Review Article
Arginine and Nitric Oxide Pathways in Obesity-Associated Asthma

Fernando Holguin
Division of Pulmonary, Allergy & Critical Care, Asthma Institute, University of Pittsburgh, Pittsburgh, PA 15213, USA
Correspondence should be addressed to Fernando Holguin; holguinf@upmc.edu
Received 20 February 2013; Accepted 2 April 2013

Obesity is a comorbidity that adversely affects asthma severity and control by mechanisms that are not fully understood. This review will discuss evidence supporting a role for nitric oxide (NO) as a potential mechanistic link between obesity and late-onset asthma (>12 years). Several studies have shown that there is an inverse association between increasing body mass index (BMI) and reduced exhaled NO. Newer evidence suggests that a potential explanation for this paradoxical relationship is related to nitric oxide synthase (NOS) uncoupling, which occurs due to an imbalance between L-arginine (NOS substrate) and its endogenous inhibitor, asymmetric dimethyl arginine (ADMA). The review will propose a theoretical framework to understand the relevance of this pathway and how it may differ between early and late-onset obese asthmatics. Finally, the paper will discuss potential new therapeutic approaches, based on these paradigms, for improving the respiratory health of obese subjects with asthma.

1. Introduction
Although obesity is associated with less asthma control, greater risk of asthma exacerbations, and reduced inhaled corticosteroid efficacy, whether in fact these conditions are causally related remains uncertain [1]. However, by defining the phenotypical aspects of this relationship, some potential new mechanistic links have been uncovered. Using cluster analyses, obesity has been shown to be an important factor among female patients whose asthma occurs after childhood and have less atopy [2, 3]. This cluster has also been associated with lower airway eosinophils and exhaled nitric oxide (eNO) [3, 4]. The inverse association between body mass index (BMI) and eNO [5] may be explained by an imbalance between L-arginine and one of its methylated products known as asymmetric dimethyl arginine (ADMA) [6, 7]. L-arginine is the substrate that nitric oxide synthase (NOS) uses to generate NO. However, L-arginine is methylated to ADMA, which is an endogenous inhibitor of all NOS isoforms [8]. In addition, ADMA can uncouple NOS and preferentially generate anion superoxide instead of NO [9]. Therefore, conditions favoring a lower L-arginine/ADMA balance would in theory contribute to reducing NO airway bioavailability and increasing airway oxidative stress.

This phenomenon has been more extensively studied in the vasculature, as a mechanism leading to endothelial dysfunction or impaired NO-mediated vasodilatation [10]. However, in the lung, the L-arginine/ADMA balance is just beginning to surface as a potential explanation to understand metabolic diseases such as obesity and how these conditions can affect airway function. This review will discuss the role of L-arginine, arginases, and ADMA on airway NO metabolism in relation to obesity and propose a theoretical framework, by which an L-arginine/ADMA imbalance in obesity may explain why obesity leads to worsened respiratory symptoms in some asthmatics.

2. Exhaled Nitric Oxide and Arginine Metabolism; Implications to Obesity and Asthma
Historically, eNO has been studied as a biomarker of eosinophilic airway inflammation and as a potential biomarker to monitor response to inhaled corticosteroids. Given that high eNO levels track with asthma exacerbations and poor control, it is believed that increased NO could potentially have a causative role in asthma severity, and
Indeed, this may be the case, if one considers the NO can lead to the formation of more reactive nitrogen species (RNS) in the setting of airway oxidative stress [11]. However, it is unknown whether elevated NO levels per se have any causative role in worsening asthma severity. In fact, NO inhibition lacks clinical benefits in humans, and NO inhalation has been reported to prevent, not to induce, bronchial hyperresponsiveness [12–15]. These results may be explained on the basis that NO, which is constitutively produced by the airway epithelium, contributes to maintaining adequate airway function; for example, NO has been implicated in ciliary beating, maintaining bronchodilation through the formation of s-nitrosothiol compounds, and in bactericidal functions [16, 17]. Also, reduced eNO is associated with several chronic lung diseases [18–20]. Therefore, having reduced eNO in obesity, far from being beneficial, may indicate a major detrimental derangement of NO metabolism [21].

Reduced NO bioavailability in obesity and asthma could be potentially explained by several mechanisms, including (a) reduced NOS substrate (L-arginine), (b) endogenous NOS inhibition, (c) combination of (a) and (b), and (d) increased consumption of airway NO into other RNS. Increased arginase expression may reduce L-arginine bioavailability. Arginases are intracellular catabolic enzymes that metabolize arginine into ornithine and urea and have been shown to be higher among patients with asthma [22]. Compared to healthy controls, subjects with asthma have been found to have reduced plasma arginine levels and increased arginase activity, which have been associated with reduced FEV1 and greater airway obstruction [23]. Whether obesity could potentially exacerbate this phenomenon is unknown; however, the fact that increased BMI has been associated with increased arginase expression suggests that this might be possible [24].

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 nitric oxide synthase (NOS) isoforms. ADMA is synthesized from L-arginine by protein-arginine methyltransferases (PRMTs) and degraded into monoo- or dimethylamine and citrulline by dimethylarginine dimethylaminohydrolase (DDAH). Conditions such as obesity, metabolic syndrome, and diabetes have been associated with increased PRMT activity and/or reduction in DDAH function. The combination of these enzymatic changes could potentially explain why obesity in asthma contributes to higher ADMA levels [25, 26]. Citrulline can be subsequently recycled into L-arginine [8]. By competing with L-arginine, ADMA uncouples NOS causing electrons flowing from the NADPH reductase domain to the oxygenase domain to be diverted into molecular oxygen rather than to L-arginine [9]. Under uncoupling conditions, NOS generates superoxide, which correlates with airway oxidative stress in murine OVA models [27]. In stimulated murine airway epithelial cells, administration of ADMA reduces nitrite production while increasing superoxide levels in a dose-dependent manner [9]. 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, while the reduced compliance was linked to increased collagen deposition. Interestingly, these findings occurred in the absence of increases in traditional biomarkers of allergic airway inflammation [28].

Evidence that ADMA is associated with reduced eNO in humans is supported by one study showing that sputum ADMA and the L-arginine/ADMA ratio are associated with eNO (r = −0.5319 and 0.500, resp., both P < 0.05) in adults and children; in addition, sputum ADMA levels were higher in asthmatics versus controls [29]. Plasma ADMA and the L-arginine/ADMA ratio have been found to have similar associations with eNO among late onset asthmatics participating in the Severe Asthma Research Program (SARP) study [7]; however, whether increased airway or plasma ADMA levels have any impact on the respiratory systems is uncertain. Murine OVA models show that pretreatment with ADMA enhances allergic airway inflammation, bronchial hyperresponsiveness, and airway remodeling [9, 28]. These experiments would suggest that increased ADMA plays a role during acute allergic inflammation; yet, the SARP study suggests that reductions in plasma L-arginine/ADMA are associated with reduced FEV1, quality of life, and more frequent respiratory symptoms. Based on these results, it could be theorized that lower L-arginine/ADMA balance and its effects on airway NO bioavailability could not only enhance airway inflammation during acute exacerbations but also affect airway function more chronically.

Another mechanism potentially contributing to reduced eNO in obesity is airway oxidative stress, which can lead to the formation of RNS and therefore lower the NO fraction that is actually measured [30]. Compared to healthy controls, subjects with asthma have greater concentration of airway oxidative stress biomarkers, which appear to increase in relation to BMI [5, 31, 32]. While this has been demonstrated in exhaled breath condensates and bronchoalveolar lavage, an interaction between asthma and obesity has not been observed in a larger study population using plasma F2-isoprostanes as biomarkers of systemic oxidative stress [33]. It is therefore possible that in subjects with asthma, obesity increases airway and not systemic oxidative stress. The sources for increased airway oxidative stress in relation to BMI are unknown and seem to be (at least at baseline) independent of the number of inflammatory cells present in the airway. Two potential sources may involve a lower L-arginine/ADMA balance leading to the preferential formation of anion superoxide from epithelial NOS and increased airway leptin levels, which have been associated with greater airway levels of proinflammatory cytokines [34].

3. Arginine and Nitric Oxide Metabolism in Obesity and Asthma, Unique to a Phenotype?

Recent studies have confirmed that asthma is not a single disease but rather a heterogeneous group of clinical entities with different risk factors, varying response to therapies, degree of lung function impairment, and healthcare use, to
name a few [35]. While every asthma phenotype has the potential of being obese, the relationship between obesity and asthma may differ. For example, obesity seems to be mostly associated with subjects whose asthma occurs after childhood and have less atopy. This phenotype shows a higher degree of healthcare utilization, with mild lung function impairment and lower eNO [2, 3]. Results from these cluster analyses lead us to hypothesize that the L-arginine/ADMA balance and its relation to NO would differ between the ages of onset asthma phenotypes. Indeed, there were remarkable differences between subjects with childhood (<12 years) versus later (≥12 years) onset asthma. Among later onset asthmatics only, the inverse association between eNO and BMI was partly explained by the L-arginine/ADMA ratio; also, in this phenotype, lower L-arginine/ADMA ratios were associated with poorer asthma-related quality of life, reduced FEV₁, and more frequent respiratory symptoms, such as wheezing, dyspnea, and chest tightness [7]. Interestingly, there were no associations with cough, sputum production, or increased healthcare utilization. Although this is a cross-sectional study and causation cannot be established, it could be speculated that in this later onset phenotype, lower L-arginine/ADMA leads to reduced airway NO bioavailability, which in turn causes an “airway dysfunction syndrome”, characterized primarily by impaired bronchial dilation without increased sputum production or cough.

Why there are differences in L-arginine and NO metabolism across age of asthma onset phenotypes is unknown. It is possible that obesity in later onset asthma is associated with changes in L-arginine methylation or arginases expression that are not seen in the early onset phenotype. Alternatively, obesity induces similar changes in both groups; however, in the early onset phenotype other mechanisms (i.e., eosinophilic inflammation, atopy) drive asthma severity and override any effects resulting from the obesity—mediated changes in L-arginine—NO metabolism (see Figure 1).

4. Treatment Options

If L-arginine/ADMA is indeed one of the mechanistic pathways by which obesity affects asthma, this could open the door to new therapeutic options. L-arginine can effectively overcome the effects of ADMA on NOS and thus could become an additional treatment for obese late onset asthmatics, particularly for those that have lower or normal eNO and are not highly eosinophilic. Supplementation with L-arginine has been shown to increase eNO in children and adults and to reduce airway inflammation and bronchial hyper-responsiveness in murine ovalbumin sensitization models [36–39]. In addition, L-arginine supplementation can prevent NOS uncoupling [27]. Unfortunately, its use as a therapeutic modality is limited, given its extensive first pass metabolism in the liver and intestine [40]. This is perhaps why one study found only modest improvements in FEV₁ in asthmatics after 1 week of L-arginine supplementation [41]. Also, because L-arginine catabolism by arginase generates ornithine, a precursor to proline and polyamine, supplementation with L-arginine could theoretically generate collagen synthesis and promote subepithelial fibrosis and airway remodeling. As an alternative, citrulline, a precursor of L-arginine, could be
substituted for L-arginine. Citrulline is a nonessential amino acid but essential to detoxify and remove ammonia from muscle and liver cells. It is not subjective to extensive first pass metabolism by gut bacteria or liver arginases and can increase L-arginine levels in a dose-dependent manner [40]. Supplementation with citrulline at a dose of 3 g/BID for 1 week improved the L-arginine/ADMA ratio from 186 ± 278 to 278 ± 14 [40] and rose the levels of L-arginine from 79 (SD ± 8) to 421 ± 65 μmol; in comparison, supplementation with an equivalent L-arginine dose for the same period of time increased L-arginine from 84 ± 9 to 283 ± 51 μmol. These results illustrate that L-citrulline is more effective in increasing arginine plasma levels.

5. Summary

Obesity may lead to abnormalities in the balance of L-arginine and ADMA in obese subjects who acquired asthma beyond childhood. These changes have been associated with increased respiratory symptoms, reduced lung function, and poorer asthma-related quality of life. In addition, murine models support a role for ADMA in enhancing allergic inflammation, oxidative and nitrosative stress. Taken together, these results suggest that the L-arginine/ADMA balance and its effects on NO bioavailability could play a role in obesity-mediated airway disease. These findings are potentially encouraging as they could offer new avenues of treatment—phenotype specific—and are also expanding our ability to characterize asthma beyond quantifying the degree of airway eosinophilic inflammation.

References

[1] A. E. Dixon, F. Holguin, A. Sood et al., “An official American Thoracic Society Workshop report: obesity and asthma,” Proceedings of the American Thoracic Society, vol. 7, no. 5, pp. 325–335, 2010.

[2] W. C. Moore, D. A. Meyers, S. E. Wenzel et al., “Identification of asthma phenotypes using cluster analysis in the severe asthma research program,” American Journal of Respiratory and Critical Care Medicine, vol. 181, no. 4, pp. 315–323, 2010.

[3] P. Haldar, I. D. Pavord, D. E. Shaw et al., “Cluster analysis and clinical asthma phenotypes,” American Journal of Respiratory and Critical Care Medicine, vol. 178, no. 3, pp. 218–224, 2008.

[4] E. R. Sutherland, E. Goleva, T. S. King et al., “Cluster analysis of obesity and asthma phenotypes,” PLoS One, vol. 7, no. 5, Article ID e36631, 2012.

[5] S. Komakula, S. Khatri, J. Mermis et al., “Body mass index is associated with reduced exhaled nitric oxide and higher exhaled 8-isoprostanes in asthmatics,” Respiratory Research, vol. 8, p. 32, 2007.

[6] F. Holguin, S. A. A. Comhair, S. L. Hazen, W. R. Powers, J. Trudeau, and S. E. Wenzel, “Reduced L-arginine/ADMA as a potential mechanism to explain increased symptom severity and reduced atopy in late onset obese asthmatics,” American Journal of Respiratory and Critical Care Medicine, vol. 187, no. 2, pp. 153–159, 2013.

[7] F. Holguin, S. A. Comhair, S. L. Hazen et al., “An association between L-arginine/asymmetric dimethyl arginine balance, obesity, and the age of asthma onset phenotype,” American Journal of Respiratory and Critical Care Medicine, vol. 187, no. 2, pp. 153–159, 2013.

[8] C. T. L. Tran, J. M. Leiper, and P. Vallance, “The DDAH/ADMA/NOS pathway,” Atherosclerosis Supplements, vol. 4, no. 4, pp. 33–40, 2003.

[9] S. M. Wells and A. Holian, “Asymmetric dimethylarginine induces oxidative and nitrosative stress in murine lung epithelial cells,” American Journal of Respiratory Cell and Molecular Biology, vol. 36, no. 5, pp. 520–528, 2007.

[10] A. J. Pope, K. Karupiah, and A. J. Cardoum, “Role of the PRMT-DDAH-ADMA axis in the regulation of endothelial nitric oxide production,” Pharmacological Research, vol. 60, no. 6, pp. 461–465, 2009.

[11] S. Ghosh and S. C. Erzurum, “Nitric oxide metabolism in asthma pathophysiology,” Biochim Biophys Acta, vol. 1810, no. 11, pp. 1008–1016, 2011.

[12] D. Singh, D. Richards, R. G. Knowles et al., “Selective inducible nitric oxide synthase inhibition has no effect on allergen challenge in asthma,” American Journal of Respiratory and Critical Care Medicine, vol. 176, no. 10, pp. 988–993, 2007.

[13] R. M. Kacmarek, R. Ripple, B. A. Cockrill, K. J. Bloch, W. M. Zapol, and D. C. Johnson, “Inhaled nitric oxide: a bronchodilator in mild asthmatics with methacholine-induced bronchospasm,” American Journal of Respiratory and Critical Care Medicine, vol. 153, no. 1, pp. 128–135, 1996.

[14] R. H. Brown, E. A. Zerhouni, and C. A. Hirshman, “Reversal of bronchoconstriction by inhaled nitric oxide: histamine versus methacholine,” American Journal of Respiratory and Critical Care Medicine, vol. 150, no. 1, pp. 233–237, 1994.

[15] M. Hogman, S. Wei, C. Frostell, H. Arnberg, and G. Hedenstierna, “Effects of inhaled nitric oxide on methacholine-induced bronchoconstriction: a concentration response study in rabbits,” European Respiratory Journal, vol. 7, no. 4, pp. 698–702, 1994.

[16] B. Gaston, J. M. Drazen, J. Loscalzo, and J. S. Stanler, “The biology of nitrogen oxides in the airways,” American Journal of Respiratory and Critical Care Medicine, vol. 149, no. 2, pp. 538–551, 1994.

[17] B. Gaston, J. Reilly, J. M. Drazen et al., “Endogenous nitrogen oxides and bronchodilator S-nitrosothiols in human airways,” Proceedings of the National Academy of Sciences of the United States of America, vol. 90, no. 23, pp. 10957–10961, 1993.

[18] H. Grasemann, S. Al-Saleh, J. A. Scott et al., “Asymmetric dimethylarginine contributes to airway nitric oxide deficiency in patients with cystic fibrosis,” American Journal of Respiratory and Critical Care Medicine, vol. 183, no. 10, pp. 1363–1368, 2011.

[19] M. Pifferi, D. Caramella, A. M. Cangiotti, V. Ragazzò, P. Macchia, and A. L. Boner, “Nasal nitric oxide in atypical primary ciliary dyskinesia,” Chest, vol. 131, no. 3, pp. 870–873, 2007.

[20] R. E. Girgis, M. A. Qureshi, J. Abrams, and P. Sverdlow, “Decreased exhaled nitric oxide in sickle cell disease: relationship with chronic lung involvement,” American Journal of Hematology, vol. 72, no. 3, pp. 177–184, 2003.

[21] B. J. Nevin and K. J. Broadley, “Nitric oxide in respiratory diseases,” Pharmacology and Therapeutics, vol. 95, no. 3, pp. 259–293, 2002.

[22] A. Lara, S. B. Khatri, Z. Wang et al., “Alterations of the arginine metabolism in asthma,” American Journal of Respiratory and Critical Care Medicine, vol. 178, no. 7, pp. 673–681, 2008.
R. C. Benson, K. A. Hardy, and C. R. Morris, “Arginase and arginine dysregulation in asthma,” Journal of Allergy, vol. 2011, Article ID 736319, 12 pages, 2011.

O. Y. Kim, S. M. Lee, J. H. Chung, H. J. Do, J. Moon, and M. J. Shin, “Arginase I and the very low-density lipoprotein receptor are associated with phenotypic biomarkers for obesity,” Nutrition, vol. 28, no. 6, pp. 635–639, 2012.

J. H. Lee, G. H. Park, Y. K. Lee, and J. H. Park, “Changes in the arginine methylation of organ proteins during the development of diabetes mellitus,” Diabetes Research and Clinical Practice, vol. 94, no. 1, pp. 111–118, 2011.

Q. Sun, X. Yang, B. Zhong et al., “Upregulated protein arginine methyltransferase 1 by IL-4 increases eotaxin-1 expression in airway epithelial cells and participates in antigen-induced pulmonary inflammation in rats,” Journal of Immunology, vol. 188, no. 7, pp. 3506–3512, 2012.

T. Ahmad, U. Mabalirajan, B. Ghosh, and A. Agrawal, “Altered asymmetric dimethyl arginine metabolism in allergically inflamed mouse lungs,” American Journal of Respiratory Cell and Molecular Biology, vol. 42, no. 1, pp. 3–8, 2010.

S. M. Wells, M. C. Buford, C. T. Migliaccio, and A. Holian, “Elevated asymmetric dimethylarginine alters lung function and induces collagen deposition in mice,” American Journal of Respiratory Cell and Molecular Biology, vol. 40, no. 2, pp. 179–188, 2009.

J. A. Scott, M. L. North, M. Rafii et al., “Asymmetric dimethylarginine is increased in asthma,” American Journal of Respiratory and Critical Care Medicine, vol. 184, no. 7, pp. 779–785, 2011.

T. A. Nguyen, J. Woo-Park, M. Hess et al., “Assaying all of the nitrogen oxides in breath modifies the interpretation of exhaled nitric oxide,” Vascular Pharmacology, vol. 43, no. 6, pp. 379–384, 2005.

R. Fernandez-Boyanapalli, E. Goleva, C. Kolakowski et al., “Obesity impairs apoptotic cell clearance in asthma,” Journal of Allergy and Clinical Immunology, vol. 131, no. 4, pp. 1041–1047, 2013.

F. Holguin and A. Fitzpatrick, “Obesity, asthma, and oxidative stress,” Journal of Applied Physiology, vol. 108, no. 3, pp. 754–759, 2010.

A. Sood, C. Qualls, A. Arychnyn et al., “Obesity-asthma association: is it explained by systemic oxidant stress?” Chest, vol. 136, no. 4, pp. 1055–1062, 2009.

N. L. Lugogo, J. W. Hollingsworth, D. L. Howell et al., “Alveolar macrophages from overweight/obese subjects with asthma demonstrate a proinflammatory phenotype,” American Journal of Respiratory and Critical Care Medicine, vol. 186, no. 5, pp. 404–411, 2012.

S. E. Wenzel, “Asthma phenotypes: the evolution from clinical to molecular approaches,” Nature Medicine, vol. 18, no. 5, pp. 716–725, 2012.

I. Abuzayan and S. W. Turner, “Changes in exhaled nitric oxide after ingestion of L-arginine in children: a pilot study,” Pediatric Pulmonology, vol. 45, no. 3, pp. 236–240, 2010.

U. Mabalirajan, T. Ahmad, G. D. Leishangthem et al., “Beneficial effects of high dose of L-arginine on airway hyperresponsiveness and airway inflammation in a murine model of asthma,” Journal of Allergy and Clinical Immunology, vol. 125, no. 3, pp. 626–635, 2010.

S. A. Kharitonov, G. Lubec, B. Lubec, M. Hjelm, and P. J. Barnes, “L-Arginine increases exhaled nitric oxide in normal human subjects,” Clinical Science, vol. 88, no. 2, pp. 135–139, 1995.

J. K. Mansoor, B. M. Morrissey, W. F. Walby et al., “L-arginine supplementation enhances exhaled NO, breath condensate VEGF, and headache at 4342 m,” High Altitude Medicine and Biology, vol. 6, no. 4, pp. 289–300, 2005.

E. Schwedhelm, R. Maas, R. Freese et al., “Pharmacokinetic and pharmacodynamic properties of oral L-citrulline and L-arginine: impact on nitric oxide metabolism,” British Journal of Clinical Pharmacology, vol. 65, no. 1, pp. 51–59, 2008.

H. W. de Gouw, M. B. Verbruggen, I. M. Twiss, and P. J. Sterk, “Effect of oral L-arginine on airway hyperresponsiveness to histamine in asthma,” Thorax, vol. 54, no. 11, pp. 1033–1035, 1999.