Disponibilité réduite de la L-Arginine chez les patients asthmatiques

"Decreased arginine bioavailability and increased serum arginase activity in asthma."
Claudia R. et al. (Am J Respir Cri Care Med), publié le 7 avril 2004

L'inflammation joue un rôle central dans la pathogenèse de l'asthme. L'oxyde nitrique (NO) est l'un des médiateurs inflammatoires qui retient de plus en plus d'attention. **Le NO est produit à partir de la L-Arginine** (par la synthase NO) et est **un vasodilatateur important de la circulation bronchique**, avec des propriétés bronchodilatatrices et anti-inflammatoires.

La recherche scientifique suggère qu'une **disponibilité réduite de NO peut être à la base de l'asthme**. Cela peut être dû à une activité pathologiquement augmentée de l'enzyme arginase, qui **convertit la L-Arginine en ornithine et entrave ainsi la réserve disponible pour la synthèse de NO**.

Il existe déjà de nombreuses recherches mesurant la concentration de NO expiré en tant que paramètre de l'inflammation des voies respiratoires dans l'asthme. Dans cette étude, cependant, **les taux sanguins de L-Arginine et l'activité de l'arginase ont été étudiés** vu que la biodisponibilité réduite de l'arginine peut jouer un rôle dans la physiopathologie de l'asthme.

Résultats et Conclusion:

Les chercheurs ont observé une diminution significative des niveaux de L-Arginine chez les patients asthmatiques. Les mesures ont montré que les **patients asthmatiques n'avaient que la moitié de la quantité de L-Arginine dans le sang par rapport au groupe contrôle** (45 vs 94, p<0.0001, Image 1A). De plus, l'absorption de L-Arginine par les cellules était moins efficace, ce qui rend une **biodisponibilité relative encore plus faible de L-Arginine chez les patients asthmatiques** (0.30 vs 0.42, p<0.005).

D'autres recherches montrent que les **concentrations sériques d'arginase chez les patients asthmatiques sont 3 fois plus élevées que dans le groupe contrôle** (1.62 µmol/ml/h vs 0.51 µmol/ml/h, p<0.0001, Image 1B). Les niveaux de NO expirés étaient également significativement plus élevés chez les patients asthmatiques, ce qui est cohérent avec les données publiées précédemment.

Ces résultats indiquent que les **patients asthmatiques sont accablés par une carence systémique en L-Arginine**. Le fait que ces carences ne se limitent pas aux poumons suggère que les conséquences de ces carences peuvent également survenir dans d'autres parties du corps et que les **patients asthmatiques peuvent bénéficier d'une composante nutritionnelle dans leur traitement, comme la supplémentation en L-Arginine**.

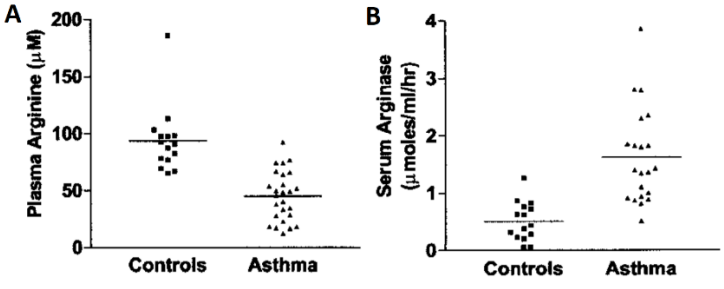

Image 1 | Disponibilité réduite de la L-Arginine chez les patients asthmatiques. (A) Les concentrations sanguines de L-Arginine sont significativement réduites chez les patients asthmatiques (p<0.0001). (B) Les taux de l'enzyme arginase sont plus de trois fois supérieurs chez les patients asthmatiques par rapport au groupe de contrôle (p<0.0001).

Kasteelhoek 12
B-8730 Beernem
T +32 50 59 88 81
F +32 50 70 56 49
info@pures.be
www.pures.be

Decreased Arginine Bioavailability and Increased Serum Arginase Activity in Asthma

Claudia R. Morris, Mirjana Poljakovic, Lisa Lavrisha, Lorenzo Machado, Frans A. Kuypers, and Sidney M. Morris, Jr.

Department of Emergency Medicine and Pediatric Clinical Research Center, Children's Hospital and Research Center at Oakland, Oakland; Children's Hospital Oakland Research Institute, Oakland, CA; and Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA

Recent studies suggest that a nitric oxide (NO) deficiency and elevated arginase activity may play a role in the pathogenesis of asthma. Although much attention has been directed toward measurements of exhaled NO in asthma, no studies to date have evaluated levels of plasma arginase or arginine, the substrate for NO production, in patients with asthma. This study, therefore, measured amino acid levels, arginase activity, and nitric oxide metabolites in the blood of patients with asthma, as well as NO in exhaled breath. Although levels of virtually all amino acids were reduced, patients with asthma exhibited a striking reduction in plasma arginine levels compared with normal control subjects without asthma (45 ± 22 vs. 94 ± 29 μM, $p < 0.0001$), and serum arginase activity was elevated (1.6 ± 0.8 vs. 0.5 ± 0.3 μmol/ml/hour, asthma vs. control, $p < 0.0001$). High arginase activity in patients with asthma may contribute to low circulating arginine levels, thereby limiting arginine bioavailability and creating a NO deficiency that induces hyperreactive airways. Addressing the alterations in arginine metabolism may result in new strategies for treatment of asthma.

Keywords: asthmatic; L-arginine; amino acids; nitric oxide; nitric oxide synthase

Inflammation plays a central role in the pathogenesis of asthma (1, 2), a major disease that is characterized by a variable degree of airflow obstruction, bronchial hyperresponsiveness, and airway remodeling (1, 3, 4). Much of the inflammation can be attributed to helper T cell type 2 cytokine activation (5, 6), the degree of which strongly correlates to disease severity (7). One of the inflammatory mediators that has received considerable attention in asthma is nitric oxide (NO), which is produced from arginine by NO synthases (8). NO is an important vasodilator of the bronchial circulation (9), with both bronchodilatory (10–12) and antiinflammatory properties (13). Recent studies suggest that asthma may be a condition of decreased NO bioavailability (14–18), rather than overproduction as a result of inflammation (19–22). This may occur in part as a result of pathologically elevated activity of arginase (23–25), an enzyme that hydrolyzes arginine to ornithine and urea. Arginase expression is induced by helper T cell type 2 cytokines (26–29), making it a potentially important enzyme to study in asthma. As both arginase and NO synthase use arginine as a common substrate, arginase may play a role in regulating NO synthesis by modulating arginine availability (27–29). In addition, arginase generates ornithine that can serve as a precursor for synthesis of proline and polyamines (30), which can contribute to airway remodeling in chronic asthma by supporting collagen synthesis and cell proliferation, respectively (16, 25). Increased arginase activity or expression has been reported in lungs of guinea-pig (24) and murine (25) models of allergic asthma, and in lungs of individuals with asthma (25). The recent discovery of "asthma signature genes" through microarray analysis identified overexpression of several genes involved in arginine metabolism, including arginase (25), thus, supporting a role for arginase and its metabolic products in asthma.

Much attention has been directed toward measurements of exhaled NO in asthma, as it is widely regarded as a marker of airway inflammation, with high levels found in both children and adults with asthma (21, 22, 31–33). NO synthase-dependent generation of S-nitrosothiols, potentially the largest pool of NO bioactivity in the lung (34, 35), has also been studied in asthma as a noninvasive biomarker of nitrosative stress (36, 37). However, no studies of individuals with asthma to date have evaluated circulating levels of arginine, the substrate for NO synthase, together with arginese activity. Since decreased arginine bioavailability may play a role in the pathophysiology of asthma by contributing to an endogenous NO deficiency, we measured amino acid levels and arginase activity in the blood of patients with asthma, as well as NO in exhaled breath. Our results support a role for reduced arginine bioavailability and increased arginase activity in asthma.

Some results of this study have been previously reported in abstract form (38, 39), and some of the control data were included among control data in another manuscript (40).

METHODS

Patients

Twenty-six patients with a history of asthma in varying stages of exacerbation were enrolled in the study. Asthma was defined by a past history of at least three episodes of wheezing. An asthma exacerbation was characterized by a worsening of asthma symptoms, which included one or more of the following: wheezing, tachypnea, shortness of breath, cough, retractions, and/or hypoxia. No patients were on systemic steroids at the time of enrollment, and no patients were receiving intravenous crystalloid fluid at the time of the study blood draw. Only 5 of 26 patients were using controller medication, four of whom were on a regimen of daily inhaled steroid (fluticasone propionate). Two of these patients were also on the leukotriene inhibitor montelukast sodium, one of whom had a history of a previous admission to the intensive care unit and intubation for life-threatening asthma. One patient was on montelukast monotherapy, and all patients reported using albuterol as a rescue medication within 24 hours of presentation.

Treatment at the time of enrollment varied from albuterol administration via meter dose inhaler and use of inhaled steroids, to continuous albuterol nebulization (5 to 20 mg/hour), nebulized ipatropium bromide (0.25–0.5 mg), and oral steroids (2 mg/kg prednisolone) as clinically warranted. Patients not tolerating oral prednisolone due to emesis or respiratory distress received intravenous methylprednisolone (2 mg/kg)

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Correspondence and requests for reprints should be addressed to Claudia R. Morris, M.D., Department of Emergency Medicine, Children's Hospital and Research Center at Oakland, 747 52nd Street, Oakland, CA 94609. E-mail: claudiamorris@comcast.net

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or intravenous dexamethasone (0.3–0.5 mg/kg). All patients admitted to the hospital received systemic steroids and at least 2 hours of continuous nebulized albuterol in the emergency department. Steroids were continued throughout the hospital admission, and albuterol was used as needed. One patient decompensated shortly after arriving on the ward and was subsequently transferred to the intensive care unit. The mean age was 14 ± 12 years, with a median age of 10 years, and a range of 2 to 52 years. Fourteen patients were male, and 16 patients were admitted to the hospital for status asthmaticus. Four families agreed to daily venipuncture during hospitalization. Fifteen individuals without asthma were enrolled as normal control subjects for comparison. Patients with a history of allergies, atopy, nocturnal cough, coughing with upper respiratory infections, or any chronic medical condition were excluded from participation as a normal control. The mean age of normal control subjects, nine of whom were male, was 14 ± 9 years, with a median age of 12 years and a range of 2 to 34 years. All control subjects provided blood samples and 14 subjects provided exhaled NO samples. The study protocol was approved by the Institutional Review Board at Children's Hospital and Research Center at Oakland, and informed consent was obtained for all patients.

Study Design

Patients at least two years of age with asthma, defined by a past history of three or more episodes of wheezing, were recruited for this study from the Emergency Department or Pediatric Clinical Research Center at Children's Hospital and Research Center at Oakland during an acute exacerbation of symptoms. Patients enrolled had a range of disease activity and degree of exacerbation, from mild to severe. Treatment was based on the severity of symptoms, and was not altered by enrollment in this study. Clinical improvement was characterized by the ability to wean from continuous nebulized albuterol to intermittent treatments at least three hours apart, normalized oxygen saturation on room air, and/or improved clinical respiratory status warranting discharge from the hospital. Exhaled NO samples were obtained in triplicate from all patients mature enough to properly perform the maneuver described below, or whose respiratory distress did not prohibit an accurate sample (n = 22). Blood was collected for determinations of amino acids, arginase activity, and NO metabolite levels. Daily levels were followed in consenting patients admitted to the hospital. Information on signs, symptoms, asthma triggers, and medication use was obtained on patients by the study nurse. Routine asthma care was not modified as a result of study participation.

Amino Acid Measurement

Plasma amino acids were quantified at the Molecular Structure Facility, University of California, Davis, California. Proteins were removed from plasma samples by precipitation with sulfosalicylic acid. Plasma amino acids were separated by ion exchange chromatography on a Beckman model 6300 amino acid analyzer (Beckman, Fullerton, CA) using a lithium citrate buffer system, and quantified by a post-column ninhydrin detection system, using methods recommended by the manufacturer.

Arginase Activity

Arginase activity was determined as the conversion of [^{14}C]guanidino-**1**-arginine to [^{14}C]urea, which was converted to $^{14}CO_2$ by urease and trapped as $Na_2{}^{14}CO_3$ for scintillation counting, as described previously (41). Briefly, 50À aliquots of serum samples were incubated for 10 minutes at 55°C in complete assay mixtures lacking arginine. The reaction was initiated by addition of labeled arginine, and incubation was continued at 37°C for 2 hours. The reaction was terminated by heating at 100°C for 3 minutes. Samples were incubated with urease at 37°C for 45 minutes, and $Na_2{}^{14}CO_3$ was trapped on NaOH-soaked filters following acidification of the samples with HCl to volatilize the $^{14}CO_2$.

NO Metabolite Measurement

The formation of NO metabolites was measured by determination of its stable end products in serum; nitrite (NO_2^-) and nitrate (NO_3^-) in J.mol/liter, according to manufacturer's instructions using Sievers NOAnalysis software for liquid sampling (Sievers Instruments, Boulder, CO), as previously described (42).

Exhaled NO

NO was measured in exhaled air, using microprocessor-based chemiluminescent NO_x analytical instrumentation (Sievers Instruments, Inc.), as previously described (43, 44). Subjects inhaled to total lung capacity, and then exhaled to residual volume into the Teflon tube, which enters into a 1.5 liter Mylar balloon (Sievers Instruments, Inc.) at a pressure of +20 mm Hg. Exhalation at this expiratory pressure without a nose clip is a maneuver that closes the velum of the posterior nasopharynx and excludes contamination by nasal NO (45). Balloons are then connected to the Sievers NO Analyzer to measure the content of NO in the trapped air sample by luminescence as above. The results are expressed as ppb. Triplicate balloon samples were obtained from each patient. Time between collection and measurement did not affect results up to a 24-hour period. The mean ± SD was determined for each set of three balloons.

Statistical Analysis

Results are expressed as means ± SD. The unpaired and paired Student's *t* test and Pearson correlation were used when appropriate to evaluate statistical significance. A p value $\leq$ 0.05 was considered statistically significant (46).

RESULTS

Amino Acid Levels

Reductions occurred in plasma levels of many amino acids in patients with asthma experiencing an acute exacerbation of respiratory symptoms (Table 1). Strikingly, the greatest decrease was in plasma levels of arginine, which were approximately half those of normal control subjects (45 ± 22 J.M vs. 94 ± 29 J.M; p < 0.0001) (Figure 1A).

As arginine, ornithine, and lysine are taken up by cells via the same y^+ transport system (47), the ratio of arginine to ornithine plus lysine, i.e., arginine/ornithine + lysine, provides an index of relative arginine availability at any given plasma arginine

TABLE 1. PLASMA AMINO ACIDS IN NORMAL CONTROLS VERSUS PATIENTS WITH ASTHMA

	Concentration (J.M)			
Amino Acid	Controls (n = 15)	Asthma (n = 26)	% Control	p Value
Ornithine	64 ± 21	49 ± 24	77	NS
Citrulline	30 ± 6	21 ± 10	70	0.002
Proline	195 ± 66	144 ± 73	74	0.03
Hydroxyproline	29 ± 14	19 ± 9	66	0.02
Lysine	162 ± 33	112 ± 57	69	0.004
Glutamic Acid	55 ± 29	40 ± 16	73	0.04
Glutamine	554 ± 86	466 ± 148	84	0.04
Glycine	251 ± 64	186 ± 103	74	0.03
Alanine	369 ± 104	292 ± 96	79	0.02
Valine	223 ± 52	161 ± 51	72	< 0.001
Aspartic Acid	9 ± 6	7 ± 1	78	0.04
Threonine	136 ± 29	99 ± 58	73	0.02
Isoleucine	66 ± 20	48 ± 23	73	0.01
Leucine	126 ± 32	96 ± 45	76	0.03
Tyrosine	72 ± 15	52 ± 20	72	0.002
Histidine	75 ± 10	57 ± 20	79	0.003
Cysteine	22 ± 13	20 ± 16	90	NS
Asparagine	35 ± 15	41 ± 18*	118	NS
Serine	107 ± 32	89 ± 64	83	NS
Tryptophan	45 ± 10	37 ± 15	82	NS
Methionine	25 ± 6	20 ± 13	80	NS
Phenylalanine	57 ± 13	56 ± 17	98	NS

Definition of abbreviation: NS = not significant.

Concentrations of amino acids are expressed as means ± SD.

% control values are the percent of the control for the asthma group.

* n = 25.

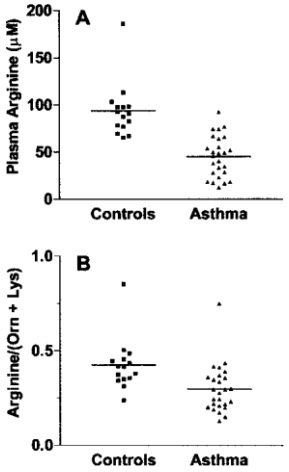

Figure 1. Plasma arginine concentration and relative arginine availability in normal control subjects compared with patients with asthma. (*A*) Plasma arginine concentration (J.M) was low in patients with asthma experiencing an acute exacerbation (n = 26) compared with normal control subjects without asthma (n = 15; p < 0.0001). (*B*) Values of relative arginine availability, expressed as plasma arginine/ornithine + lysine, in normal control subjects and patients with asthma differed significantly (p < 0.05). Mean values are indicated by the *horizontal lines*.

concentration. Relative arginine availability also was significantly lower in patients with asthma compared with normal control subjects (0.30 ± 0.13 vs. 0.42 ± 0.14, p < 0.005) (Figure 1B), further limiting arginine availability in the asthma group.

Plasma levels of ornithine (Table 1), a product of arginine catabolism, were generally lower in patients with asthma relative to control subjects, and relative ornithine availability, ornithine/arginine + lysine, was somewhat higher in patients with asthma than in control subjects (0.25 ± 0.07 for control subjects and 0.34 ± 0.17 for patients with asthma), but neither of these trends reached statistical significance. On the other hand, citrulline, the precursor of endogenous arginine synthesis, was significantly reduced in patients with asthma relative to normal control subjects (Table 1), possibly contributing to the decrease in plasma arginine levels in these patients.

Arginase Activity

Serum arginase activity was increased significantly in patients with asthma versus normal control subjects (1.62 ± 0.83 J.mol/ml/hour vs. 0.51 ± 0.34 J.mol/ml/hour, p < 0.0001) (Figure 2) Although plasma arginine levels declined and plasma arginase activities increased in patients with asthma as a group, no significant correlations between arginase activity and arginine, ornithine, or other amino acids were identified when values for individual patients were analyzed.

NO

Consistent with previously published data (21, 22, 31–33, 44), exhaled NO levels in patients with asthma were significantly higher than those of normal control subjects (36 ± 18 ppb, n = 22 vs. 16 ± 8 ppb, n = 14; p < 0.001), despite the paradoxical decline in plasma arginine levels. There was no significant difference in NO metabolites in the serum of patients with asthma versus normal control subjects (29.8 ± 27 J.M, n = 23 vs. 27.2 ± 9 J.M, n = 13).

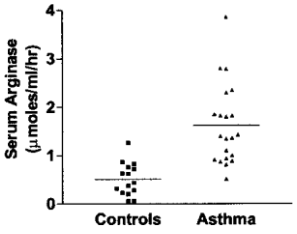

Figure 2. Serum arginase activities (J.mol/ml/hour) in normal control subjects (n = 15) and patients with asthma (n = 21). Values are significantly different (p < 0.0001). Mean values are indicated by the *horizontal lines*.

Admission versus Discharge

Ten patients were acutely treated and managed as outpatients, and 16 patients were admitted to the hospital for respiratory distress and status asthmaticus. Both groups had similar arginine, ornithine, and arginase activity levels. Proline concentrations were significantly lower in the group of patients with asthma admitted to the hospital versus those discharged to home (120 ± 79 J.M vs. 182 ± 46 J.M, p = 0.04). All other amino acids measured, except cysteine (12 ± 13 J.M, n = 12 vs. 31 ± 14 J.M, n = 9; p = 0.005) and lysine (93 ± 52 J.M, n = 16 vs. 144 ± 52 J.M, n = 10; p = 0.02) (admit vs. discharge) were similar in both groups.

Sequential amino acid data was obtained on four of the admitted patients. Plasma arginine levels were low on the day of admission and consistently increased by discharge from the hospital in all patients (Figure 3A). There were no consistent changes in relative arginine availability; two patients had clear increases, whereas two other patients exhibited no change (Figure 3B). Progressive increases in most individual amino acids occurred during hospitalization. Serial arginase activities were available for two patients and showed substantial declines into the normal range by discharge in each case (Figure 3C), as serum NO_x levels increased (26.6 to 37.2 J.M in Patient 1, and 23.9 to 70.7 J.M in Patient 4).

DISCUSSION

This is the first report of a systemic arginine deficiency and elevated serum arginase activity in asthma. Because arginine deficiency is not confined to pulmonary tissue in this disease, this suggests that consequences of such a deficiency also are not confined to the lungs. It is important to note that relative arginine availability, as well as absolute arginine availability, is reduced in patients with asthma experiencing an acute clinical exacerbation. Thus, the arginine to ornithine + lysine ratio also should be

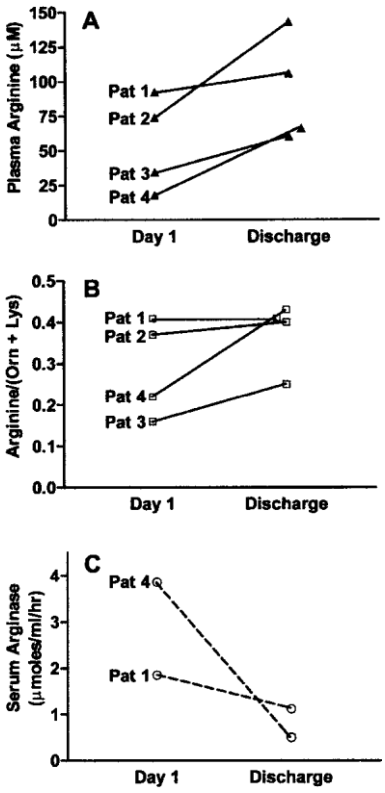

Figure 3. (*A*) Changes in plasma arginine concentration, (*B*) relative arginine availability, and (*C*) serum arginase activity, in patients with asthma between admission (Day 1) and the day of discharge from the hospital (Discharge). Levels normalized as the patients improved clinically. High arginase activity in patients with asthma may contribute to low circulating arginine levels, thereby limiting arginine bioavailability and potentially exacerbating a NO deficiency that induces hyperreactive airways.

taken into account when evaluating overall arginine availability. We cannot, however, conclude that intracellular pools of arginine are everywhere reduced in asthma because the status of arginine transport activity in patients with asthma is unknown. In fact, expression of the arginine transporter CAT-2 (cationic amino acid transporter) is increased in murine models of asthma (25), suggesting that, at least in some cells, increased uptake may offset reductions in circulating arginine levels. Consistent with this notion, one study found that intracellular levels of arginine in airway epithelial cells of patients with asthma were over threefold higher than in cells of healthy control subjects; however, plasma arginine levels were not determined in this study (48).

With recent studies supporting the role of a NO deficiency in airway hyperreactivity (14, 15), our findings have significant clinical relevance and represent a new focus for asthma research (16). Decreased arginine levels may reflect substrate depletion owing to increased demand for NO in asthmatics to maintain basal bronchodilator tone while compensating for increased NO consumption during oxidative stress, combined with an inflammatory-mediated induction of arginase activity during exacerbations. However, the data also illustrate a biological paradox in that exhaled NO increases in acute asthma despite elevated arginase activity and decreases in circulating arginine levels. Increased levels of exhaled NO could reflect metabolism of arginine from a compartmentalized pool in which arginine content is not reflected by circulating arginine levels, as suggested by increases in intracellular arginine levels in the study noted above (48). An alternate explanation for increased levels of exhaled NO independent of arginine availability is nonenzymatic generation of NO from nitrite due to airway acidification in asthmatics (49).

Reduced arginine availability may also contribute to lung injury by promoting formation of cytotoxic reactive NO species such as peroxynitrite. As arginine levels decline, NO synthase itself can begin to generate superoxide in lieu of NO (50), thereby favoring NO consumption via the generation of peroxynitrite that could induce lung injury (50–52). This reduction in bioavailability of NO via formation of species such as peroxynitrite could be further amplified by the rapid loss of superoxide dismutase activity during the asthmatic response (53, 54). Such a model would also help explain reduced NO bioavailability in the face of increased expression of inducible NO synthase in asthma (55).

Increased catabolism of arginine via arginase in asthmatic lungs may not only compromise the ability to synthesize NO, but also may contribute to airway remodeling through increased production of ornithine, a precursor for synthesis of proline and polyamines (30). These downstream products of arginase activity may play a role in the pathogenesis of asthma by promoting collagen synthesis and cell proliferation (16, 25), processes that occur in airway wall thickening and airway remodeling (1, 3, 4, 56). The increased production of ornithine also may be reflected in the increased levels of polyamines found in peripheral blood of asthmatics (57).

Although our present data on this point is anecdotal, it is of interest to note that plasma arginase activity declined significantly with treatment and improvement of symptoms. Additional studies are needed to determine whether measurements of plasma arginase activity will provide a useful marker for underlying metabolic disorder and efficacy of treatment for this disease. Although the patients were not controlled for diet, it is also interesting to note that an increase in serum NO metabolites occurred by discharge, despite the antiinflammatory effects of steroid therapy, a treatment that suppresses inducible NO synthase activity that patients with asthma routinely receive during hospitalization. It is possible that the increase in NO metabolites is a reflection of decreased arginase activity and improved arginine bioavailability.

The arginase activity present in serum probably does not accurately reflect whole body arginase activity or that compartmentalized in the lungs, since the arginases are intracellular enzymes that appear in circulation only after cell damage or cell death. The cell types that contribute to the elevated plasma arginase in asthmatics have not been identified. However, as arginase is induced in monocytes in response to helper T cell type 2 cytokines (27, 28), we speculate that these cells are one likely source of the elevated arginase in serum, consistent with the localization of arginase expression within macrophages in lungs of asthmatic mice (25).

Although the greatest amino acid deficiency occurred for arginine, deficiencies of virtually all other amino acids also occurred in the group with asthma, similar to previous findings (58). A poor nutritional status at the time of analysis due to illness could contribute to these deficiencies. However, it is also possible that altered amino acid metabolism occurs during an asthma exacerbation, perhaps as a consequence of inflammation. Concentrations of amino acids rose during hospitalization, suggesting that the altered amino acid profile occurs during the acute event. Thus, patients with asthma may benefit from more focused attention on a nutritional component of treatment. Although the concept of dietary factors influencing the pathogenesis of asthma is not novel (59), the impact of multiple amino acid deficiencies on asthma severity warrants further investigation.

It is worth noting that asthma occurs in 30–70% of patients with sickle cell disease (60, 61), but is often unrecognized and untreated by clinicians. Relevant to the present study, we have recently demonstrated that elevated arginase activity and a low arginine to ornithine ratio also are associated with pulmonary complications of this disease (40), another inflammatory condition in which decreased arginine bioavailability contributes to a NO deficiency (62–65). In sickle cell disease, an arginine deficiency likely develops as a result of increased substrate demand for NO synthesis, perhaps coupled with increased arginase activity. Although an explanation for the high incidence of asthma in sickle cell disease has not been identified, we suggest that decreased arginine bioavailability, elevated arginase activity, and a NO deficiency may result in an asthma-like syndrome in sickle cell disease. Further investigation of the asthmatic condition in sickle cell disease may provide greater insight into the pathophysiology of asthma itself.

Asthma is estimated to affect 15 million people in the United States, and it is the most frequent reason for preventable childhood hospitalizations, costing billions of dollars annually (1, 3). It is a complex syndrome with many clinical phenotypes that likely involve a multitude of mechanisms, influenced also by genetic and environmental factors (4). As the incidence of asthma rises to "epidemic levels" (66), new insights into the pathogenesis of asthma are needed to identify new therapeutic intervention strategies. New therapies that maximize both arginine and NO bioavailability, including the use of arginase inhibitors and arginine supplementation, warrant exploration.

Conflict of Interest Statement: C.R.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; M.P. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; L.L. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; L.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; F.A.K. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; S.M.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this article.

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