitrosative stress. The ability of L-citrulline to prevent the effects of ADMA-mediated NOS uncoupling was only effective under conditions where L-arginine bioavailability was reduced.

ADMA is an endogenous, competitive, non-selective inhibitor of all NOS isoforms, is metabolized by the enzyme dimethyl-argininedimethyl-aminohydrolase (DDAH), and is produced by the lungs and readily detectable in the airways (10, 22). Although much of what is known about the health effects of increased ADMA has been related to the cardiovascular system, several experimental and clinical studies are showing that this arginine derivative is

also relevant to asthma and other airway diseases. In LA-4 stimulated lung epithelial cells, ADMA has been shown to increase the production of superoxide anion and peroxynitrate in a dose dependent manner and in OVA sensitized murine models, pretreatment with ADMA potentiates allergic inflammation while reducing nitrite concentration in the bronchoalveolar lavage (BAL) fluid. Even in the absence of induced airway inflammation, altering the balance between L-arginine and ADMA can lead to changes in airway structure and function; when continuously infused through an osmotic pump in mice, it reduced nitrites while increasing airway resistance and collagen deposition (8). These results suggest that chronically elevated ADMA can play a role in chronic obstructive airway disease. Furthermore, in mice, metabolic syndrome induced by high fructose or fat diet lowered exhaled NO, increased ADMA and arginase, and reduced L-arginine, while producing oxidative and nitrosative stress, however without airway cellular inflammation (23). These results highlight the possibility that the metabolic syndrome and related obesity can affect NO metabolism in the airways.

Subjects with asthma have greater sputum ADMA levels when compared to healthy controls and a controlled allergen exposure results in an increase in sputum ADMA after 7 and 24 hours (10, 24). Asthmatic children also have higher airway condensate ADMA levels when compared to healthy controls a difference not modified by use of inhaled corticosteroids (25). The L-arginine/ADMA balance, and its effects on airway NO metabolism, may be particularly relevant to an obese asthma phenotype characterized by high BMI and having late (after childhood) disease onset and reduced FeNO (26). Together, the results from clinical and experimental studies suggest that alterations in the L-arginine/ADMA balance could impair NOS activity leading to airways dysfunction and remodeling in asthma.

Our study shows that in HBECs, L-citrulline can be recycled into L-arginine and under conditions of limited L-arginine bioavailability, is effective in preventing ADMA-mediated NO reductions in HBECs and on reducing the formation of 3-NT. A similar mechanism was demonstrated in a murine macrophage cell line (RAW 264.7), in which L-citrulline restored NO production when L-arginine bioavailability was limited (27). While this result potentially implies that HBECs have the capacity to upregulate the enzymatic machinery that metabolizes L-citrulline into L-arginine, it may also just primarily reflect the fact that L-citrulline supplementation has a larger effect on the L-arginine/ADMA ratio at lower substrate concentrations. Interestingly, although L-citrulline reduced nitrosative stress, it failed to reduce oxidative stress. These differences may be related to the fact that there are many other cellular sources that can generate reactive oxygen species independently of NOS. These results, suggest that L-citrulline, could have therapeutic value for asthmatics whose airway dysfunction is driven by having a reduced L-arginine/ADMA balance.

Supplementation with L-arginine increases FeNO in children and adults and reduces airway inflammation and bronchial hyperresponsiveness in murine ovalbumin sensitization models (4, 28–30). In addition, L-arginine supplementation prevents NOS uncoupling (31). Unfortunately, L-arginine supplementation as a therapeutic modality is limited, given its extensive first pass metabolism in the liver and intestine (32). This is perhaps why one study found only modest improvements in FEV₁ in asthmatics after 1 week of L-arginine supplementation (33). Citrulline is a non-essential amino acid, but essential to detoxify and

remove ammonia from muscle and liver cells. L-Citrulline is FDA approved and commercially available as an amino acid supplement. It is not subjected to extensive first pass metabolism by gut bacteria or liver arginases and increases L-arginine levels in a dose dependent manner (32). More importantly, citrulline at a dose of 3 g/BID for 1 week, has been shown to substantially increase the plasma L-arginine/ADMA ratio at doses tolerated without side effects(32). In an experimental model of isolated tracheal preparation, L-citrulline was shown to restore impaired iNANC-mediated bronchodilation (2).

Some limitations must be considered when interpreting the results of this study. First, this is a relative small sample of mild to moderate asthmatics and therefore results may not be generalizable. Second, *in vitro* experiments using stimulated HBECs may not necessarily reflect how changes in L-arginine/ADMA affect these cells *in vivo*, which may explain why we did not observe differences between epithelial cells from asthmatics and controls. However, experimental animal studies do suggest that changes in this metabolic pathway impair NOS activity, which ultimately affect lung function; whether or not it can be reversed by L-citrulline supplementation, remains to be determined (8). Third, we did not assess the potential role for L-citrulline or the effect of IL-13 and IFN γ on the activity of DDAH. Although some inflammatory cytokines have been shown potentiate the effects of ADMA through enhanced DDAH degradation, these results have been demonstrated in transgenic mice and submerged BEAS-2B cell lines (34, 35); therefore these findings may not directly be comparable to primary human cells grown in air-liquid-interface. Nonetheless, further studies are necessary to understand the contribution of this enzyme to our *in vitro* system.

Conclusions

An imbalance characterized by greater ADMA and reduced L-arginine leads to greater NOS uncoupling, reduced NO bioavailability and more nitrosative stress in airway epithelial cells. This may explain why some asthmatics have reduced exhaled NO and increased asthma morbidity. L-citrulline supplementation could be a precision medicine strategy to restore airway NO and prevent the downstream effects resulting from NOS inhibition and uncoupling on certain asthma phenotypes.

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FIGURE 1.

In primary human bronchial epithelial cells, IL-13 + IFN γ increase NOS2 protein expression and Nitric Oxide metabolites (NOx)

FIGURE 2.

ADMA decreases the production of NO metabolites in primary human bronchial epithelial cells without affecting NOS2 expression.

Footnote: NOx (nitrate + nitrite) in IL-13 + IFN γ (10 ng each) stimulated primary human airway epithelial cells of asthmatic subjects in air-liquid interface, treated with ADMA for 48h prior to harvest (n=11 asthmatics and n=5 controls)

FIGURE 3.

The ADMA dose – response effect on the production of nitric oxide metabolites in primary human bronchial airway epithelial cells from asthmatics and healthy controls

FIGURE 4.

Addition of L-citrulline to prevent ADMA – mediated reduction of NO metabolites in primary human airway epithelial exposed to high (100 μM) or low (25 μM) L-arginine cell media concentrations.

FIGURE 5.

Effect of L-citrulline treatment on the concentration of argininosuccinate synthase in primary human bronchial epithelial cells

Footnote: Cytoplasmic ASS concentration [red fluorescence] in cells exposed to L-citrulline cultured in media containing 25 μM L-arginine.

FIGURE 6.

The effect of L-citrulline treatment on the protein concentration of argininosuccinate synthase of primary human bronchial epithelial cells exposed to low and physiological-range L-arginine media concentrations

Footnote: Argininosuccinate synthase [ASS] protein in primary airway epithelial cells exposed to increasing L-citrulline concentrations and ADMA with 25 μM L-arginine media; (D) Same as [C] but with a media containing 100 μM L-arginine;

FIGURE 7.

ADMA effect on H₂O₂ concentration in primary human airway epithelial cells

Footnote: H₂O₂ (nM/L) in primary human airway epithelial cells stimulated with ADMA (100, 250, and 500 μM for 48 hours), n=4 asthmatics, *p<0.02, one-way ANOVA.

FIGURE 8.

Effect of L-citrulline on nitrotyrosine formation in primary human airway epithelial cells treated with ADMA

Footnote: ADMA increases 3-Nitrotyrosine (3-NT) in primary epithelial cells isolated from healthy (n=6/group) and asthmatic subjects (n=6/group). L-Citrulline reduces ADMA induced 3-NT. *p<0.001, one-way ANOVA.

FIGURE 9.

Effect of ADMA (100µM, 500 µM) or L-citrulline (1600 µM) on LDH concentrations in primary human airway epithelial cells.

Footnote: n=3 asthmatic subjects

TABLE 1

Clinical and demographic characteristics of asthmatics and healthy controls participating in the bronchoscopy study.

	Asthma n=11	Controls n=6	p
Age	31 (19 – 50)	39 (21 – 52)	0.2
Sex (% Female)	64	34	0.2
BMI	31 (21 – 42)	29 (24 – 33)	0.2
FEV ₁ L	3 (2.5 – 3.6)	3.8 (2.9 – 4.7)	0.1
FEV ₁ % predicted	90 (80 – 99)	97 (81 – 113)	0.2
FVC L	3.9 (3.3 – 4.6)	4.6 (3.7 – 5.7)	0.09
FVC% predicted	98 (90 – 105)	96 (82 – 110)	0.7
FEV ₁ /FVC	76 (72 – 80)	79 (72 – 87)	0.6
FeNO ppb	24 (15 – 52)	15 (14 – 18)	0.07
ACT	19 (14 – 23)	N/A	N/A
Inhaled steroids (%)	30%	N/A	N/A
SABA (%)	100%	N/A	N/A

Footnote:

BMI: Body mass index

FEV₁: Forced exhalation in one second

FVC: Forced vital capacity

FeNO: Fraction of exhaled nitric oxide

ACT: Asthma control test

SABA: Short acting beta agonist