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Effect of Arginase Inhibition on Pulmonary L-Arginine Metabolism in Murine *Pseudomonas* Pneumonia

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**Abstract**

**Rationale:** Infection of the lung with *Pseudomonas aeruginosa* results in upregulation of nitric oxide synthases (NOS) and arginase expression, and both enzymes compete for L-arginine as substrate. Nitric oxide (NO) production may be regulated by arginase as it controls L-arginine availability for NOS. We here studied the effect of systemic arginase inhibition on pulmonary L-arginine metabolism in *Pseudomonas* pneumonia in the mouse.

**Methods:** Mice (C57BL/6, 8–10 weeks old, female) underwent direct tracheal instillation of *Pseudomonas* (PAO-1)-coated agar beads and were treated by repeated intra-peritoneal injections of the arginase inhibitor 2(S)-amino-6-boronohexanoic acid (ABH) or PBS until lungs were harvested on day 3 of the infection. L-arginine metabolites were quantified using liquid chromatography-tandem mass spectrometry, NO metabolites nitrate and nitrite by Griess reagent and cytokines by ELISA.

**Results:** NO metabolite concentrations (48.5 ± 2.9 vs. 10.9 ± 2.3 μM, p < 0.0001), as well as L-ornithine (29.6 ± 1.7 vs. 2.3 ± 0.4 μM, p < 0.0001), the product of arginase activity, were increased in *Pseudomonas* infected lungs compared to naïve controls. Concentrations of the NOS inhibitor asymmetric dimethylarginine (ADMA) were also increased (0.44 ± 0.02 vs. 0.16 ± 0.01 μM, p < 0.0001). Arginase inhibition in the infected animals resulted in a significant decrease in L-ornithine (14.6 ± 1.6 μM, p < 0.001), increase in L-arginine concentration (p < 0.001), L-arginine/ADMA ratio (p < 0.001), L-arginine availability for NOS (p < 0.001), and NO metabolite concentrations (67.3 ± 5.7 μM, p < 0.05). Arginase inhibitor treatment also resulted in an increase in NO metabolite levels in animals following intratracheal injection of LPS (p = 0.015). Arginase inhibition was not associated with an increase in inflammatory markers (IFN-γ, IL-1β, IL-6, MIP-2, KC or TNF-α) in lung. Concentrations of the L-ornithine-dependent polyamines putrescine, spermidine and spermine were increased in *Pseudomonas* infected lungs (p < 0.001, respectively) but were unaffected by ABH treatment.

**Conclusions:** Systemic arginase inhibition with ABH during *Pseudomonas* pneumonia in mice results in an increase in pulmonary NO formation but no pro-inflammatory effect.

**Introduction**

Infection of the lung with bacteria leads to increased expression of the inducible nitric oxide synthase (iNOS) or NOS2 and NO production [1–3], as does intra-tracheal instillation of lipopolysaccharide (LPS) [4,5]. NO production from NOS depends on the availability of substrate and co-factors, as well as the presence of endogenous inhibitors including asymmetric dimethylarginine (ADMA) [6]. In the context of lung infection with *P. aeruginosa*, an opportunistic pathogen frequently causing infections in patients with chronic lung diseases including chronic obstructive pulmonary disease (COPD), bronchiectasis or cystic fibrosis (CF), evidence suggests that relative airway NO deficiency may have negative effects for the host. Studies in CF patients for instance have shown that low levels of airway NO are a risk factor for acquisition of this pathogen [7]. In addition, in a rat model of chronic *P. aeruginosa* lung infection, supplementation with L-arginine reduced the pro-inflammatory cytokine interleukin (IL)−1β in airways, inhibited neutrophil recruitment, and ameliorated lung tissue damage, while pharmacological inhibition of NOS in this model significantly worsened lung damage [3].

Arginase is an enzyme that converts L-arginine to urea and L-ornithine. The two isoforms of arginase are expressed in a number of tissues including the lung and are thought to reduce NO
production from NOS by limiting the availability of substrate L-arginine [6,8,9]. Thus arginase may represent a target for interventions aiming to increase L-arginine availability for NOS and NO production. Inhibition of arginase in animal models of allergic airway inflammation, for instance, resulted in anti-inflammatory effects and abrogation of airway remodeling and hyperresponsiveness to methacholine in these animals, presumably by increasing L-arginine availability for NOS and increased NO formation [10–12]. Data on whether inhibition of arginase can increase NO production in the context of bacterial infection in vivo are currently lacking. We therefore studied the effects of chronic systemic arginase inhibition on the pulmonary L-arginine metabolism in a mouse model of chronic *P. aeruginosa* lung infection.

**Methods**

The experiments were approved by the institutional Animal Care Committee and were conducted in accordance with the guidelines of the Canadian Council for Animal Care.

**Mice and infection protocol**

Eight to ten week old female C57BL/6 mice purchased from Charles River Laboratories (Charles River, Oakville, Quebec, Canada) were housed in a pathogen-free environment and received autoclaved food and water in the laboratory animal services at our institution. Agarose beads embedded with *Pseudomonas aeruginosa* (mPAO1) were made following a published protocol [13] and modified by us, and beads were injected into the airways after intubation under direct vision as previously described [13] in anaesthetized mice (ketamine 150 mg/kg and xylazine 10 mg/kg administered intraperitoneally). A final *P. aeruginosa* dose of 2x10⁶ CFU in a volume of 40–50 μl was injected into the trachea. Infected mice were treated with a total of 4 i.p. injections of PBS or 100 μg of the arginase inhibitor 2(S)-amino-6-boronohepxanoic acid (ABH) dissolved in 0.3 ml of PBS at 24, 48, 60 and 70 hours following the instillation of PAO-1. Body weight was monitored daily, before and for 3 days following the infection. At 72 hours post infection mice were anaesthetized, blood was drawn by intracardial puncture and organs were harvested. Uninfected not ABH treated mice were used as controls.

A different group of animals (male BALB/c mice, 8 weeks old) underwent an established LPS pneumonia protocol [14]. Anaesthetized mice were instilled with 50 μg of LPS from E. coli O111:B4 (Sigma) and treated with i.p. injections of PBS (n = 8) or ABH (n = 8) similar as above, immediately before, and 12, 24, 36 and 48 hrs post instillation of LPS. Lungs were harvest immediately after the last injection of ABH or PBS and processed on ice. Lysis buffer (25 mM Tris-HCl, pH 7.4, 1% TritonX100, 10% glycerol) containing protease inhibitors (Complete, Mini, EDTA-free plus 2 μM EDTA, Roche Applied Science) was added (2.5 ml/g lung). Tissue was homogenized using high-speed homogenizer (Polytron PT 1200E, Kinematica, Switzerland) for 20 min, supernatants aliquoted and stored at −80°C until further analyses.

Liquid chromatography-tandem mass spectrometry (LC/MS/MS) to measure L-arginine, L-ornithine, L-citrulline, ADMA and the L-ornithine derived polyamines putrescine, spermidine and spermine was performed in supernatant of organ homogenates as previously described [15,16]. NO metabolites in plasma and in lung homogenates from the LPS pneumonia model were quantified with help of a chemiluminescence analyzer (Eco Physics CLD 88 sp, Durnten, Switzerland), while Griess reagent (Cayman, Ann Arbor, MI) was used for nitrate and nitrite measurements in lung homogenates of animals infected with *Pseudomonas*. Arginase activity was measured by conversion of L-arginine to ornithine in vitro, as previously described [17]. A multi-analyte panel enzyme-linked immunosorbeny assay (ELISA) was used to determine the concentrations of interferon-γ (IFN-γ), interleukin-1 beta (IL-1β), IL-6, macrophage inflammatory protein 2 (MIP-2), keratinocyte chemoattractant (KC), and tumor necrosis factor-alpha (TNF-α) in supernatant of lung homogenates (Millipore, Billerica, MA, USA).

All results are expressed as the mean ± standard error of the mean (SEM). Binary comparisons were made with two-tailed student’s t-test or Mann-Whitney test, where appropriate. Comparisons of three groups were performed by one-way ANOVA with Turkey’s multiple comparison or Kruskal-Wallis test with Dunn’s multiple comparison post hoc test, where appropriate. P-values < 0.05 were considered statistically significant. Statistical analyses were conducted using GraphPad Prism 4.0c (Graphpad Software Inc., La Jolla, CA USA).

**Results**

*P. aeruginosa* lung infection resulted in significant weight loss but no mortality in animals. Weight loss following infection did not differ significantly (ANOVA) between ABH and PBS treated mice on day 1 (5.9±0.9 vs. 5.4±0.4%), day 2 (10.1±0.9 vs. 9.4±1.2%) or day 3 (7.5±1.7 vs. 6.7±1.9%). There was no difference in lung wet weights between ABH and PBS treated infected mice (0.167±0.017 g vs. 0.193±0.007 g, p = 0.244).

*Pseudomonas* infection resulted in a significant increase in L-arginine concentrations (28.4±1.4 vs 17.5±0.7 μM, p<0.0001) and a more pronounced increase in L-ornithine (29.6±1.7 vs 23.3±0.4 μM, p<0.0001) in lung homogenates of infected mice compared to non-infected controls. Consequently, the L-arginine/ L-ornithine ratio, an index of L-arginine availability for intracellular NOS [18,19], was significantly reduced by *P. aeruginosa* infection (Figure 1). Treatment with the arginase inhibitor ABH, resulted in a significant decrease in L-ornithine (14.6±1.6 μM, p<0.0001), the product of arginase activity, but increase in its precursor L-arginine (38.9±2.2 μM, p = 0.001). The L-arginine/ L-ornithine ratio increased significantly with ABH treatment but did not normalize (Figure 1). L-citrulline concentrations were higher in the infected animals compared to non-infected controls (47.2±4.1 vs. 6.3±0.4 μM, p<0.0001) but not different in PBS vs ABH treated animals (49.5±9.2 μM).

*P. aeruginosa* infection also resulted in a significant increase in the concentration of the competitive NOS inhibitor ADMA (0.44±0.02 vs. 0.16±0.01 μM, p<0.0001) and a decrease in the L-arginine/ADMA ratio, an index of NOS impairment. ABH treatment did not affect ADMA concentrations in the lung but restored the L-arginine/ADMA ratio to normal (Figure 2).

Plasma NOx concentrations were higher in *Pseudomonas* infected PBS treated animals than controls (41.8±5.6 vs. 27.5±2.1 μM, p = 0.02) but not different from ABH treated animals (43.9±7.5 μM). NOx (nitrate+nitrite) concentrations were significantly increased in infected lungs compared to controls (48.5±2.9 vs. 10.9±2.3 μM, p<0.0001) and further increased with ABH treatment (67.3±5.7 μM, p = 0.01) (Figure 3). A significant increase in lung NO metabolite concentrations following arginase inhibition was also seen in the LPS pneumonia model (ABH vs. PBS: 26.0±5.7 vs. 10.1±0.8 μM, p = 0.01) (Figure 3).

NOx concentrations in liver homogenates were not different between the ABH or PBS treatment groups (23.1±3.2 vs. 24.1±5.2 μM) but ABH resulted in a significant increase in
As arginase 1 is the predominant isoform expressed in liver while arginase 2 is the predominant arginase isoform expressed in kidney, the differences in NOx concentration between the two organs is suggestive of arginase 2 specificity of ABH.

Arginase activity measured in lung homogenates in-vitro confirmed an increase in infected mice (48.8 ± 20.8 vs. 15.0 ± 2.3 mU/mg protein, p < 0.005). The expression of both arginase isoforms was increased in lung of infected mice using Western blot analysis (data not shown). Concentrations of the L-ornithine derived polyamines putrescine, spermidine and spermine were significantly higher in infected mice but not different between PBS and ABH treated animals (Table 1). Cytokine concentrations for the three groups are shown in Table 2. ABH treatment did not have an effect on cytokine concentrations.

**Discussion**

We here show that *Pseudomonas* infection of the lung resulted in a significant increase in lung tissue L-ornithine, the product of arginase activity. While there was also an increase in L-arginine concentration in the lung following infection, the availability of L-arginine for NOS, expressed as L-arginine/ornithine ratio, was lower in infected mice than in non-infected controls. In addition, we observed an increase in the concentration of the competitive NOS inhibitor ADMA in infected lungs. Systemic application of an arginase inhibitor resulted in increased L-arginine availability.
isotopes, that infection of the mouse lung with 
Pseudomonas may promote infection [24]. We have recently shown using stable 
limitation of NO production during infections with this bacterium 
sensitive to NO- and nitrite-mediated killing, and therefore, 
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A similar increase in lung NOx after ABH treatment was seen in mice after intratracheal instillation of lipopolysaccharide (LPS) (p = 0.015, t-test). A similar increase in lung NOx after ABH treatment was seen in mice after intratracheal instillation of lipopolysaccharide (LPS) (p = 0.015, t-test).

c and C. pylori, which induces arginase 
activity as a strategy to limit L-arginine availability for host 
NOS in order to reduce NO-mediated host defense and facilitate 
persistent gastric mucosal infection [23]. Pseudomonas is highly 
sensitive to NO- and nitrite-mediated killing, and therefore, 
limitation of NO production during infections with this bacterium 
may promote infection [24]. We have recently shown using stable 
isotopes, that infection of the mouse lung with P. aeruginosa resulted in a significant increase in the expression and activity of NOS 2 but also arginase 1 and arginase 2 [25]. In the current study we demonstrated that systemic arginase inhibition enhances pulmonary NO production using an established model of 
P. aeruginosa infected animals.

**Table 1.** Concentrations (μmol/L) of the L-ornithine derived polyamines putrescine, spermidine and spermine in lung homogenates of naïve control and Pseudomonas infected mice treated with PBS or arginase inhibitor ABH.

|                | Putrescine | Spermidine | Spermine |
|----------------|------------|------------|----------|
| Control        | 4.9±1.2    | 4.0±1.2    | 2.6±0.7  |
| Pseudomonas    | 34.2±2.4   | 39.6±2.3   | 13.6±0.8 |
| PBS            | 34.2±2.4   | 39.6±2.3   | 13.6±0.8 |
| ABH            | 28.7±2.9   | 37.5±3.7   | 12.6±0.8 |

* Concentrations were significantly different between groups (p<0.001, ANOVA). All three polyamines were lower in controls compared to infected animals but not different between PBS or ABH treatment group (n=6-8/group). 
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for NOS and increased NO production. Increased NO production induced by arginase inhibition was not associated with an increase in pro-inflammatory cytokines in P. aeruginosa infected animals.

Arginase and NOS both compete for L-arginine as substrate and an important role of arginase is thought to be the regulation of 
substrate/inhibitor) an index reflecting NOS impairment. Effects 
inhibitor ADMA and a decrease in L-arginine/ADMA ratio (NOS 
significant increase in the concentration of the competitive NOS 
production in the infected and ABH treated animals were not 
caused by effects of the arginase inhibitor on Pseudomonas, as similar 
increases in NO following the administration of ABH were also 
seen in animals with intratracheal LPS instillation. Gender 
disparity exists in certain aspects of the nitric oxide pathway 
[26–29], and it is therefore worth mentioning that the infected 
animals in our experiments were females while the LPS treated 
were male mice. As arginase inhibition resulted in increases in NO 
production in both groups, it can be speculated that the effect of 
ABH on pulmonary NO production in the mouse is gender 
independent. However, further studies will be needed to assess 
whether treatment with arginase inhibitors may result in different 
physiological responses in males and females.

Our results also showed that infection of the lung resulted in a 
significant increase in the concentration of the competitive NOS 
inhibitor ADMA and a decrease in L-arginine/ADMA ratio (NOS 
substrate/inhibitor) an index reflecting NOS impairment. Effects 
of ADMA in the context of respiratory infections with Pseudomonas were previously studied using human nasal epithelial cells. Pre-
icubation with ADMA significantly reduced Pseudomonas-induced 
epithelial damage, loss of ciliated cells and bacterial adherence to 
the cultured respiratory mucosa in-vitro [30]. While these data

**Table 2.** Cytokine concentrations (ng/g) in lung homogenates of naïve control and Pseudomonas infected mice treated with PBS or arginase inhibitor ABH.

|                | Control (n=6) | PBS (n=14) | ABH (n=15) |
|----------------|--------------|------------|------------|
| IFN-γ          | 0.04±0.01    | 3.50±0.83  | 2.54±0.52  |
| IL-1β          | 8.54±0.96    | 15.51±3.02 | 12.26±2.37 |
| IL-6           | 9.97±0.51    | 52.27±5.67 | 65.09±12.1 |
| MIP-2          | 0.15±0.01    | 59.46±7.30 | 44.29±3.67 |
| KC             | 4.52±1.43    | 31.84±5.90 | 29.28±3.38 |
| TNF-α          | 0.05±0.01    | 7.61±1.41  | 5.48±0.83  |

Concentrations were significantly different between groups (p<0.001, Kruskal-Wallis test). All cytokines with the exception of IL-1β were lower in controls compared to infected animals not different between PBS and ABH treated animals.

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Figure 3. Nitric oxide metabolite (NOx) concentrations in mouse lung homogenates of untreated (naïve) control as well as Pseudomonas infected mice treated with PBS or the arginase inhibitor 2(S)-amino-6-boronohexanoic acid (ABH). Concentrations were significantly different between groups (p<0.0001, ANOVA). NOx levels in lung were lower in naïve compared to Pseudomonas infected and PBS treated mice (∗; p<0.001, Mann-Whitney test). ABH treatment resulted in a significant increase in ABH vs. PBS treated animals (p<0.05, Mann-Whitney test). A similar increase in lung NOx after ABH treatment was seen in mice after intratracheal instillation of lipopolysaccharide (LPS) (p = 0.015, t-test). doi:10.1371/journal.pone.0090232.g003
provided evidence that ADMA was associated with beneficial effects, it was unclear in that study whether the observed effects of ADMA were direct or mediated through inhibition of NOS, as ADMA did not cause a significant change in nitrite concentration in the culture medium [30]. In our experiments ABH treatment did not result in a change in ADMA concentration in the infected lungs, but normalized the ratio of L-arginine/ADMA with a concomitant increase in NO production.

Dosing of the arginase inhibitor was based on previous publications on systemic use of ABH in rodents [31,32]. The half-life of ABH in C57BL/6 mice was previously reported to be approximately 8 hours [33]. There was no evidence that repeated systemic application of the arginase inhibitor was harmful to the animals. Although Pseudomonas resulted in significant weight loss in the animals on day 1 and day 2 of the infection, there were no differences in weight loss between the groups of ABH or PBS treated animals. There was also no significant difference in cytokine concentrations in lung homogenates when comparing ABH and PBS treated mice, suggesting that neither ABH nor the increase in NO production in the lung had a pro-inflammatory effect. Previous work has shown that mice deficient for NOS2 had impaired clearance of Pseudomonas from the lung 18 h after infection [34]. The present study focused on the effect of arginase inhibition on NO production; whether the increase in NO formation would lead to altered defense against Pseudomonas was not assessed, but should be investigated in future studies.

L-ornithine, the product of arginase activity, is substrate for collagen formation but also for polyamine biosynthesis. Polyamines are important in cell repair and also act as NOS inhibitors [6,9,16]. Arginase activity and polyamine levels are significantly increased in models of asthma and in the guinea pig for instance, the increase in putrescine was prevented by pharmacological blockade of arginase [11]. In our experiments, Pseudomonas infection also resulted in a significant increase in polyamine concentrations in the lung. Arginase inhibition reduced L-ornithine formation by approximately 50% but there was no effect on polyamine concentrations in the lung. This could likely be due to the fact that ornithine availability for ornithine decarboxylase (ODC), the first and rate-limiting step in L-ornithine dependent polyamine biosynthesis [35] remained sufficient for polyamine production.

An alternative strategy to increase concentration and availability of L-arginine for NOS is supplementation of its substrate. A previous study using a chronic model of Pseudomonas infection of the lung in rats had shown that L-arginine given in drinking water resulted in lower IL-1β concentrations in BAL fluid in the L-arginine treated compared to the untreated group, whereas VEGF was increased. L-arginine supplementation has the potential disadvantage of providing substrate for NOS but also arginase enzymatic activity, which may result in unwanted effects of L-ornithine derived metabolites. For instance, generation of spermine by ODC inhibits iNOS translation and NO-mediated H. pylori killing [36,37]. Studies in humans with CF or asthma, both conditions associated with increased arginase activity and relative NO deficiency, have shown that the effect of systemic L-arginine supplementation on pulmonary NO formation is moderate and limited by increased formation of the NOS inhibitor ADMA, a product of protein degradation [38,39]. Ultimately, one approach does not exclude the other and the combination of both may have the highest likelihood of addressing the relative NO deficiency in P. aeruginosa infection.

In conclusion systemic ABH used in the early phase of acute P. aeruginosa lung infection at doses effective to significantly reduce arginase activity in the lung resulted in increased pulmonary NO production. The role of pharmacological inhibition of arginase for treatment of lung infections deserves further investigation.

**Author Contributions**
Conceived and designed the experiments: HG FR AM NP. Performed the experiments: HH DD PG AM. Analyzed the data: FR HG PG AM. Contributed reagents/materials/analysis tools: HG. Wrote the paper: FR HG AM.

**References**

1. Tsai WC, Surieter RM, Zisman DA, Wilkowski JM, Bucknell KA, et al. (1997) Nitric oxide is required for effective innate immunity against Klebsiella pneumoniae. Infect Immun 65: 1870–7.

2. Nathan C, Shiloh MU (2000) Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. Proc Natl Acad Sci USA 97: 8481–8.

3. Hopkins N, Gunning Y, O’Croinin DF, Laffey JG, Mc Laughlin P (2006) Anti-inflammatory effect of augmented nitric oxide production in chronic lung injury. J Pathol. 209:198–205.

4. Buttery LD, Evans TJ, Springall DR, Carpenter A, Cohen J, et al. (1994) Immunonochemical localization of inducible nitric oxide synthase in endotoxic-treated rats. Lab Invest 71: 555–64.

5. Liu SF, Barnes JF, Evans TW (1997) Time course and cellular localization of lipopolysaccharide-induced nitric oxide synthase messenger RNA expression in the rat in vivo. Crit Care Med 25: 512–18.

6. Rakic K, Warnken M (2010) L-arginine metabolic pathways. The Open Nitric Oxide Journal 2: 9–19.

7. Graessmann H, Ratjen F (2012) Nitric oxide and L-arginine deficiency in cystic fibrosis. Curr Pharm Des 18:726–36.

8. Maarssingh H, Tio MA, Zaagama J, Meurs H (2003) Arginase attenuates inhibitory nonadrenergic noncholinergic nerve-induced nitric oxide generation and airway smooth muscle relaxation. Respir Res 6:23.

9. North ML, Marsden PA, Graessmann H, Scott JA (2009) Functionally important role for arginase 1 in the airway hyperresponsiveness of asthma. Am J Physiol Lung Cell Mol Physiol 296:L911–20.

10. Guibaud C, Martin P, Houle D, Boghady ML, Guert MC, et al. (2005) Cystic fibrosis lung disease following infection with Pseudomonas aeruginosa in C57 knockout mice using novel non-invasive direct pulmonary infection technique. Lab Anim 39:336–32.

11. Douda DN, Jackson R, Graessmann H, Palaniyar N (2011) Innate immune collectin surfactant protein D simultaneously binds both neutrophil extracellular traps and carbohydrate ligands and promotes bacterial trapping. J Immunol 187:1856–65.

12. Scott JA, North ML, Rafii M, Huang H, Pencharz P, et al. (2011) Asymmetric dimethylarginine is increased in asthma. Am J Respir Crit Care Med 184:779–85.

13. North ML, Graessmann H, Khanna N, Inman MD, Gauvreau GM, et al. (2013) Increased ornithine-derived polyamines cause airway hyperresponsiveness in a mouse model of asthma. Am J Respir Cell Mol Biol 48:694–702.

14. Belik J, Shehaz D, Pan J, Graessmann H (2008) Developmental changes in arginase expression and activity in the lung. Am J Physiol Lung Cell Mol Physiol 294:L498–504.

15. Morris CR, Kato GJ, Poljakovic M, Wang X, Blackwelder WC, et al. (2005) Dysregulated arginine metabolism, hemolysis-associated pulmonary hypertension, and mortality in sickle cell disease. JAMA 294:81–90.

16. Tang WH, Wang Z, Cho L, Brennan DM, Hazen SL (2008) Diminished global arginine metabolism, hemolysis-associated pulmonary hypertension, and mortality in sickle cell disease. JAMA 294:81–90.

17. Nathan C, Xie QW (1994) Nitric oxide synthases: roles, tools, and controls. Cell 73:457–65.

18. Schmidt HH, Walter U (1994) NO at work. Cell 78:919–25.

19. Gaston B, Drazen JM, Loscalzo J, Stamler JS (1994) The biology of nitrogen oxides in the airways. Am J Respir Crit Care Med 149:530–51.
23. Lewis ND, Asim M, Barry DP, Singh K, de Sablet T, et al. (2010) Arginase II restricts host defense to Helicobacter pylori by attenuating inducible nitric oxide synthase translation in macrophages. J Immunol 184:2572–82.

24. Yoon SS, Karabulut AC, Lipscomb JD, Henning RJ, Lymar SV, et al. (2007) Two-pronged survival strategy for the major cystic fibrosis pathogen, Pseudomonas aeruginosa, lacking the capacity to degrade nitric oxide during anaerobic respiration. EMBO J 26:3662–72.

25. Mehl A, Huang H, Jaekl T, Ratjen F, Grasemann H (2010) Pseudomonas infection of the lung results in increased arginase and NOS activity. Pediatr Pulmonol Suppl 33:134 (abstract).

26. Sun J, Picht E, Ginsburg KS, Bers DM, Steenbergen C, et al. (2006) Hypercontractile female hearts exhibit increased S-nitrosylation of the L-type Ca2+ channel alpha1 subunit and reduced ischemia/reperfusion injury. Circ Res 98:403–11.

27. Wang-Rosenke Y, Mika A, Khadzhynov D, Loof T, Neumayer HH, et al. (2012) Impact of biological gender and soluble guanylate cyclase stimulation on renal recovery after relief of unilateral ureteral obstruction. J Urol 188:316–23.

28. Sun J, Picht E, Ginsburg KS, Bers DM, Steenbergen C, et al. (2006) Hypercontractile female hearts exhibit increased S-nitrosylation of the L-type Ca2+ channel alpha1 subunit and reduced ischemia/reperfusion injury. Circ Res 98:403–11.

29. Grasemann H, Storm van’s Gravesande K, Buscher R, Devriendt J, Ratjen F (2003) Effects of sex and of gene variants in constitutive nitric oxide synthases on exhaled nitric oxide. Am J Respir Crit Care Med 167(8):1113–6.

30. Dowling RB, Newton R, Robichaud A, Cole PJ, Barnes PJ, et al. (1998) Effect of inhibition of nitric oxide synthase on Pseudomonas aeruginosa infection of respiratory mucosa in vitro. Am J Respir Cell Mol Biol 19:950–8.

31. Kim JH, Bugaj LJ, Oh YJ, Bivalacqua TJ, Ryoo S, et al. (2009) Arginase inhibition restores NOS coupling and reverses endothelial dysfunction and vascular stiffness in old rats. J Appl Physiol 107(4):1249–57.

32. Shatanawi A, Romero MJ, Iddings JA, Chandra S, Umapathy NS, et al. (2011) Angiotensin II-induced vascular endothelial dysfunction through RhoA/Rho kinase/p38 mitogen-activated protein kinase/arginase pathway. Am J Physiol Cell Physiol: C1181–92.

33. Xu L, Hilliard B, Carmody RJ, Tsahary G, Shin H, et al. (2003) Arginase and autoimmunity inflammation in the central nervous system. Immunology 110:141–8.

34. Yu H, Naar SZ, Deretic V (2000) Innate lung defenses and compromised Pseudomonas aeruginosa clearance in the malnourished mouse model of respiratory infections in cystic fibrosis. Infect Immun 68:2142–7.

35. Palanimurugan R, Schell H, Hofmann K, Dohmen RJ (2004) Polyamines regulate their synthesis by inducing expression and blocking degradation of ODC-antizyme. EMBO J 23:4657–67.

36. Chaturvedi R, de Sablet T, Coburn LA, Hobert AP, Wilson KT (2012) Arginine and polyamines in Helicobacter pylori-induced immune dysregulation and gastric carcinogenesis. Amino Acids 42:627–40.

37. Grasemann H, Storm van’s Gravesande K, Buscher R, Devriendt J, Ratjen F (2003) Effects of sex and of gene variants in constitutive nitric oxide synthases on exhaled nitric oxide. Am J Respir Crit Care Med 167(8):1113–6.

38. Dowling RB, Newton R, Robichaud A, Cole PJ, Barnes PJ, et al. (1998) Effect of inhibition of nitric oxide synthase on Pseudomonas aeruginosa infection of respiratory mucosa in vitro. Am J Respir Cell Mol Biol 19:950–8.

31. Kim JH, Bugaj LJ, Oh YJ, Bivalacqua TJ, Ryoo S, et al. (2009) Arginase inhibition restores NOS coupling and reverses endothelial dysfunction and vascular stiffness in old rats. J Appl Physiol 107(4):1249–57.

32. Shatanawi A, Romero MJ, Iddings JA, Chandra S, Umapathy NS, et al. (2011) Angiotensin II-induced vascular endothelial dysfunction through RhoA/Rho kinase/p38 mitogen-activated protein kinase/arginase pathway. Am J Physiol Cell Physiol: C1181–92.

33. Xu L, Hilliard B, Carmody RJ, Tsahary G, Shin H, et al. (2003) Arginase and autoimmunity inflammation in the central nervous system. Immunology 110:141–8.

34. Yu H, Naar SZ, Deretic V (2000) Innate lung defenses and compromised Pseudomonas aeruginosa clearance in the malnourished mouse model of respiratory infections in cystic fibrosis. Infect Immun 68:2142–7.

35. Palanimurugan R, Schell H, Hofmann K, Dohmen RJ (2004) Polyamines regulate their synthesis by inducing expression and blocking degradation of ODC-antizyme. EMBO J 23:4657–67.

36. Chaturvedi R, de Sablet T, Coburn LA, Hobert AP, Wilson KT (2012) Arginine and polyamines in Helicobacter pylori-induced immune dysregulation and gastric carcinogenesis. Amino Acids 42:627–40.

37. Grasemann H, Storm van’s Gravesande K, Buscher R, Devriendt J, Ratjen F (2003) Effects of sex and of gene variants in constitutive nitric oxide synthases on exhaled nitric oxide. Am J Respir Crit Care Med 167(8):1113–6.

38. Dowling RB, Newton R, Robichaud A, Cole PJ, Barnes PJ, et al. (1998) Effect of inhibition of nitric oxide synthase on Pseudomonas aeruginosa infection of respiratory mucosa in vitro. Am J Respir Cell Mol Biol 19:950–8.

31. Kim JH, Bugaj LJ, Oh YJ, Bivalacqua TJ, Ryoo S, et al. (2009) Arginase inhibition restores NOS coupling and reverses endothelial dysfunction and vascular stiffness in old rats. J Appl Physiol 107(4):1249–57.