An Association between L-Arginine/Asymmetric Dimethyl Arginine Balance, Obesity, and the Age of Asthma Onset Phenotype

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Rationale: Increasing body mass index (BMI) has been associated with less fractional exhaled nitric oxide (F_{ENO}). This may be explained by an increase in the concentration of asymmetric dimethyl arginine (ADMA) relative to L-arginine, which can lead to greater nitric oxide synthase uncoupling.

Objectives: To compare this mechanism across age of asthma onset groups and determine its association with asthma morbidity and lung function.

Methods: Cross-sectional study of participants from the Severe Asthma Research Program, across early- (<12 yr) and late- (>12 yr) onset asthma phenotypes.

Measurements and Main Results: Subjects with late-onset asthma had a higher median plasma ADMA level (0.48 μ M, [interquartile range (IQR), 0.35–0.7] compared with early onset, 0.37 μ M [IQR, 0.29–0.59], $P = 0.01$) and lower median plasma L-arginine (late onset, 52.3 [IQR, 43–61] compared with early onset, 51 μ M [IQR 39–66]; $P = 0.02$). The log of plasma L-arginine/ADMA was inversely correlated with BMI in the late- ($r = -0.4$, $P = 0.0006$) in contrast to the early-onset phenotype ($r = -0.2$, $P = 0.07$). Although F_{ENO} was inversely associated with BMI in the late-onset phenotype ($P = 0.02$), the relationship was lost after adjusting for L-arginine/ADMA. Also in this phenotype, a reduced L-arginine/ADMA was associated with less IgE, increased respiratory symptoms, lower lung volumes, and worse asthma quality of life.

Conclusions: In late-onset asthma phenotype, plasma ratios of L-arginine to ADMA may explain the inverse relationship of BMI to F_{ENO}. In addition, these lower L-arginine/ADMA ratios are associated with reduced lung function and increased respiratory symptom frequency, suggesting a role in the pathobiology of the late-onset phenotype.

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AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

In subjects with asthma, obesity has been associated with increased severity of respiratory symptoms, greater healthcare use, and reduced responsiveness to corticosteroids. However, this obese late-onset phenotype does not fit in the classic paradigm of increased allergic inflammation. Rather, obesity has been associated with reduced exhaled nitric oxide (NO), less airway eosinophilia, and increased airway oxidative stress.

What This Study Adds to the Field

In subjects with late-onset asthma, obesity is associated with a lower plasma L-arginine/asymmetric dimethyl arginine (ADMA) and partly explains why exhaled NO is inversely related to body mass index. Moreover, in the late- but not early-onset asthma reduced plasma L-arginine/ADMA ratios are associated with continuous respiratory symptoms, less allergic inflammation, reduced asthma quality of life, and lower lung volumes.

Keywords: asthma; obesity; age of asthma onset; ADMA; arginine

The extent to which obesity affects the health of people with asthma, and by what mechanisms, remains largely undetermined. Although adiposity imposes an important load on the respiratory system, beyond worsening dyspnea, there is little evidence that obesity affects asthma by increasing traditional biomarkers of airway inflammation or bronchial hyperresponsiveness. In fact, increasing body mass index (BMI) has been paradoxically associated with reduced levels of fractional exhaled nitric oxide (F_{ENO}) and sputum eosinophils (1–4). In addition, cluster analyses identify obesity as part of a phenotype characterized by less atopy and adult-onset asthma, with additional analyses suggesting obesity may be causally related to asthma among those with adult-onset asthma with less atopy (5, 6). In contrast, in those with childhood-onset more atopic asthma, the asthma appears to be causative for the weight gain (7). This difference in relationship among early- and late-onset asthma was conditionally confirmed in a study of obese subjects with asthma undergoing bariatric surgery (8). Loss of body weight improved respiratory

symptoms in most subjects, yet bronchial hyperresponsiveness was reduced only among those with later-onset disease with lower IgE levels. Taken together, these results suggest obesity may affect asthma differently depending on the underlying phenotype.

One potential mechanism linking obesity as a causal mechanism for asthma is through interactions with NO pathways. Although low FE_{NO} levels could be due to many factors, the formation of asymmetric dimethyl arginine (ADMA), a product of posttranslational methylation of L-arginine, could play an important role through its ability to inhibit all NO synthase (NOS) isoforms (9). Sputum levels of ADMA in asthma have been reported to inversely correlate with FE_{NO} (10). Furthermore, ADMA has been shown to be higher in obese subjects and in those with the metabolic syndrome (11, 12). Because obesity appears to be predominantly associated with a late-onset asthma phenotype, we hypothesized that reduced L-arginine/ADMA ratios would be an important determinant of the inverse relationship of BMI to FE_{NO} in subjects with late-onset asthma. Moreover, we hypothesized that reductions in L-arginine/ADMA would associate with lower $FEV_1\%$ and less asthma-related quality of life and allergic inflammation in the late- but not early-onset phenotype. This research has been previously published as an abstract (13).

METHODS

The study population consisted of participants aged 18 years or older from the multicenter Severe Asthma Research Program (SARP) study who met criteria for asthma and had plasma levels of L-arginine and ADMA measured. All subjects signed an informed consent, and all the

participating sites had institutional review board approval. Asthma diagnosis was based on having either a 12% increase in FEV_1 after short-acting bronchodilator or a methacholine dose at which an FEV_1 drop greater than 20% from baseline occurred of 16 mg/ml or less. The SARP study has been previously described in detail (5, 7). For purposes of this study, mild and moderate asthma were analyzed together as “not severe” asthma. Age of onset was dichotomized into early childhood (<12 yr) and adolescent/late-onset (≥ 12 yr) phenotypes (7, 14).

Clinical Data

Variables included healthcare use and respiratory symptoms in the 3 months preceding enrollment. Subjects also completed the Juniper Asthma Quality of Life Questionnaire (AQLQ) (15). Inflammatory biomarkers included IgE and FE_{NO} (see the online supplement for full methods).

Plasma L-Arginine and ADMA

Quantification of plasma L-arginine and ADMA levels were performed at the Cleveland site (S.C.E.) and the Pittsburgh site (R.W.P.) using high-performance liquid chromatography and as previously described (16–18). Arginine and methylated forms have been reported previously for some of the cohort (18). The L-arginine/ADMA ratio was chosen as the best indicator of the availability of substrate to enzyme.

Statistical Analysis

To determine the association between the plasma L-arginine, ADMA, and the L-arginine/ADMA ratios with the BMI and the BMI categories, across the age of asthma onset phenotypes, the data were log-transformed and correlated to BMI. The Fisher r -to- z transformation

TABLE 1. STUDY POPULATION

	All (N = 155)	Late-Onset Asthma (N = 76 [49%])	Early-Onset Asthma (N = 79 [51%])	P Value*
Median age (range), yr	40 (18–69)	47 (20–69)	30 (18–66)	
Sex, % female	73	76	69	0.3
Race, %				
White	63	70	57	0.04
Black	27	26	22	
Other	10	4	11	
BMI, mean (95% CI)	31 (29–32)	32 (29–34)	31 (28–32)	0.3
BMI categories				
Lean	28	29	26	0.9
Overweight	26	25	26	
Obese	46	45	47	
Mild to moderate asthma, %	51	49	54	
Severe asthma, %†	49	51	46	0.6
Age onset, mean (95% CI)	16 (14–19)	31 (28–32)	4 (3–6)	<0.001
Asthma duration, mean (95% CI), yr	23 (20–25)	16 (13–18)	29 (27–32)	<0.001
Atopy, %‡	84	42	59	<0.001
Inhaled steroids, %	89	93	84	0.08
High-dose inhaled steroids, %	47	47	45	0.8
LABA, %	73	81	65	0.02
Systemic steroids, %	30	28	20	0.2
FEV_1 , L, mean (95% CI)	2.2 (2.1–2.4)	2 (1.8–2.2)	2.4 (2.2–2.6)	0.01
$FEV_1\%$, mean (95% CI)	69 (66–73)	68 (63–73)	71 (66–76)	0.4
FVC, L, mean (95% CI)	3.2 (3.1–3.4)	3 (2.8–3.2)	3.4 (3.1–3.7)	0.01
FVC%, mean (95% CI)	83 (80–85)	81 (77–84)	83 (79–88)	0.3
FEV_1/FVC , mean (95% CI)	60 (66–70)	67 (63–70)	69 (66–72)	0.2
PC_{20} , mg/ml, median (IQR)	1 (0.4–4.7)	0.9 (0.3–4)	1.2 (0.5–5.9)	0.2
IgE, IU/ml, median (IQR)	137 (32–345)	57 (20–225)	173 (73–491)	<0.001
Sputum eosinophils, %, median (IQR)	1.2 (0.25–5.75)	3.3 (0.4–6.4)	0.7 (0.2–3.7)	0.2
L-arginine/ADMA**	118 (110–126)	108 (98–119)	127 (114–140)	0.02

Definition of abbreviations: ADMA = asymmetric dimethyl arginine; BMI = body mass index; CI = confidence interval; IQR = interquartile range; LABA = long-acting beta agonist; PC_{20} = methacholine dose at which an FEV_1 drop >20% from baseline occurred.

Bold indicates $P < 0.05$.

* P values represent comparisons between age of asthma onset phenotypes.

† Severe indicates patients meet American Thoracic Society severity classification.

‡ Atopy defined as having at least one positive (>3 mm) skin test reaction to allergens.

** Geometric mean (95% CI).

Figure 1. Distribution of the L-arginine/asymmetric dimethyl arginine (ADMA) ratio by body mass index categories in subjects with late- and early-onset asthma. Dashed line corresponds to the median L-arginine/ADMA for the entire study population (121.9). Bonferroni comparisons for late onset: obese to lean, $P = 0.003$; overweight to obese, $P = 0.2$; overweight to lean, $P = 0.4$. An outlier is identified as a black dot outside the box plots.

was used to compare correlation coefficients. The association between FE_{NO} with L-arginine, ADMA, and the L-arginine/ADMA ratio were also determined using a similar approach. The L-arginine/ADMA ratios were also compared across normal weight ($BMI \leq 25$), overweight ($25 < BMI < 30$), or obese ($BMI > 30$) subjects with asthma.

A multivariable linear regression analysis was used to determine the association between BMI and log-transformed FE_{NO} by age of asthma onset phenotypes. Similarly, a multivariable linear regression model was also fitted to determine the association between L-arginine/ADMA ratios with the lung volumes (FEV_1 in liters, $FEV_1\%$, FVC in liters, FVC%, and FEV_1/FVC), IgE, and the total AQLQ score. To determine whether or not lower L-arginine/ADMA ratios are associated with adverse health outcomes, a multivariable logistic analysis was modeled to determine the association between having a plasma L-arginine/ADMA ratio below the median distribution in each age of onset category, with healthcare use and respiratory symptoms defined as having occurred at least two times per week or more in the preceding 3 months before enrollment. Selection of model's covariables was done through backward elimination, based on having either a $P \leq 0.1$ or a change in the model's estimate greater than or equal to 10%. *A priori* covariables included: demographics, use of inhaled or systemic steroids, asthma severity phenotype, prebronchodilator FEV_1 , asthma duration, and atopy. An interaction term between the age of asthma onset groups or obesity was evaluated in the multivariable models. Statistical analyses were conducted using Stata 12.0 SE, (College Station, TX).

RESULTS

Study Population

The study population consisted of 155 adult subjects with asthma, with a median age of 40 years (range 18–70 yr), of whom 73% were women and 49% had severe asthma. A large proportion of the participants were obese (47%). The median age for asthma onset was 10 years (range, 0–59 yr), with median asthma duration of 22 years (range, 1–59 yr). Table 1 shows the study population divided into late- (≥ 12 yr of age) and early-onset asthma (< 12 yr of age) categories. In general, subjects with late-onset asthma were older and had a shorter duration of asthma. Subjects with late-onset asthma also had lower FEV_1 and FVC, were less atopic, and had lower IgE levels.

Plasma L-Arginine/ADMA Levels, BMI, and FE_{NO}

Subjects with late-onset asthma had a higher median plasma ADMA (late onset, $0.48 \mu M$ [interquartile range (IQR), 0.35 – 0.7] compared with early onset, $0.37 \mu M$ [IQR, 0.29 – 0.59]; $P = 0.01$) and lower median plasma L-arginine (late onset, 52.3 [IQR, 43 – 61] vs. early onset, $51 \mu M$ [IQR 39 – 66]; $P = 0.8$). The plasma L-arginine/ADMA was also lower in late onset ($n = 76$; median, 109 ; IQR, 81 – 138) compared with early onset ($n = 79$; median, 121 ; IQR, 94 – 178) ($P = 0.02$). L-Arginine/ADMA (log) was inversely correlated to BMI in the late-onset group ($r = -0.4$, $P = 0.0006$) and to a much lesser extent in the early-onset group ($r = -0.2$, $P = 0.07$). In addition, these correlation coefficients were significantly different from each other ($P < 0.001$). In the late-onset phenotype, the ratio of L-arginine/ADMA decreased in relation to the BMI categories (P for trend < 0.001), with obese subjects with late-onset asthma having significantly lower levels as compared with early-onset obese subjects (Figure 1). The L-arginine/ADMA ratio levels were not different across BMI categories in the early-onset phenotype.

The log-transformed ADMA, L-arginine, and L-arginine/ADMA were correlated with the log of FE_{NO} (respectively, $r = -0.20$, $P = 0.01$; $r = 0.32$, $P = 0.001$; $r = 0.39$, $P = 0.001$) for the entire study population. Compared with nonobese subjects with asthma, obese subjects had different ADMA- FE_{NO} correlations ($P = 0.001$) (respectively, $r = 0.02$, $P = 0.8$; $r = -0.4$, $P = 0.0006$) and L-arginine/ADMA- FE_{NO} correlations ($P = 0.01$) (respectively, $r = 0.23$, $P = 0.01$; $r = 0.53$, $P < 0.001$). However, there were no significant differences between these correlations across the age of asthma onset phenotypes (data not shown).

Multivariable Regression Analysis of FE_{NO} on BMI and Adjustment for Arginine/ADMA Ratio

After adjusting for current inhaled corticosteroid use, duration of asthma, and prebronchodilator FEV_1 , the log- FE_{NO} was inversely and linearly associated with BMI among late- ($\beta = -0.03$; 95% confidence interval [CI], -0.04 to -0.003 ; $P = 0.02$) but not early-onset asthma phenotypes ($\beta = -0.002$; 95% CI, -0.02 to 0.02 ; $P = 0.8$) (age of asthma onset interaction with BMI on FE_{NO} , $P = 0.03$) (Figure 2). However, after adjusting for plasma L-arginine/ADMA levels, this association in the late-onset phenotype was no longer significant ($\beta = -0.01$; 95% CI, -0.04 to 0.005 ; $P = 0.2$). In contrast, among the early-

Figure 2. Association between log-transformed exhaled nitric oxide and body mass index by age of asthma onset phenotype. Open circles and dashed line = early onset. Closed circles and continuous line = late onset. The interaction between BMI and age of asthma onset on the log-exhaled nitric oxide (eNO) was significant, with $P = 0.03$.

onset phenotype, adjusting for L-arginine/ADMA did not significantly change the FE_{NO} -BMI association (see Table E1 in the online supplement).

Multivariable Regression Analysis of Plasma L-Arginine/ADMA with Biomarkers of Allergic Inflammation

Among the late-onset phenotype, there was a linear association between L-arginine/ADMA and serum IgE ($\beta = 1.01$; 95% CI, 0.28–1.7; $P = 0.007$) after adjusting for high-dose inhaled steroids, systemic corticosteroid use (oral or injected), sex, atopy, and race. However, using the same covariables, there was no significant association among the subjects with early-onset asthma ($\beta = 0.4$; 95% CI, -0.4 to 1.1 ; $P = 0.4$) (Table E2). The interaction between L-arginine/ADMA with the age of asthma onset or obesity, respectively, was $P = 0.6$ and $P = 0.7$.

Multivariable Regression Analysis of Plasma L-Arginine/ADMA with Lung Volumes

Both $FEV_1\%$ and FVC% were linearly associated with the plasma L-arginine/ADMA in subjects with late-onset asthma after adjusting for inhaled steroid use, sex, atopy, years of asthma duration, and age (Figures 3 and 4). The age of asthma onset or obesity were significant interactions with the L-arginine/ADMA ratio on the $FEV_1\%$ model ($P = 0.04$ for both). This means that among the subjects with late-onset asthma, a lower L-arginine/ADMA ratio was associated with a lower $FEV_1\%$ (Figure 3) in contrast to the subjects with early-onset asthma, in whom this association was in the opposite direction. However, the interaction between obesity and L-arginine/ADMA revealed a distinct pattern. Among obese subjects with asthma, higher L-arginine/ADMA ratio was associated with lower $FEV_1\%$, whereas the opposite was observed for the nonobese (Figure E1). It is unclear why an opposite relationship was seen in the obesity–L-arginine/ADMA interaction on $FEV_1\%$. Given the cross-sectional nature of the study, it is possible that other factors associated with obesity are also determining this relationship. As shown in Figure 4, lower FVC% was associated with lower L-arginine/ADMA among the late-onset asthma phenotype, which was in contrast to the early-onset phenotype (interaction between age of asthma onset and L-arginine/ADMA on FVC%, $P = 0.01$); however, the interaction between obesity

Figure 3. Adjusted linear regression of $FEV_1\%$ with the log of L-arginine/asymmetric dimethyl arginine (ADMA) by age of asthma onset phenotype. Open circles and dashed line = early onset. Closed circles and continuous line = late onset. Interaction of L-arginine/ADMA and age of asthma onset on $FEV_1\%$ was significant, with $P = 0.04$.

Figure 4. Adjusted linear regression of FVC with the log of L-arginine/asymmetric dimethyl arginine (ADMA) by age of asthma onset phenotype. Open circles and dashed line = early onset. Closed circles and continuous line = late onset. Interaction of L-arginine/ADMA and age of asthma onset on FVC% was significant, with $P = 0.01$.

with L-arginine/ADMA was not significant ($P = 0.2$) (see Table E3 for regression analyses on other lung function parameters).

Multivariable Regression Analysis of Plasma L-Arginine/ADMA and Asthma Quality of Life

There was a significant association between plasma L-arginine/ADMA with the total average AQLQ score only in late-onset asthma after adjusting for $FEV_1\%$ predicted, use of inhaled or systemic steroids, and sex ($P = 0.007$). Figure 5 shows the adjusted linear slope of the regression model for early and late phenotypes. The interaction between the age of asthma onset or obesity with L-arginine/ADMA on AQLQ was, respectively, $P = 0.06$ and $P = 0.3$.

Multivariable Regression Analysis of Plasma L-Arginine/ADMA and Respiratory Symptoms and Healthcare Use

Figure 6 shows the odds ratios and 95% CI for having at least two respiratory symptoms/week or greater, in relation to a plasma L-arginine/ADMA ratio below the median in each age of onset category. Subjects with late-onset asthma with lower L-arginine/ADMA had increased odds for greater frequency of wheezing ($P < 0.001$), dyspnea ($P = 0.03$), and chest tightness ($P = 0.003$), while adjusting for age, sex, asthma severity, and prebronchodilator $FEV_1\%$. In the late-onset phenotype, the interaction between obesity and having an L-arginine/ADMA below the median distribution on wheezing was $P = 0.05$. No significant associations were observed for the early-onset phenotype. Having an L-arginine/ADMA below the median was not predictive of increased healthcare use (asthma-related hospitalizations, emergency department or intensive care unit admissions; data not shown) in either age of asthma onset phenotype.

DISCUSSION

In this cross-sectional study of subjects with moderate to severe asthma, increasing BMI was inversely associated with FE_{NO} in the late-onset phenotype; moreover, this association was partly

Figure 5. Adjusted linear regression of the total asthma quality of life questionnaire score with the log of L-arginine/asymmetric dimethyl arginine (ADMA) by age of asthma onset phenotype. Open circles and dashed line = early onset. Closed circles and continuous line = late onset. Interaction between the L-arginine/ADMA and age of asthma onset on Asthma Quality of Life Questionnaire (AQLQ) was not significant, with $P = 0.06$.

determined by the relative proportion of L-arginine to ADMA. Unlike the early-onset phenotype, lower L-arginine/ADMA ratios in the late-onset phenotype were significantly associated with reduced lung volumes, less allergic inflammation, increased frequency of respiratory symptoms, and poorer asthma-related quality of life, although interactions were not always significant.

By competing with L-arginine, ADMA uncouples all NOS isoforms, causing electrons flowing from the NADPH (nicotinamide adenine dinucleotide phosphate reduced) reductase domain to the oxygenase domain to be diverted into molecular oxygen rather than to L-arginine (19). Uncoupling shifts NOS from producing NO to preferentially making superoxide (Figure 7), which can lead to the formation of nitrogen oxide radicals (20). In murine models, ADMA levels correlate with peroxynitrate formation (9). Furthermore, in stimulated murine airway epithelial cells with LPS, IFN- γ , and tumor necrosis factor- α , Wells and colleagues observed that administration of ADMA reduced nitrite production while increasing superoxide levels in a dose-dependent manner (19). Continuous ADMA infusion for 2 weeks also increased airway resistance and reduced lung compliance *in vivo* in mice. This increased airway resistance was attributed to reduced NO bioavailability. Interestingly, these findings occurred in the absence of increases in traditional biomarkers of allergic airway inflammation (21). Subjects with asthma have been reported to have higher airway ADMA levels compared with healthy control subjects and to be inversely correlated with exhaled NO ($r = -0.5$; $P < 0.01$) (10), suggesting ADMA can reduce NO airway bioavailability. In a prior report of serum arginine bioavailability in this cohort, subjects with asthma were found to have alterations in methylated forms of arginine, yet still maintained greater arginine bioavailability as compared with healthy individuals. Arginine bioavailability was related to airflow obstruction in subjects with severe asthma, supporting the concept of a severe asthma metabolic phenotype (18).

Obesity, with or without the metabolic syndrome, has been associated with higher plasma ADMA levels, and concomitantly asthma has been linked to having lower L-arginine plasma levels (12, 18, 22, 23); it is therefore possible that the combination of these conditions synergistically results in even lower L-arginine/

ADMA ratios. Therefore, as BMI increases in subjects with asthma, a reduced L-arginine/ADMA results in greater airway epithelial iNOS uncoupling. This may explain why several studies have found lower FE_{NO} levels in obese subjects with asthma and potentially why higher BMI has been associated with greater airway oxidative stress (1–4). This mechanism may be potentially constrained to the airways, as biomarkers of systemic oxidative stress have been found not to explain the obesity–asthma association (24). Alternatively, higher arginase expression and activity, which have been respectively described in association with obesity or asthma, could also contribute to obese subjects with asthma having lower FE_{NO} (18, 25).

NO is constitutively produced in the airway epithelium and plays a role in maintaining airway patency (26, 27); therefore, reduced NO bioavailability could potentially lead to increased airway resistance, which may explain why lower L-arginine/ADMA was strongly associated with lower lung volumes, wheezing, tightness, and dyspnea in the late-onset phenotype. Why these relationships are not seen in the early-onset phenotype is unclear; however, one possibility may be that other mechanisms (i.e., greater Th2-driven inflammation/remodeling) affect lung function and respiratory symptoms to a greater degree than changes in L-arginine/ADMA. However, the observation that this ratio does not similarly impact the early-onset phenotype argues against a purely correlative association with obesity itself. The results of a recent study by Sutherland and colleagues further support our results by showing that among two obese asthma phenotype clusters, the ones with the lowest eNO levels were also those with later onset asthma and lower IgE levels (28).

These results have significant clinical implications. If L-arginine/ADMA-mediated changes in NO metabolism are in fact a mechanistic pathway between obesity and late-onset asthma, it could lead to new phenotype-specific therapeutic options. For example, L-arginine, which can prevent ADMA-mediated NOS uncoupling, has been shown to increase FE_{NO} in subjects with asthma and could have therapeutic potential (9, 29–32). Similar studies in the cardiovascular literature have also shown that using L-arginine is an effective way to reduce ADMA–NOS uncoupling, leading to improvements in endothelial NO production and function (33, 34). Although L-arginine supplementation has been shown to induce modest improvements in lung function (35), this level of clinical effectiveness

Figure 6. Association between having an L-arginine/asymmetric dimethyl arginine (ADMA) below the median distribution with the odds of having increased respiratory symptom frequency, by age of asthma phenotype. * $P < 0.001$; † $P < 0.05$; ‡ $P < 0.01$. The interaction between L-arginine/ADMA and obesity on increased wheezing was $P = 0.05$, only for the late-onset phenotype.

Figure 7. Schematic representation of L-arginine–nitric oxide (NO) metabolism and NO synthase (NOS) uncoupling by asymmetric dimethyl arginine (ADMA). ADMA is one of three methylated analogs of L-arginine occurring through posttranslational modification; however, ADMA is the only one that can competitively inhibit all NOS isoforms. ADMA is synthesized from L-arginine by protein-arginine methyltransferases (PRMT) and degraded into mono- or dimethylamine and citrulline by dimethylarginine dimethylaminohydrolase (DDAH), whose activity can be reduced in obesity and the metabolic syndrome (41). Citrulline can be subsequently recycled into L-arginine (11). By competing with L-arginine, ADMA uncouples NOS, causing electrons flowing from the NADPH (nicotinamide adenine dinucleotide phosphate reduced) reductase domain to the oxygenase domain to be diverted into molecular oxygen rather than to L-arginine (19). Under uncoupling conditions, NOS generates superoxide, which correlates with airway oxidative stress in murine ovalbumin models (9).

may be due to its extensive first-pass intestinal and hepatic metabolism, which may limit achieving adequate therapeutic levels (36). L-Citrulline has been shown to more effectively raise L-arginine levels and could therefore offer an attractive alternative supplementation (36). Additionally, data from this study would argue that this approach would only improve the health of obese subjects with late-onset asthma, a group not selected for previous studies.

Several limitations should be considered when interpreting the results from this study. This is a cross-sectional analysis, and therefore a causative relationship cannot be established. In addition, the study population was composed of subjects for whom SARP centers provided samples for ADMA and L-arginine quantification. Subsequent subjects from Pittsburgh were added based on availability of banked biospecimens; therefore, this is not a randomly selected population. However, it is unlikely that the selection of the study population was systematically biased on BMI or age of asthma onset. Plasma ADMA levels may not be representative of the airway levels; however, the lung has been shown to a significant source of ADMA and an important contributor to plasma levels (37). This would suggest that plasma levels could potentially inform lung levels. Given the limited sample size and roughly similar sex distribution between age of asthma onset phenotypes, we were unable to perform an analysis by sex. However, in the multivariable analysis, female sex was not found to be a strong confounder. Although a significant interaction between obesity and age of asthma onset was observed in the relation of FEV₁% and L-arginine/ADMA, it is unclear why this interaction was in the opposite direction with obesity. It may potentially imply that other factors confound this association. Further studies are needed to determine the effects of L-arginine/ADMA on FEV₁% across BMI categories. Also, given the lack of results from healthy control subjects, it is not possible to determine the relative contribution of obesity versus asthma on the L-arginine/ADMA balance and NO metabolism in relation to the outcomes presented on this study.

In conclusion, we have identified a potential mechanism involving NO metabolism that may biologically explain how obesity leads to asthma and its severity in late onset disease. Although further mechanistic studies are required, these results could have important therapeutic implications. Clinical trials with L-citrulline or L-arginine to restore airway NO metabolism are needed to determine whether they can improve asthma control in a specific asthma phenotype (38–40).

Author disclosures are available with the text of this article at www.atsjournals.org.

References

- Komakula S, Khatri S, Mermis J, Savill S, Haque S, Rojas M, Brown L, Teague GW, Holguin F. Body mass index is associated with reduced exhaled nitric oxide and higher exhaled 8-isoprostanes in asthmatics. *Respir Res* 2007;8:32.
- Berg CM, Thelle DS, Rosengren A, Lissner L, Torén K, Olin AC. Decreased fraction of exhaled nitric oxide in obese subjects with asthma symptoms: data from the population study INTERGENE/ADONIX. *Chest* 2011;139:1109–1116.
- Barros R, Moreira A, Fonseca J, Moreira P, Fernandes L, de Oliveira JF, Delgado L, Castel-Branco MG. Obesity and airway inflammation in asthma. *J Allergy Clin Immunol* 2006;117:1501–1502.
- Liu L, Teague WG, Erzurum S, Fitzpatrick A, Mantri S, Dweik RA, Bleeker ER, Meyers D, Busse WW, Calhoun WJ, et al. Determinants of exhaled breath condensate pH in a large population with asthma. *Chest* 2011;139:328–336.
- Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, D'Agostino R Jr, Castro M, Curran-Everett D, Fitzpatrick AM, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *Am J Respir Crit Care Med* 2010;181:315–323.
- Halder P, Pavord ID, Shaw DE, Berry MA, Thomas M, Brightling CE, Wardlaw AJ, Green RH. Cluster analysis and clinical asthma phenotypes. *Am J Respir Crit Care Med* 2008;178:218–224.
- Holguin F, Bleeker ER, Busse WW, Calhoun WJ, Castro M, Erzurum SC, Fitzpatrick AM, Gaston B, Israel E, Jarjour NN, et al. Obesity and asthma: an association modified by age of asthma onset. *J Allergy Clin Immunol* 2011;127:1486–1493.e2.
- Dixon AE, Pratley RE, Forgiione PM, Kaminsky DA, Whittaker-Leclair LA, Griffes LA, Garudathri J, Raymond D, Poynter ME, Bunn JY, et al. Effects of obesity and bariatric surgery on airway hyperresponsiveness, asthma control, and inflammation. *J Allergy Clin Immunol* 2011;128:508–515.e1–2.
- Ahmad T, Mabalirajan U, Ghosh B, Agrawal A. Altered asymmetric dimethyl arginine metabolism in allergically inflamed mouse lungs. *Am J Respir Cell Mol Biol* 2010;42:3–8.
- Scott JA, North ML, Rafii M, Huang H, Pencharz P, Subbarao P, Belik J, Grasemann H. Asymmetric dimethylarginine is increased in asthma. *Am J Respir Crit Care Med* 2011;184:779–785.
- Tran CT, Leiper JM, Vallance P. The DDAH/ADMA/NOS pathway. *Atheroscler Suppl* 2003;4:33–40.
- McLaughlin T, Stuhlinger M, Lamendola C, Abbasi F, Bialek J, Reaven GM, Tsao PS. Plasma asymmetric dimethylarginine concentrations are elevated in obese insulin-resistant women and fall with weight loss. *J Clin Endocrinol Metab* 2006;91:1896–1900.
- Holguin F, Khatri S, Serpil E, Powers RW, Trudeau J, Wenzel SE. Reduced L-arginine/ADMA as a potential mechanism to explain increased symptom severity and reduced atopy in late onset obese asthmatics. *Am J Respir Crit Care Med* 2012;185:A2197.
- Miranda C, Busacker A, Balzar S, Trudeau J, Wenzel SE. Distinguishing severe asthma phenotypes: role of age at onset and eosinophilic inflammation. *J Allergy Clin Immunol* 2004;113:101–108.
- Juniper EF, Svensson K, Mork AC, Stahl E. Measuring health-related quality of life in adults during an acute asthma exacerbation. *Chest* 2004;125:93–97.
- Teerlink T, Nijveldt RJ, de Jong S, van Leeuwen PA. Determination of arginine, asymmetric dimethylarginine, and symmetric dimethylarginine in human plasma and other biological samples by high-performance liquid chromatography. *Anal Biochem* 2002;303:131–137.

17. Speer PD, Powers RW, Frank MP, Harger G, Markovic N, Roberts JM. Elevated asymmetric dimethylarginine concentrations precede clinical preeclampsia, but not pregnancies with small-for-gestational-age infants. *Am J Obstet Gynecol* 2008;198:112.e1–7.
18. Lara A, Khatri SB, Wang Z, Comhair SA, Xu W, Dweik RA, Bodine M, Levison BS, Hammel J, Bleeker E, *et al.* Alterations of the arginine metabolome in asthma. *Am J Respir Crit Care Med* 2008;178:673–681.
19. Wells SM, Holian A. Asymmetric dimethylarginine induces oxidative and nitrosative stress in murine lung epithelial cells. *Am J Respir Cell Mol Biol* 2007;36:520–528.
20. Antoniadou C, Shirodaria C, Leeson P, Antonopoulos A, Warrick N, Van-Assche T, Cunningham C, Tousoulis D, Pillai R, Ratnatunga C, *et al.* Association of plasma asymmetrical dimethylarginine (ADMA) with elevated vascular superoxide production and endothelial nitric oxide synthase uncoupling: implications for endothelial function in human atherosclerosis. *Eur Heart J* 2009;30:1142–1150.
21. Wells SM, Buford MC, Migliaccio CT, Holian A. Elevated asymmetric dimethylarginine alters lung function and induces collagen deposition in mice. *Am J Respir Cell Mol Biol* 2009;40:179–188.
22. Kocak H, Oner-Iyidogan Y, Gurdol F, Oner P, Esin D. Serum asymmetric dimethylarginine and nitric oxide levels in obese postmenopausal women. *J Clin Lab Anal* 2011;25:174–178.
23. Palomo I, Contreras A, Alarcon LM, Leiva E, Guzman L, Mujica V, Icaza G, Diaz N, Gonzalez DR, Moore-Carrasco R. Elevated concentration of asymmetric dimethylarginine (ADMA) in individuals with metabolic syndrome. *Nitric Oxide* 2011;24:224–228.
24. Sood A, Steffes M, Smith L, Holvoet P, Thyagarajan B, Jacobs D. Obesity-asthma association: is it explained by systemic oxidant stress? *Chest* 2009;136:1055–1062.
25. Kim OY, Lee SM, Chung JH, Do HJ, Moon J, Shin MJ. Arginase I and the very low-density lipoprotein receptor are associated with phenotypic biomarkers for obesity. *Nutrition* 2012;28:635–639.
26. Kacmarek RM, Ripple R, Cockrill BA, Bloch KJ, Zapol WM, Johnson DC. Inhaled nitric oxide. A bronchodilator in mild asthmatics with methacholine-induced bronchospasm. *Am J Respir Crit Care Med* 1996;153:128–135.
27. Sanna A, Kurtansky A, Veriter C, Stanescu D. Bronchodilator effect of inhaled nitric oxide in healthy men. *Am J Respir Crit Care Med* 1994;150:1702–1704.
28. Sutherland ER, Goleva E, King TS, Lehman E, Stevens AD, Jackson LP, Stream AR, Fahy JV, Leung DY. Cluster analysis of obesity and asthma phenotypes. *PLoS ONE* 2012;7:e36631.
29. Abuzayan I, Turner SW. Changes in exhaled nitric oxide after ingestion of L-arginine in children: a pilot study. *Pediatr Pulmonol* 2010;45:236–240.
30. Mabalirajan U, Ahmad T, Leishangthem GD, Joseph DA, Dinda AK, Agrawal A, Ghosh B. Beneficial effects of high dose of L-arginine on airway hyperresponsiveness and airway inflammation in a murine model of asthma. *J Allergy Clin Immunol* 2010;125:626–635.
31. Kharitonov SA, Lubec G, Lubec B, Hjelm M, Barnes PJ. L-arginine increases exhaled nitric oxide in normal human subjects. *Clin Sci (Lond)* 1995;88:135–139.
32. Mansoor JK, Morrissey BM, Walby WF, Yoneda KY, Juarez M, Kajekar R, Severinghaus JW, Eldridge MW, Schelegle ES. L-arginine supplementation enhances exhaled NO, breath condensate VEGF, and headache at 4,342 m. *High Alt Med Biol* 2005;6:289–300.
33. Ochiai M, Hayashi T, Morita M, Ina K, Maeda M, Watanabe F, Morishita K. Short-term effects of L-citrulline supplementation on arterial stiffness in middle-aged men. *Int J Cardiol* 2012;155:257–261.
34. Boger RH. Asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, explains the “L-arginine paradox” and acts as a novel cardiovascular risk factor. *J Nutr* 2004;134:2842S–2847S; discussion 2853S.
35. de Gouw HW, Verbruggen MB, Twiss IM, Sterk PJ. Effect of oral L-arginine on airway hyperresponsiveness to histamine in asthma. *Thorax* 1999;54:1033–1035.
36. Schwedhelm E, Maas R, Freese R, Jung D, Lukacs Z, Jambrecina A, Spickler W, Schulze F, Boger RH. Pharmacokinetic and pharmacodynamic properties of oral L-citrulline and L-arginine: impact on nitric oxide metabolism. *Br J Clin Pharmacol* 2008;65:51–59.
37. Bulau P, Zakrzewicz D, Kitowska K, Leiper J, Gunther A, Grimminger F, Eickelberg O. Analysis of methylarginine metabolism in the cardiovascular system identifies the lung as a major source of ADMA. *Am J Physiol Lung Cell Mol Physiol* 2007;292:L18–L24.
38. Adcock IM, Barnes PJ. Molecular mechanisms of corticosteroid resistance. *Chest* 2008;134:394–401.
39. Barnes PJ. Corticosteroid resistance in airway disease. *Proc Am Thorac Soc* 2004;1:264–268.
40. Barnes PJ, Ito K, Adcock IM. Corticosteroid resistance in chronic obstructive pulmonary disease: Inactivation of histone deacetylase. *Lancet* 2004;363:731–733.
41. Lee JH, Park GH, Lee YK, Park JH. Changes in the arginine methylation of organ proteins during the development of diabetes mellitus. *Diabetes Res Clin Pract* 2011;94:111–118.