

Altered L-arginine metabolism in children with controlled asthma

Leszek Kraj, M.D.,¹ Marta Krawiec, M.D.,² Marek Koter, Ph.D.,¹ Wojciech Graboń, M.D.,¹ Grażyna Kraj, M.D.,² Małgorzata Chołojczyk, M.Sc.,¹ Marek Kulus, Ph.D.,² and Anna Barańczyk-Kuźma, Ph.D.¹

ABSTRACT

Decreased level of L-arginine may lead to airway hyperresponsiveness, inflammation, and airway remodeling. Changes in L-arginine metabolism were observed earlier in adult asthmatic patients. Studies on L-arginine metabolism in children with bronchial asthma are limited. Because biosynthesis of L-arginine is insufficient in growing children, its potential metabolic alterations may have important clinical implications. This study was designed to evaluate L-arginine metabolism in children with well-controlled asthma. The studies were conducted on blood serum of 30 asthmatic and 20 healthy children (control group). Levels of L-arginine and its metabolic products, L-citrulline and L-ornithine, were measured by HPLC. Arginase activity was determined spectrophotometrically. Disease severity was evaluated by the asthma control test (ACT) and the level of nitric oxide (NO) in exhaled air. In asthmatic children L-arginine concentration was significantly lowered, whereas arginase activity was unchanged when compared with the healthy group. However, L-ornithine and L-citrulline levels were significantly increased. There was no correlation between arginase activity, amino acids levels, ACT scores, and exhaled NO. In children with chronic, well-controlled asthma L-arginine metabolism is altered. Given that L-arginine is absolutely essential for children, our findings may be of particular importance for the management of children with nonexacerbated asthma. They may also help to develop new therapeutic strategies targeted at L-arginine metabolism in the future.

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Bronchial asthma is a chronic inflammatory disease characterized by reversible bronchial obstructive reactions, airway hyperresponsiveness, inflammation, and remodeling.¹ The disease is characterized by periodic aggravation, symptomless periods controlled pharmacologically, and remissions with no need for medications. Many studies indicate that L-arginine plays an important role in all of these features and is considered as one of the asthma markers in adult patients.^{2–5} Pharmacologic modification of L-arginine metabolic pathways has been proposed as a plausible treatment strategy in the disease.⁶ L-Arginine is crucial for endogenous biosynthesis of ornithine, polyamines, nitric oxide (NO), proline, glutamate, creatine, and agmatine.⁷ It may stimulate secretion of insulin, glucagon, prolactin, and growth hormone.^{7,8} Moreover, as an immunomodulating amino acid, L-arginine improves the effectiveness of lymphocytes T, stimulates the activity of macrophages, natural killer and lymphokine activated killer cells, and enables phagocytosis by

granulocytes.⁹ In healthy adults the biosynthesis of endogenous L-arginine from L-citrulline is sufficient but it is inadequate during the period of growth. Thus, for growing children L-arginine is classified as the essential amino acid.¹⁰ It is also considered to be conditionally essential in some pathological states (inflammation, injuries, burns, and sepsis) when its supplementation is recommended.¹⁰ The particularly significant role of L-arginine in bronchial asthma results from its involvement in biosynthesis of polyamines and NO, a bronchodilator but also a mediator of inflammation.^{11–13} Increased activity and/or expression of the enzymes associated with L-arginine catabolism, such as arginase and inducible NO synthase (iNOS) were observed in bronchi of asthmatic patients.^{14,15} These enzymes are induced simultaneously by a number of factors and their activity is regulated by availability of a common substrate—L-arginine.^{3,16} NOS splits L-arginine to NO and L-citrulline. Increased iNOS activity resulting in high levels of exhaled NO was observed both in adult^{5,17,18} and pediatric asthma patients.^{19–22} Arginase hydrolyzes L-arginine to L-ornithine, which can be further metabolized to L-proline (involved in collagen synthesis), and polyamines (responsible for cellular proliferation). It is known that patients with asthma exacerbation have an increased arginase activity in the blood serum with a concomitant decrease in L-arginine level.²³ Today, the studies on L-arginine metabolism in bronchial asthma have

From the ¹Departments of Biochemistry and ²Pediatric Pneumonology and Allergy, Medical University of Warsaw, Poland

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Address correspondence to Anna Barańczyk-Kuźma, M.D., Ph.D., Department of Biochemistry, Medical University of Warsaw, 02-097 Warsaw, Banacha 1, Poland
E-mail address: akuzma@wum.edu.pl

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Table 1 Demographic data of studied children

Characteristics	Asthmatic Children (n = 30)	Control Children (n = 20)
Age (yr)	10.6 ± 3.8 (5–17)	11.7 ± 2.9 (6–14)
Sex, male/female	18: 12	11: 9
BMI (kg/m ²)	17.9 ± 5.0	19.3 ± 5.0
Weight (kg)	37.8 ± 22.2	47.7 ± 19.7
ACT (points)	21.4 ± 3.3	—

ACT = asthma control test; BMI = body mass index.

been focused mainly on adults but not pediatric patients; therefore, the data on L-arginine metabolism in asthmatic children are limited.^{22,23} Interestingly, arginase activity declined significantly when the proper treatment of exacerbation was applied. Thus, the aim of our study was to evaluate L-arginine metabolism in children with nonexacerbated, well-controlled asthma.

MATERIALS AND METHODS

A total number of 50 children were recruited from the outpatient clinic. The study group included 30 children with chronic, nonexacerbated bronchial asthma (based on Global Initiative for Asthma criteria). Twenty-three children were treated with inhaled steroids (controller medication), one additionally with an antileukotriene drug and long active β agonists; seven received no asthma treatment. The control group consisted of 20 healthy matched children (Table 1). Studies were approved by the Institutional Bioethical Committee (KB12/2009). Written informed consent was obtained from all children's parents. The quantitative analysis of amino acids was performed by HPLC.²⁴ Arginase activity was determined spectrophotometrically from the amount of ornithine released during the reaction.²⁵ NO in exhaled air was measured according to recommendations of the American Thoracic Society.²⁶ Statistical analysis was performed using routine methods. Asthma control test (ACT) is a simple questionnaire of five questions concerning the presence of asthma symptoms.

RESULTS

The levels of amino acids in blood serum of individual children are shown in Fig. 1. The mean level of L-arginine in asthmatic children was significantly lower, whereas the levels of L-citrulline and L-ornithine were significantly higher compared with controls. Serum arginase activity was similar in both groups (Fig. 2). The mean value of exhaled NO in the whole group of asthmatic children was 19.0 ± 12.9 ppb. In asthmatic children no significant correlation was found between arginase activity and L-arginine, L-ornithine, or L-citrulline levels. There was also no significant correlation

between clinical parameters of ACT scores, exhaled NO, body mass index, amino acids levels, and arginase activity (Table 2).

DISCUSSION

Because of insufficient biosynthesis of L-arginine in growing children and in inflammatory states, knowledge of its metabolism in asthmatic children is of particular importance. Because it was found that in children with active asthma L-arginine metabolism is altered, we focused our studies on L-arginine and its metabolites in children with well-controlled asthma. To date, the studies on serum L-arginine and exhaled NO levels were performed by Riccioni *et al.*²¹ in children without asthma exacerbation for a month. Lara *et al.*²⁷ studied L-arginine metabolism in nonexacerbated asthma in a small pediatric group ($n = 30$) added to the group of 202 adults. Comparing the results to the control group formed of adult cases was a major limitation of the study. Our findings considering decreased serum L-arginine level in asthmatic children are consistent with the observations of others.^{21,22} The reason for decreased serum L-arginine levels in acute and asymptomatic asthma may be an elevated expression of arginase in respiratory tract epithelium and in the inflammatory infiltrate macrophages.^{3,28,29} A significant increase of serum arginase activity was observed in 26 children with asthma in various stages of exacerbation.²³ In our patients with well-controlled asthma, arginase activity was unchanged but the level of L-ornithine, produced by arginase, was increased. L-Ornithine is a substrate for two enzymes—ornithine aminotransferase and decarboxylase. Ornithine aminotransferase participates in conversion of ornithine to proline used for collagen synthesis. Ornithine decarboxylase starts synthesis of polyamines regulating cellular growth, proliferation, and differentiation.^{8,30} The increased polyamine concentration was previously observed in asthma patients.³¹ Thus, L-ornithine is crucial for biosynthesis of compounds involved in pathogenesis of parenchymal remodeling in lungs. During asthma exacerbation, the level of L-ornithine was found unchanged, despite the substantial increase of serum arginase activity.²³ However, its level in nonexacerbated disease was significantly higher whereas arginase activity remained unchanged. It is possible that during suppressed inflammation less ornithine is used for biosynthesis of proline and polyamines involved in pulmonary parenchymal remodeling.¹³ Surprisingly, contrary to Morris *et al.*²³ and Lara *et al.*²⁷ who observed a decreased or unchanged L-citrulline level in patients with asthma, we found an increased serum level of this amino acid in children with asymptomatic, well-managed asthma. L-Citrulline is a nonprotein amino acid and its systemic level comprehensibility only depends on endogenous me-

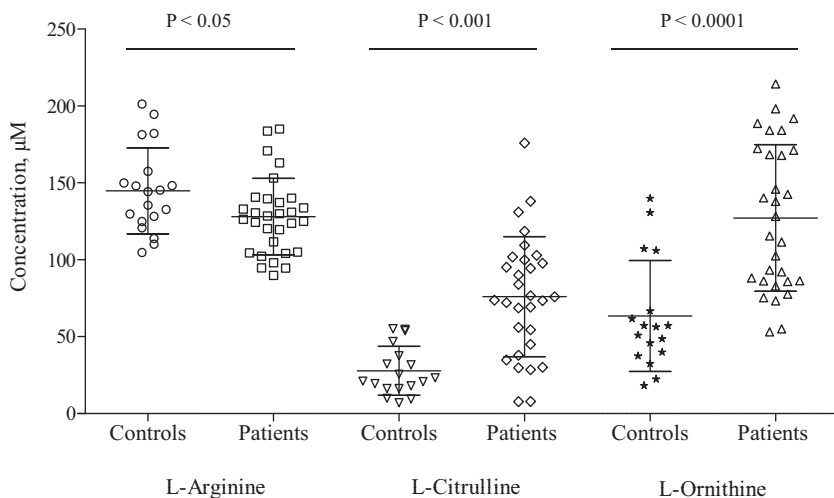


Figure 1. Levels of amino acids in blood serum of individual asthmatic and control children.

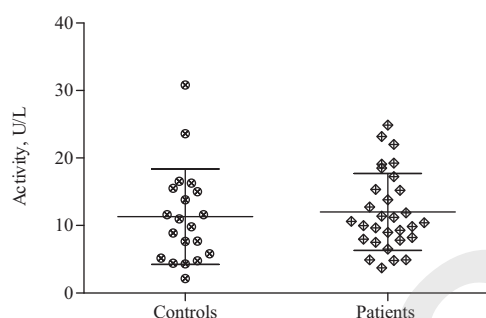


Figure 2. Arginase activity in blood serum of individual asthmatic and control children.

Table 2 Correlation between biochemical and clinical parameters in asthmatic children

Parameter	ACT Scores	Exhaled NO	BMI
L-Arginine	−0.003 (NS)	0.04 (NS)	−0.145 (NS)
L-Citrulline	−0.048 (NS)	−0.202 (NS)	−0.034 (NS)
L-Ornithine	−0.015 (NS)	−0.085 (NS)	0.146 (NS)
Arginase	−0.024 (NS)	−0.02 (NS)	−0.151 (NS)

Results are expressed as R values in Spearman's rank correlation test.

ACT = asthma control test; BMI = body mass index; NO = nitric oxide; NS = nonsignificant.

tabolism. The majority of L-citrulline is synthesized from L-glutamine in the small intestine and converted to L-arginine in the kidneys.³² L-Citrulline can also be synthesized from L-arginine by NOS. iNOS is transcriptionally regulated by proinflammatory stimuli with the ability to produce large amounts of NO and L-citrulline.³ It is up-regulated in asthmatic lungs and increased amounts of NO are exhaled.^{14,17,22} In our patients, the increased level of L-citrulline was not accompanied by exhaled NO, similar to healthy subjects.^{21,22} Therefore, increased L-citrulline level may be caused by its impaired conversion to L-arginine by the L-citrulline-L-arginine cycle and/or increased synthesis from L-arginine by iNOS. The expression of the L-citrulline-L-arginine cycle enzymes has not been studied in asthma. The amount of L-arginine formed from L-citrulline is insufficient in growing children.³² Because serum L-arginine level in asthmatic children is lowered, oral L-arginine supplementation should be considered.^{21,22} Unfortunately, orally administered L-arginine is largely taken up by the liver. In contrast, hepatic L-citrulline uptake is low, thus supplemented L-citrulline could be considered as an L-arginine precursor by-passing the liver.³³ We have shown that increased levels of L-citrulline were accompanied by decreased

levels of L-arginine; thus, the L-citrulline supplementation might be ineffective in children with well-controlled asthma. These findings give new insight on potential L-citrulline supplementation to restore a systemic L-arginine pool in pediatric patients with asthma.

In conclusion, nonexacerbated pediatric asthma is associated with altered L-arginine metabolism but the amino acids profile differs from that observed for acute asthma in adults. Reduced systemic L-arginine availability should be taken into account in nutritional and pharmacologic management of pediatric patients with chronic, well-managed asthma.

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