

Review

The biochemistry of asthma[☆]

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ABSTRACT

Background: Asthma is not one disease. Different patients have biochemically distinct phenotypes.

Scope of review: Biomarker analysis was developed to identify inflammation in the asthmatic airway. It has led to a renewed interest in biochemical abnormalities in the asthmatic airway. The biochemical determinants of asthma heterogeneity are many. Examples include decreased activity of superoxide dismutases; increased activity of eosinophil peroxidase, S-nitrosoglutathione reductase, and arginases; decreased airway pH; and increased levels of asymmetric dimethyl arginine.

Major conclusions: New discoveries suggest that biomarkers such as exhaled nitric oxide reflect complex airway biochemistry. This biochemistry can be informative and therapeutically relevant.

General significance: Improved understanding of airway biochemistry will lead to new tests to identify biochemically unique subpopulations of patients with asthma. It will also likely lead to new, targeted treatments for these specific asthma subpopulations. This article is part of a Special Issue entitled Biochemistry of Asthma.

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1. Overview

1.1. Background

Asthma is broadly defined as reversible airway smooth muscle contraction associated with lung inflammation. It affects over 300 million people worldwide [1,2]. Asthma management has been substantially improved with the use of anti-inflammatory therapies: as many as 85% of asthma patients are reasonably well controlled on inhaled corticosteroids, cysteinyl leukotriene receptor antagonists and/or injections of anti-IgE [2]. However, conventional asthma therapies – particularly use of higher dose corticosteroids – have associated morbidities [1–3]. Further, approximately 15% of patients continue to have significant disabling and/or life-threatening symptoms despite intensive use of these interventions; these patients are considered to have severe asthma [2–4]. Therefore, there is interest in understanding specific biochemical targets that may be amenable to more effective and less toxic therapy, particularly in the case of severe asthma [2–5].

1.2. Immunology

Understanding of asthma immunology has advanced substantially over the last three decades. The biology of lymphocytes, eosinophils, mast cells, basophils, airway epithelial cells, dendritic cells and airway smooth muscle cells has been worked out in murine models and – to some extent – validated in human airway tissues [2,5,6]. However,

murine models of asthma are genetically and phenotypically homogeneous. Therefore, they imperfectly reflect the complexity of human asthma [7]. For example, a clinical trial with anti-interleukin (IL)5 – predicted to be highly efficacious based on murine models – lacked pulmonary efficacy in human asthma [8]. Thus, there is interest in finding novel pathways of more direct relevance to human asthma.

Asthma can be viewed as a rash of the airways that leads to bronchoconstriction. Both asthma and skin rashes can be treated using topical corticosteroid therapy. The efficacy of the topical therapy can be evaluated on the skin by simple inspection or by performing a relatively minor biopsy. In the Severe Asthma Research Program and related programs, we can similarly evaluate the airway visually – and perform biopsies – using a bronchoscope [4]. However, bronchoscopy is not practical in clinical day-to-day asthma management [2].

Because we cannot routinely inspect the airway, clinicians measure airflow obstruction as a surrogate marker of asthmatic airway inflammation. Forced expiratory volume at 1 s (FEV₁), for example, is an important marker for asthmatic airflow obstruction [2]. However, FEV₁ and other available measures of airflow obstruction do not directly measure airway inflammation [9]. Indeed, measures of airway inflammation have not routinely been available in clinic. Therefore, corticosteroid and other anti-inflammatory therapies have to be used without a specific, direct marker of their anti-inflammatory efficacy. This has been problematic because failure to wean these medications can result in side effects; and because excessive weaning can result in life-threatening exacerbations [2,9,10].

1.3. Biochemical markers

To address this difficulty, biochemical markers of airway inflammation have recently been developed. These include measures of the

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fraction of exhaled NO (FE_{NO}), exhaled carbon monoxide (CO), exhaled breath condensate (EBC) pH and other EBC analytes [9–16]. These markers show modest promise as tests for inflammation that could be used to adjust corticosteroid dosing [15,16]. Biochemically, the results of these biomarker analyses have been assumed to report simple, mono-enzymatic effects downstream of specific immune pathways [9,15,16]. However, we have come to understand that the expression of these gene products is exceptionally complex and is not directly reported by any one of these exhaled biomarkers. As a result of these new insights, asthmatic airway biochemistry has recently re-emerged as an exciting new field of study revealing new paradigms and new targets for therapy. Because human asthma is genetically and phenotypically heterogeneous, these proposed new therapies are anticipated to be targeted to specific asthma patients. As such, they may both be effective and prevent toxicities associated with non-specific therapies such as corticosteroids.

2. Heterogeneity of asthma

2.1. Immunological heterogeneity

Asthma is not one disease [2–6,17]. It represents a syndrome of airway inflammation associated with bronchoconstriction. This syndrome encompasses pathology downstream of a variety of different immunological processes and biochemical abnormalities. For example, different patients have different inflammatory responses (i.e., high IgE, low IgE, increased airway eosinophil levels, or increased airway neutrophil levels) [5,6]. Diverse biochemical responses are associated with these diverse inflammatory responses. This heterogeneous biochemistry can lead to abnormal levels of biochemical markers. Examples in exhaled air include high exhaled NO in patients with airway eosinophils [18]; and low breath condensate pH in patients with airway neutrophils [19]. Indeed, there is now evidence for additional asthma populations with a range of biomarkers such as by high urinary bromotyrosine levels in patients with eosinophilia [20]. As we will see, however, within each of these broad subphenotypes is substantial overlap. Indeed, it has not been possible to identify a unifying “asthma pathophysiology” or “asthma gene.” There are a large number of genes that have single nucleotide polymorphisms (SNPs) abnormally distributed in the asthma population. Thus, various signals that affect inflammatory cascades and downstream biochemical airway responses differ in different patients. Indeed, it is increasingly apparent that there are different responses not only in different patients, but in different regions of the airway [21–23]. In this way, airway rashes again recapitulate skin rashes: different causes are associated with both different immune responses and different anatomic distributions. A key difference between asthma and dermatitis is that inflammation in the airway can cause life-threatening airway narrowing by signaling both airway wall thickening and constriction of the underlying smooth muscle. Note also that there are a number of important biochemical pathways specifically associated with inflammatory cascades relevant to asthma, including those involved in leukotriene, lipoxin, resolvins and complement cascades. These have been reviewed extensively elsewhere (see summaries [1,2,24,25]) and will not be the focus of this review.

3. Immunological and biochemical views of the causes of the asthma epidemic

Accounting for epidemiology is often the first step toward understanding the pathophysiology of a disease or syndrome. It is therefore important to ask why it is that asthma incidence has increased dramatically in the past four decades [1,2]. If specific inflammatory and biochemical genes underlie asthma pathophysiology, were not these genes present in the population in the early twentieth century? Why was asthma not more prevalent then? Commonly, three answers are given. I favor the third. First – and most

commonly discussed – is the hygiene hypothesis [1,2,26,28]; and the corollary hypothesis that alterations in gut flora lead to asthma [26]. As the world's population has become less rural, people have become less commonly exposed to aerosolized endotoxin from livestock; and more exposed to antibiotics. This results in a tendency for children to have skewing of the normal T-helper (Th)1 lymphocyte response to endotoxin toward a Th2 lymphocyte response more characteristic of asthma. Critics of this type of hypothesis have presented counterexamples, arguing against the correlation on historical grounds, geographical grounds and immunological grounds [26,27,29]. The second hypothesis is that environmental air pollution has caused an abnormal immune and biochemical response because of increased oxidative, nitrosative and particulate stress on the airway [30,31]. Again, there are significant counterexamples [29,31]. Indeed, regions with some of the worst asthma have less air pollution. The third hypothesis was based initially on an epidemiological observation. In the International Study of Asthma and Allergy in Childhood (ISAAC) collaborative study, asthma rates worldwide have paralleled acetaminophen usage rates [32,33]. Recent birth cohort data have confirmed this observation [29]. Hunt and coworkers proposed a biochemical mechanism by which acetaminophen might increase asthma risk. The airway is acidified biochemically during intercurrent viral infections [34,35]. This acidification converts paracetamol to nitroparacetamol, a potent hapten. It is precisely in the context of viral infections that acetaminophen is given for fever control. Thus, a hapten is created in the airway that may augment the local response (in dendritic cells, bronchus-associated lymphoid tissue, epithelial cells, etc.) to bystander antigens.

4. Biochemical phenotypes of asthma: Chemistry underlying heterogeneity

Whatever the underlying reason(s) for the asthma epidemic, abnormal airway immunology characteristic of asthma has occurred on the background of human genetic heterogeneity. Different gene regulatory effects upstream and downstream of various immune responses lead to patient-specific biochemical diseases of the airway epithelium. These responses vary with the underlying genotype of the patient. Here, I will focus my discussion on specific examples that may be particularly relevant to clinical phenotyping and to novel, targeted therapies. Abnormalities in these target enzyme systems may be identified clinically by an array of different biomarkers. It is most meaningful to think in terms of the target enzyme abnormality and the panel of biomarker changes that identifies this abnormality. Therefore, this section will be organized according to biochemistry rather than biomarkers. In contrast, previous individual biomarkers have previously been conceptualized to reflect a single enzyme disorder (for example, FE_{NO} reflecting iNOS activity).

4.1. Decreased superoxide dismutase (SOD) activity

Superoxide can be responsible for substantial airway damage. It has direct effects on cells and also reacts rapidly with NO radical to form peroxynitrite (protonated to form peroxynitrous acid, $pK_a \sim 6.9$ [36–38]). SOD isoforms initiate superoxide detoxification. Serum activity of SOD is low in patients with severe asthma and is roughly inversely correlated with FEV_1 [38] (Fig. 1). Patients with decreased SOD activity may be those who would benefit from antioxidant therapy. Importantly, trials generically treating all asthma patients with antioxidants have not been successful [39,40]. This may be because subpopulations of patients with preserved SOD activity were included (Fig. 1). Also, different antioxidants have different efficacy in different cellular and anatomic locations [41]. Stratifying patients based on SOD activity may identify a target population of asthma patients who will benefit from targeted antioxidant therapy. Decreased SOD activity can be reported by decreased serum activity

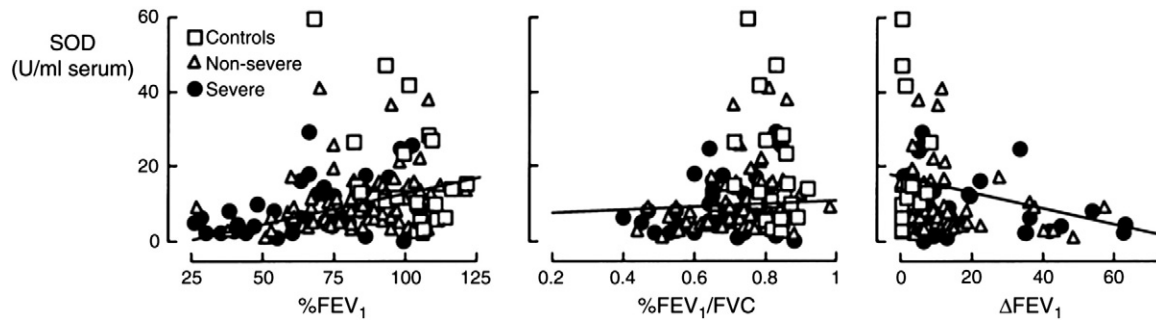


Fig. 1. Correlations of serum SOD activity with airflow (%FEV₁, FEV₁/FVC, and ΔFEV₁). SOD activity is directly correlated with %FEV₁ ($R = 0.312$, $p < 0.001$) and FEV₁/FVC ($R = 0.296$, $p < 0.001$), whereas SOD activity is inversely correlated with hyperresponsiveness, as determined by change in FEV₁ after β -agonist administration (180 μ g of albuterol; $R = -0.334$, $p = 0.001$). From reference [38].

(38), by decreased FE_{NO} and, in all likelihood by increased urinary γ -Br and nitrotyrosine levels (42; see below).

4.2. Increased eosinophil peroxidase activity

Many patients with asthma have increased levels of eosinophils in their lungs. Corticosteroids are particularly effective at decreasing levels of eosinophils; and this effect is generally believed to improve asthma outcomes [2,18]. However, identifying airway eosinophilia is a challenge. Bronchoscopy is invasive; and sputum induction can be uncomfortable and unreliable. Eosinophilic production of bromotyrosine is catalyzed by eosinophil peroxidase, which converts hydrogen peroxide and bromine to hypobromous acid (HOBr) [42]. In pH-dependent fashion, HOBr can be cytotoxic [34,42]. Thus, increased eosinophil peroxidase activity is a biochemical process that can injure the airway. Recently, it has been observed that bromotyrosine is increased in the urine of children with asthma and is a biomarker that predicts exacerbation [20]. Again, antioxidant therapy may be particularly useful in patients with high urinary bromotyrosine levels, particularly in conjunction with decreased circulating SOD activity [20,38]. Biochemical therapy might be targeted in this way to overlapping subpopulations identified by biomarkers.

4.3. Increased activity of S-Nitrosogluthathione (GSNO) reductase and related enzymes

S-Nitrosogluthathione is an endogenous airway smooth muscle relaxant that promotes apoptosis of inflammatory cells, increases ciliary motility and has anti-microbial properties [43–47]. Strikingly, however, GSNO levels are decreased in the airways of patients with asthma; and

levels are almost undetectable in the airways of children with asthmatic respiratory failure [48,49]. The reason for this deficiency is not primarily decreased GSNO synthesis; rather, it is increased GSNO breakdown [14,17,48,50,52]. A critical airway enzyme involved in GSNO breakdown is GSNO reductase [50–53]. The GSNO reductase-deficient mouse is protected from antigen-induced methacholine hyperresponsiveness (murine asthma [51]; Fig. 2). Moreover, GSNO reductase expression and activity are generally increased in the human asthmatic airway [23,48,52]; and SNPs associated with either loss or gain of function of GSNO reductase – including an important new SNP in the 3-untranslated region – are associated with decreased or increased asthma risk, respectively, in the human population [50–52].

That said, our preliminary evidence suggests that GSNO upregulation is not homogeneous in a given human asthmatic airway [23]. Different studies, including our own, have found lack of uniform changes in the expression and activity of GSNO reductase in the airways of different asthma patients [23,52]. We have also recently observed increased expression and activity of the alternative GSNO catabolic enzyme, carbonyl reductase 1 [57] in the human airway [58]. These S-nitrosothiol catabolic enzymes appear to work together to cause GSNO depletion in the airways of some asthma patients, particularly those with severe asthma [48]. A biomarker profile suggesting increased GSNO catabolism in a given patient could include increased NO in response to an inhaled NO⁺-donor challenge (Table 1) and increased exhaled breath condensate (EBC) formic acid concentration (See, Section 4, below).

Note that endothelial NOS (eNOS) expression and activity – and GSNO activity – are regulated by estrogen and testosterone (59–60), suggesting that nitrosylation/denitrosylation signaling could be

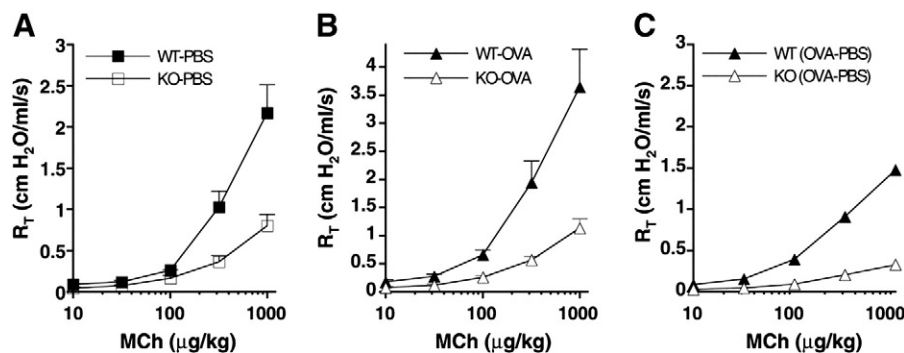


Fig. 2. Airways of GSNO^{−/−} mice are hyporeactive to methacholine (MCh) and allergen challenge. Total pulmonary resistances (R_t) of WT and GSNO^{−/−} (KO) mice after control (nonallergic; PBS) (A) and allergen (OVA) (B) treatment were determined in the absence or presence of various concentrations of MCh administered intravenously. R_t values in PBS-treated and in OVA-treated GSNO^{−/−} mice were significantly lower than in WT controls [KO PBS versus WT PBS, $P < 0.001$; KO OVA versus WT OVA, $P < 0.004$; analysis of variance (ANOVA) and post-hoc analyses at 3- to 5-MCh doses]. Data represent the mean $\pm$ SE of at least 7 to 10 mice per group. (C) The incremental effect of OVA (over PBS control) on WT and GSNO^{−/−} mice [OVA minus PBS (OVA-PBS)]. Whereas R_t of WT mice increased significantly after OVA treatment (WT PBS versus WT OVA, $P < 0.04$; ANOVA), the R_t of GSNO^{−/−} mice did not change significantly (KO PBS versus KO OVA, $P = 0.1$; ANOVA). From reference [51].

Table 1
Examples of abnormal biochemistry in the asthmatic airways.

Airway biochemistry	Phenotype biomarker		Potential targeted therapies
Decreased SOD activity [38]	Decreased FE _{NO} ^a	Decreased SOD activity [38]	Anti-oxidant and/or SOD-mimetic
Increased arginase activity [80,81]			Arginine and/or S-nitrosothiol replacement
Increased ADMA level [86] and/or decreased DDAH activity [84]			
Increased iNOS activity [15]	Increased FE _{NO} ^a [10–19,49,87]		NOS inhibitor
Decreased glutaminase activity [75]	Decreased EBC pH ^a or decreased FE _{NO} ^a on buffer challenge [78,79]		Glutamine and/or inhaled buffer
Other causes of decreased airway pH [34]			
Increased activity of GSNO reductase or other GSNO catabolic enzyme [48,50–52]	Decreased airway GSNO or increased FE _{NO} ^a on GSNO challenge [88]; or increased EBC formic acid [77].		S-nitrosylating agent and/or GSNO-R inhibitor
Increased eosinophil peroxidase [20]	Increased urinary bromotyrosine [20]		Increased corticosteroids

Note that there are a number of important biochemical pathways specifically associated with inflammatory cascades relevant to asthma – including those involved in leukotriene, lipoxin and complement cascades – that are extensively covered elsewhere [1,2,24,25] and are not the focus of this review.

^aClinically available biomarker; all others are in development.

involved in the gender discordance in asthma. However, additional work will be required to test this hypothesis.

S-nitrosothiols act through cyclic GMP-independent mechanisms to relax human airway smooth muscle [44,61]. Note in this regard that GSNO and other endogenous S-nitrosothiols do not ordinarily act as NO radical donors [44,53,61–68]. They normally cause bioactivities by covalent NO^+ transfer reactions, modifying specific cysteines in specific motifs [63,64]. These reactions are commonly stereoselective. That is, they are caused by L-isomer S-nitrosothiols but not in corresponding D isomer [66,68]. This covalent biochemistry is highly regulated in cells [64]. Recent evidence suggests that stereoselective S-nitrosylation of the myosin head at two redox-sensitive cysteines in specific, S-nitrosylation motifs prevents actin/myosin interaction and changes both cardiac and vascular smooth muscle contraction kinetics [65]. This mechanism may also be involved in the effect on airway smooth muscle.

An additional cGMP-independent mechanism by which GSNO and other cellular S-nitrosothiols protect against asthma is through preventing tachyphylaxis to β -2 adrenergic agonists [67]. GSNO is in transnitrosation equilibrium with other cellular S-nitrosothiols [50,53]. Catabolism of GSNO downstream of this transnitrosation promotes denitrosylation of a variety of important cellular proteins [53,64,67]. One of these proteins is G protein-coupled receptor kinase 2 (Grk2), which is S-nitrosylated by eNOS [67]. Grk2 S-nitrosylation prevents agonist-induced activation of Grk2 to signal β -2 adrenergic receptor internalization. Thus, GSNO reductase-deficient mice are protected from tachyphylaxis to β -2 adrenergic overstimulation [51–67]. Indeed, in the human population, SNPs in the GSNO reductase gene are more predictive of response to β -2 adrenergic agonists than are SNPs in the β -2 adrenergic agonist receptor itself among children in the emergency department. This appears to be particularly true in African American children [55].

Because S-nitrosylating agents cause human airway smooth muscle relaxation and prevent tachyphylaxis to β 2 adrenergic agonists, there is interest in developing exogenous inhaled S-nitrosylating agents and/or inhibitors S-nitrosothiol catabolism to treat asthma (Table 1). Note, however, that recent evidence suggests that inhibition of GSNO reductase could be associated with increased cancer risk in the setting of increased nitrosative stress. In the liver, GSNO reductase protects against hepatocellular carcinoma in the setting of chronic active viral hepatitis [69]. In the lung, Ras S-nitrosylation associated with chronic cigarette smoking may be a risk factor for non-small cell carcinoma if expression or activity of GSNO reductase is decreased [70]. Dose, timing and specificity are thus critically important in exploiting this biochemical pathway for asthma

treatment. It is important to target only those patients with increased GSNO reductase activity, with the goal of normalizing – not ablating – activity.

4.4. Low airway pH and nitrite protonation

The airway is acidified in association with infection [30,67–69]. Specifically, pH is low in the context of acute viral infections, active tuberculosis and chronic, cystic fibrosis-associated *Pseudomonas* infection [71–73]. As in the stomach, low pH in the airway can protect against infection. Low pH favors protonation of peroxynitrous acid, hypochlorous acid and HOBr, all with near neutral pKa's [34]. Though low pH is anti-microbial, it also can cause substantial airway injury, causing cellular necrosis, impaired airway clearance and cough [34].

Alveolar lining fluid is likely to be acidic because of high pCO_2 , high baseline activity of macrophages and extrusion of acidic lamellar bodies from type 2 pneumocytes [34,74]. Acidity may help protect the alveolus from infection. As the acidic distal airway fluid works its way proximally, it is buffered by NH_3 , HCO_3^- , proteins (like albumin) and other buffers [34,73,75]. This mechanism appears to recapitulate, at least in part, renal tubular acid buffering. Th1 cytokines can inhibit buffering by inhibiting enzymes such as glutaminase – preventing buffering in the more proximal airway [73]. They also 1) upregulate airway epithelial Duox2, which produces both H_2O_2 and protons; and 2) activate macrophages in the airway that actively acidify vacuoles (through V_{ATP} ases) that, in turn, acidify the extracellular space after macrophage necrosis [34]. Additionally, proton, chloride and bicarbonate transporters appear to be critically involved in buffering acid. Finally, an important source of acid in the airway may be the activity of GSNO reductase itself. This enzyme was originally described as an aldehyde dehydrogenase [14,76]. Using NAD^+ , it can oxidize S-formyl glutathione to form formic acid. Thus, those patients that have decreased GSNO in their airways may also be those with increased formic acid and associated low pH [14,77]. Therefore, it may be possible non-invasively to identify those patients with decreased GSNO reductase activity as those with decreased EBC pH and increased EBC formic acid [77]. Of note, a significant subpopulation of patients with severe asthma has recently been identified as matching this biochemical profile [19].

Note also that airway pH is an important determinant of FE_{NO} . Specifically, FE_{NO} decreases after inhalation of buffer [78,79; Fig. 3]. This likely reflects the low pH in the distal airway and – indeed – can serve as a challenge test to identify patients with low decreased airway pH [79]. Specifically, the pKa of nitrous acid is approximately 3.3 [34,35]. Nitrite, once protonated, rapidly evolves NO. Inhaling

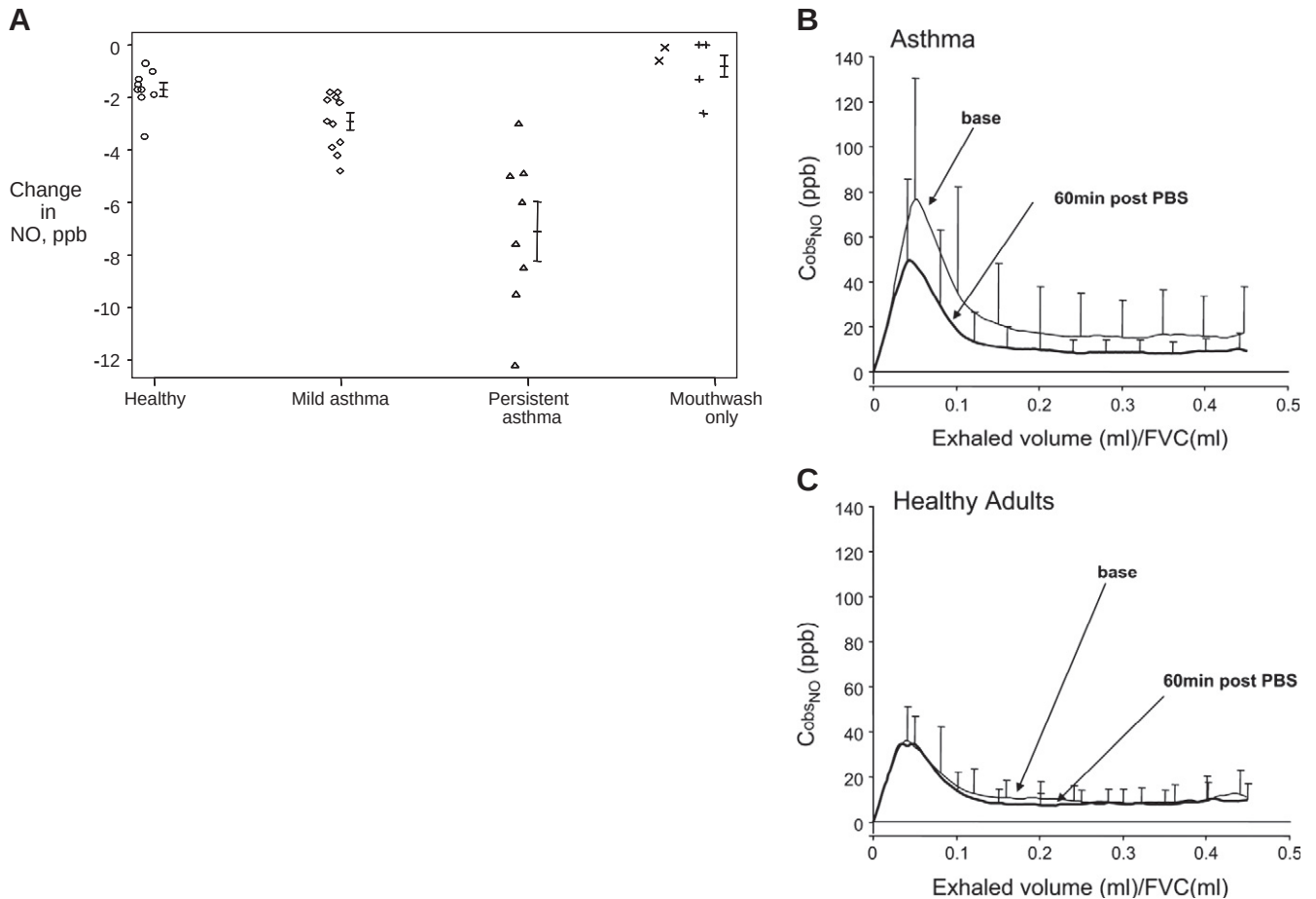


Fig. 3. Inhaled alkaline buffer decreases FE_{NO}. A. Inhaled phosphate buffered – but not buffer mouthwash – decreases FE_{NO} in a subpopulation of patients with persistent asthma (from reference [78]). Flow-dependent analysis reveals change in NO concentrate after PBS (reflecting buffering) both proximal and distal airway compartments in asthma, but no change in either compartment in healthy controls (C; from reference [79]).

buffer prevents this NO evolution, preventing loss of nitrogen oxides from the airway. Thus, a buffer challenge may be a test for low airway pH. Inhaled buffer may be a therapy for an asthma subpopulation with chronic airway damage associated with low pH (Table 1).

4.5. Increased arginase activity

Increased arginase activity is associated with worse lung function in some asthma patients [80]. This is rationalized by the paradigm that depletion of arginine results in decreased substrate availability for NOS isoforms, resulting in less S-nitrosothiol formation and increased bronchoconstriction. Arginase SNPs have been associated with human asthma [81]. Of note, S-nitrosylation of arginase 1 increases its activity [82], and S-nitrosylation of argininosuccinate synthase decreases its activity, decreasing arginine synthesis locally [83]. Thus, NOS activation may have negative feedback effects – depleting its own substrate – through effects on arginine metabolism. Specific haplotypes in the arginase 1 gene promoter – including SNP rs60689358 – have been associated with abnormal β_2 -agonist responsiveness in asthma patients [82], perhaps through a mechanism that alters NOS activity and, downstream, GRK2 S-nitrosylation [81]. Increased arginase activity has been proposed to be associated with low FE_{NO} in asthma.

4.6. Increased levels of asymmetric dimethyl arginine (ADMA)

ADMA is an endogenous arginine transport inhibitor that decreases the activity of NOS isoforms [84,85]. The lung is an important source of ADMA [85]. High ADMA levels in the airway lead to

inhibition of NOS activity, leading to airway smooth muscle constriction [86]. ADMA is broken down by dimethyl arginine dimethylhydrolase (DDAH). DDAH, in turn, is inhibited by S-nitrosylation at its active site cysteine (C-249) [84]. Thus, NOS activation can increase ADMA levels by inhibiting ADMA catabolism. This, coupled with the changes in arginine metabolism discussed above, suggests that NOS isoforms can feed back to inhibit their own activity through several mechanisms that may be disordered in asthma. Increased ADMA levels in the airway would likely be in the differential diagnosis of low FE_{NO} levels in asthma.

5. Clinical implications

Downstream of various inflammatory responses that characterize asthma, specific genotypes give rise to specific biochemical abnormalities. I anticipate these can be targeted to improve asthma outcomes and to minimize the need for corticosteroids. Novel biomarker panels will identify specific asthma subpopulations; novel treatments will target these subpopulations. However, the process of developing clinical diagnostic and management strategies will not always be simple. For example, a biomarker in use in many asthma clinics is FE_{NO} measurement; yet FE_{NO} is not a simple marker for NOS activity. That is to say, a high FE_{NO} level does not simply predict that specific NOS inhibition will be effective or corticosteroid-spacing. Indeed, there is a paradox in the nitrogen oxide biochemistry in asthma. High levels of NOS activity in asthma are associated with inflammation and airway injury; whereas low levels of NOS activity are associated with bronchoconstriction [36]. Therefore, it has always been difficult to

- [18] A. Jatakanon, S. Kharitonov, S. Lim, P.J. Barnes, Effect of differing doses of inhaled budesonide on markers of airway inflammation in patients with mild asthma, *Thorax* 54 (1999) 108–114.
- [19] L. Liu, W.G. Teague, S. Erzurum, A. Fitzpatrick, S. Mantri, R. Dweik, E.R. Bleeker, D. Meyers, W.W. Busse, W.J. Calhoun, M. Castro, K.F. Chung, D. Curran-Everett, E. Israel, W.N. Jarjour, W. Moore, S.P. Peters, S. Wenzel, J.F. Hunt, B. Gaston, National Institutes of Health/National Heart, Lung, and Blood Institute's Severe Asthma Research Program, Determinants of exhaled breath condensate pH in a large asthma population, *Chest* 139 (2011) 328–336.
- [20] S.H. Wedes, W. Wu, S.A.A. Comhair, K.M. McDowell, J.A. DiDonato, S.C. Erzurum, S.L. Hazen, Urinary bromotyrosine measures asthma control and predicts asthma exacerbations in children, *J. Pediatr.* 159 (2011) 248–255.
- [21] N.J. Tustison, T.A. Altes, G. Song, E.E. de Lange, J.P. Mugler III, J.C. Gee, Feature analysis of hyperpolarized helium-3 pulmonary MRI: a study of asthmatics versus nonasthmatics, *Magn. Reson. Med.* 63 (2010) 1448–1455.
- [22] E.E. de Lange, T.A. Altes, J.T. Patrie, J.J. Battiston, A.P. Juersvich, J.P. Mugler III, T.A. Platts-Mills, Changes in regional airflow obstruction over time in the lungs of patients with asthma: evaluation with 3He MR imaging, *Radiology* 250 (2009) 567–575.
- [23] N.V. Marozkina, W.G. Teague, D. Thompson-Batt, T. Altes, S. Erzurum, B. Gaston, S-nitrosoglutathione catabolic enzymes in severe asthma, *Am. J. Respir. Crit. Care Med.* 183 (2011) A2566.
- [24] O. Haworth, M. Cernadas, R. Yang, C.N. Serhan, B.D. Levy, Resolvin E1 regulates interleukin 23, interferon-gamma and lipoxin A4 to promote the resolution of allergic airway inflammation, *Nat. Immunol.* 9 (2008) 873–879.
- [25] O. Haworth, M. Cernadas, R. Yang, C.N. Serhan, B.D. Levy, Resolvin E1 regulates interleukin 23, interferon-gamma and lipoxin A4 to promote the resolution of allergic airway inflammation, *Nat. Immunol.* 9 (2008) 873–879.
- [26] J. Yoo, H. Tcheurekdjian, S.V. Lynch, M. Cabana, H.A. Boushey, Microbial manipulation of immune function for asthma prevention: inferences from clinical trials, *Proc. Am. Thorac. Soc.* 4 (2007) 277–282.
- [27] T.A. Platts-Mills, E. Erwin, P. Heymann, J. Woodfolk, Is the hygiene hypothesis still a viable explanation for the increased prevalence of asthma? *Source Allergy* 60 (Suppl 79) (2005) 25–31.
- [28] M.J. Ege, M. Mayer, A.C. Normand, J. Genuneit, W.O. Cookson, C. Braun-Fahrlander, D. Heederik, R. Piarroux, E. von Mutius, GABRIELA Transregio 22 Study Group, Exposure to environmental microorganisms and childhood asthma, *N. Engl. J. Med.* 364 (2011) 701–709.
- [29] A. Alemanyeh, M. Girmay, A. Atalay, J. John, G. Gail, V. Andrea, The role of acetaminophen and geohelminth infection on the incidence of wheeze and eczema: a longitudinal birth-cohort study, *Am. J. Respir. Crit. Care Med.* 183 (2011) 165–170.
- [30] G. Renzetti, G. Silvestre, C. D'Amario, E. Bottini, F. Gloria-Bottini, N. Bottini, A. Auais, M.K. Perez, G. Piedimonte, Less air pollution leads to rapid reduction of airway inflammation and improved airway function in asthmatic children, *Pediatrics* 123 (2009) 1051–1058.
- [31] D.M. Lang, M. Polansky, Patterns of asthma mortality in Philadelphia from 1969 to 1991, *N. Engl. J. Med.* 331 (1994) 1542–1546.
- [32] R. Beasley, T. Clayton, J. Crane, E. von Mutius, C.K.W. Lai, S. Montefort, A. Stewart, for the ISAAC Phase Three Study Group, Association between paracetamol use in infancy and childhood, and risk of asthma, rhinoconjunctivitis, and eczema in children aged 6–7 years: analysis from Phase Three of the ISAAC programme, *Lancet* 372 (2008) 1039–1048.
- [33] R.W. Beasley, T.O. Clayton, J. Crane, C.K. Lai, S.R. Montefort, E. Mutius, A.W. Stewart, ISAAC phase three study group. Acetaminophen use and risk of asthma, rhinoconjunctivitis, and eczema in adolescents: international study of asthma and allergies in childhood phase three, *Am. J. Respir. Crit. Care Med.* 183 (2011) 171–178.
- [34] J.F. Hunt, B. Gaston, F. Ricciardolo, Acid stress in the airway, *J. Allergy Clin. Immunol.* 84 (2004) 731–765.
- [35] J. Hunt, K. Fang, R. Malik, A. Snyder, N. Malhotra, T.A.E. Platts-Mills, B. Gaston, Endogenous airway acidification: implications for asthma pathophysiology, *Am. J. Respir. Crit. Care Med.* 161 (2000) 694–699.
- [36] H.B. Lim, T. Ichinose, Y. Miyabara, H. Takano, Y. Kumagai, N. Shimojo, J.L. Devalia, M. Sagai, Involvement of superoxide and nitric oxide on airway inflammation and hyperresponsiveness induced by diesel exhaust particles in mice, *Free Radic. Biol. Med.* 25 (1998) 635–644.
- [37] H.R. De Raev, F.B. Thunnissen, F.T. Kaneko, F.H. Guo, M. Lewis, M.S. Kavuru, M. Secic, M.J. Thomassen, S.C. Erzurum, Decreased Cu, Zn-SOD activity in asthmatic airway epithelium: correction by inhaled corticosteroid in vivo, *Am. J. Physiol.* 272 (1997) L148–154.
- [38] S.A. Comhair, K.S. Ricci, M. Arroliga, A.R. Lara, R.A. Dweik, W. Song, S.L. Hazen, E.R. Bleeker, W.W. Busse, K.F. Chung, B. Gaston, A. Hastie, M. Hew, N. Jarjour, W. Moore, S. Peters, W.G. Teague, S.E. Wenzel, S.C. Erzurum, Correlation of systemic superoxide dismutase deficiency to airflow obstruction in asthma, *Am. J. Respir. Crit. Care Med.* 172 (2005) 306–313.
- [39] P.J. Pearson, S.A. Lewis, J. Britton, A. Fogarty, Vitamin E supplements in asthma: a parallel group randomised placebo controlled trial, *Thorax* 59 (2004) 652–656.
- [40] A. Fogarty, S.A. Lewis, S.L. Scrivener, M. Antoniak, S. Pacey, M. Pringle, J. Britton, Oral magnesium and vitamin C supplements in asthma: a parallel group randomized placebo-controlled trial, *Clin. Exp. Allergy* 33 (2003) 1355–1359.
- [41] T.S. Morozkina, V.N. Sukolinskii, A.V. Strel'nikov, The selective effect of vitamin E, A, and C complexes on antioxidant protection in neoplastic and normal tissues, *Vopr. Med. Khimii* 37 (1991) 59–61.
- [42] W. Wu, M.K. Samoszuk, S.A. Comhair, M.J. Thomassen, C.F. Farver, R.A. Dweik, M.S. Kavuru, S.C. Erzurum, S.L. Hazen, Eosinophils generate brominating oxidants in allergen-induced asthma, *J. Clin. Invest.* 105 (2000) 1455–1463.
- [43] B. Gaston, J. Reilly, J.M. Drazen, J. Fackler, P. Ramdev, D. Arnette, M. Mullins, D. Sugarbaker, C. Chee, D. Singel, J. Loscalzo, J.S. Stamler, Endogenous nitrogen oxides and bronchodilator S-nitrosothiols in human airways, *Proc. Natl. Acad. Sci. U.S.A.* 90 (1993) 10957–10961.
- [44] B. Gaston, A. Doctor, D. Singel, J.S. Stamler, S-Nitrosothiol signaling in respiratory biology, *Am. J. Respir. Crit. Care Med.* 173 (2006) 1186–1193.
- [45] D. Li, G. Shirakami, X. Zhan, R. Johns, Regulation of ciliary beat frequency by the nitric oxide-cyclic guanosine monophosphate signaling pathway in rat airway epithelial cells, *Am. J. Respir. Cell Mol. Biol.* 23 (2000) 175–181.
- [46] M. Saura, C. Zaragoza, A. McMillan, R.A. Quick, C. Hohenad, J.M. Lowenstein, C.J. Lowenstein, An antiviral mechanism of nitric oxide: inhibition of a viral protease, *Immunity* 10 (1999) 21–28.
- [47] V. Venketaraman, Y.K. Dayaram, M.T. Talaue, N.D. Connell, Glutathione and nitrosoglutathione in macrophage defense against *Mycobacterium tuberculosis*, *Infect. Immun.* 73 (2005) 1886–1889.
- [48] B. Gaston, S. Sears, J. Woods, J. Hunt, M. Ponaman, T. McMahon, J.S. Stamler, Bronchodilator S-nitrosothiol deficiency in asthmatic respiratory failure, *Lancet* 351 (1998) 1317–1319.
- [49] R. Dweik, S. Comhair, B. Gaston, F. Thunnissen, C. Farver, M. Thomassen, M. Kavuru, J. Hammel, H. Abu-Soud, H.S. Erzurum, NO chemical events in the human airway during the immediate and late antigen-induced asthmatic response, *Proc. Natl. Acad. Sci. U. S. A.* 98 (2001) 2622–2627.
- [50] K. Fang, R. Johns, T. Macdonald, M. Kinter, B. Gaston, S-Nitrosoglutathione breakdown prevents airway smooth muscle relaxation in the guinea-pig, *Am. J. Physiol. Lung Cell. Mol. Biol.* 279 (2000) L716–L721.
- [51] L.D. Que, L. Liu, Y. Yan, G. Whitehead, S.H. Gavett, D.A. Schwartz, J.S. Stamler, Protection from experimental asthma by an endogenous bronchodilator, *Science* 308 (2005) 1618–1621.
- [52] L.G. Que, Z. Yang, J.S. Stamler, N.L. Lugogo, M. Kraft, M. S-nitrosoglutathione reductase: an important regulator in human asthma, *Am. J. Respir. Crit. Care Med.* 180 (2009) 226–231.
- [53] L. Liu, A. Hausladen, M. Zeng, L. Que, J. Heitman, J.S. Stamler, A metabolic enzyme for S-nitrosothiol conserved from bacteria to humans, *Nature* 410 (2001) 490–494.
- [54] H. Wu, I. Romieu, J.J. Sienra-Monge, B. Estela Del Rio-Navarro, D.M. Anderson, C.A. Jenchura, H. Li, M. Ramirez-Aguilar, I. Del Carmen Lara-Sanchez, S.J. London, Genetic variation in S-nitrosoglutathione reductase (GSNOR) and childhood asthma, *J. Allergy Clin. Immunol.* 120 (2007) 322–328.
- [55] P.E. Moore, K.K. Ryckman, S.M. Williams, N. Patel, M.L. Summar, J.R. Sheller, Genetic variants of GSNOR and ADRB2 influence response to albuterol in African-American children with severe asthma, *Pediatr. Pulmonol.* 44 (2009) 649–654.
- [56] S. Choudhry, L.G. Que, Z. Yang, L. Liu, C. Eng, S.O. Kim, G. Kumar, S. Thyne, R. Chapela, J.R. Rodriguez-Santana, W. Rodriguez-Cintrón, P.C. Avila, J.S. Stamler, E.G. Burchard, GSNOR reductase and beta2-adrenergic receptor gene-gene interaction: bronchodilator responsiveness to albuterol, *Pharmacogenet. Genomics* 20 (2010) 351–358.
- [57] R.L. Bateman, D. Rauh, B. Tavshanjan, K.M. Shokat, Human carbonyl reductase 1 is an S-nitrosoglutathione reductase, *J. Biol. Chem.* 283 (2008) 35756–35762.
- [58] N. Marozkina, M. Periasamy, D. Thompson-Batt, S. Erzurum, W.G. Teague, B. Gaston, Carbonyl reductase 1 expression and activity are increased in human asthma, *Am. J. Respir. Crit. Care Med.* 183 (2011) A2066.
- [59] E.A. Kirsch, I.S. Yuhanna, Z. Chen, Z. German, T.S. Sherman, P.W. Shaul, Estrogen acutely stimulates endothelial nitric oxide synthase in H441 human airway epithelial cells, *Am. J. Respir. Cell Mol. Biol.* 20 (1999) 658–666.
- [60] K. Brown-Steinke, K. deRonde, S. Yem, L.A. Palmer, Gender differences in S-nitrosoglutathione reductase activity in the lung, *PLoS One* 5 (2010) e14007.
- [61] B. Gaston, J.M. Drazen, A. Jansen, D.A. Sugarbaker, J. Loscalzo, J.S. Stamler, Relaxation of human bronchial smooth muscle by S-nitrosothiols *in vitro*, *J. Pharmacol. Exp. Ther.* 268 (1994) 978–984.
- [62] J.S. Stamler, E.J. Toone, S.A. Lipton, N.J. Sucher, (S)NO signals: translocation, regulation, and a consensus motif, *Neuron* 18 (1997) 691–696.
- [63] J.S. Paige, G. Xu, B. Stancevic, S.R. Jaffrey, Nitrosothiol reactivity profiling identifies S-nitrosylated proteins with unexpected stability, *Chem. Biol.* 15 (2008) 1307–1316.
- [64] M.W. Foster, D.T. Hess, J.S. Stamler, Protein S-nitrosylation in health and disease: a current perspective, *Trends Mol. Med.* 15 (2009) 391–404.
- [65] A.M. Evangelista, V.S. Rao, A.R. Filo, N.V. Marozkina, A. Doctor, D.R. Jones, B. Gaston, W.H. Guilford, Direct regulation of striated muscle myosins by nitric oxide and endogenous nitrosothiols, *PLoS One* 5 (2010) e11209.
- [66] S.J. Lewis, A. Hoque, J.N. Bates, Differentiation of L- and D-S-nitrosothiol recognition sites *in vivo*, *J. Cardiovasc. Pharmacol.* 46 (2005) 660–671.
- [67] E.J. Whalen, M.W. Foster, A. Matsumoto, K. Ozawa, J.D. Violin, L.G. Que, C.D. Nelson, M. Benhar, J.R. Keys, H.A. Rockman, W.J. Koch, Y. Daaka, R.J. Lefkowitz, J.S. Stamler, JS, Regulation of beta-adrenergic receptor signaling by S-nitrosylation of G-protein-coupled receptor kinase 2, *Cell* 129 (2007) 511–522.
- [68] A. Lipton, M. Johnson, T. Macdonald, M. Lieberman, D. Gozal, B. Gaston, S-Nitrosothiols signal the ventilatory response to hypoxia, *Nature* 413 (2001) 171–174.
- [69] W. Wei, B. Li, M.A. Hanes, S. Kakar, X. Chen, L. Liu, S-nitrosylation from GSNOR deficiency impairs DNA repair and promotes hepatocarcinogenesis, *Sci. Transl. Med.* 2 (2010) 19a13.
- [70] N.V. Marozkina, C. Wei, S. Yem, H. Wallrabe, A.S. Nagji, T. Morozkina, D.R. Jones, B. Gaston, S-Nitrosoglutathione reductase in human lung cancer, *Cancer Prev. Res.* (2011), *Am. J. Respir. Crit. Care Med.* (in press).
- [71] L. Ngamtrakulpanit, Y. Yuanlin, A. Adjei, G. Amoah, B. Gaston, J. Hunt, Identification of intrinsic airway acidification in pulmonary tuberculosis, *Glob. J. Health Sci.* 2 (2010) 106–110.

- [72] S.S. Yoon, R. Coakley, G.W. Lau, S.V. Lyman, B. Gaston, A.C. Karabulut, R.F. Hennigan, S.H. Hwang, G. Buettner, M.J. Schurr, J.E. Mortensen, J.L. Burns, D. Speert, R.C. Boucher, D.J. Hassett, Anaerobic killing of mucoid *Pseudomonas aeruginosa* by acidified nitrite under conditions characteristic of the cystic fibrosis airway, *J. Clin. Invest.* 116 (2006) 436–446.
- [73] S. Carraro, J. Doherty, K. Zaman, I.C. Gainov, R. Turner, J. Vaughan, J.F. Hunt, J. Marquez, B. Gaston, S-nitrosothiols regulate cell-surface pH buffering by airway epithelial cells during the human immune response to rhinovirus, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 173 (2006) 1186–1193.
- [74] S.J. Wadsworth, A.R. Spitzer, A. Chander, Ionic regulation of proton chemical (pH) and electrical gradients in lung lamellar bodies, *Am. J. Physiol.* 273 (1997) L427–436.
- [75] J. Hunt, E. Erwin, L. Palmer, J. Vaughan, N. Malhotra, T.A.E. Plattsmills, B. Gaston, Expression and activity of pH-regulatory glutaminase in the human airway epithelium, *Am. J. Respir. Crit. Care Med.* 165 (2002) 101–107.
- [76] D.E. Jensen, G.K. Belka, G.C. Du Bois, S-nitrosoglutathione is a substrate for rat alcohol dehydrogenase class III isoenzyme, *Biochem. J.* 331 (1998) 659–668.
- [77] R. Greenwald, A.M. Fitzpatrick, B. Gaston, N.V. Marozkina, S. Erzurum, W.G. Teague, Breath formate is a marker of airway S-nitrosothiol depletion in severe asthma, *PLoS One* 5 (2010) e11919.
- [78] B. Gaston, R. Kelly, P. Urban, L. Liu, E.M. Henderson, A. Doctor, W.G. Teague, A. Fitzpatrick, S. Erzurum, J.F. Hunt, Buffering airway acid decreases exhaled nitric oxide in asthma, *J. Allergy Clin. Immunol.* 118 (2006) 817–822.
- [79] H-W. Shin, D.A. Shelley, A. Fitzpatrick, B. Gaston, S.C. George, Airway nitric oxide is reduced following phosphate buffered saline inhalation in asthma, *J. Appl. Physiol.* 102 (2007) 1028–1033.
- [80] A. Lara, S.B. Khatri, Z. Wang, S.A. Comhair, W. Xu, R.A. Dweik, M. Bodine, B.S. Levison, J. Hammel, E. Bleecker, W. Busse, W.J. Calhoun, M. Castro, K.F. Chung, D. Curran-Everett, B. Gaston, E. Israel, N. Jarjour, W. Moore, S.P. Peters, W.G. Teague, S. Wenzel, S.L. Hazen, S.C. Erzurum, National Heart, Lung, and Blood Institute's Severe Asthma Research Program, Alterations of the arginine metabolome in asthma, *Am. J. Respir. Crit. Care Med.* 178 (2008) 673–681.
- [81] A.A. Litonjua, J. Lasky-Su, K. Schneiter, K.G. Tantisira, R. Lazarus, B. Klanderman, J.J. Lima, C.G. Irvin, S.P. Peters, J.P. Hanrahan, S.B. Liggett, G.A. Hawkins, D.A. Meyers, E.R. Bleecker, C. Lange, S.T. Weiss, ARG1 is a novel bronchodilator response gene: screening and replication in four asthma cohorts, *Am. J. Respir. Crit. Care Med.* 178 (2008) 688–694.
- [82] L. Santhanam, H.K. Lim, H.K. Lim, V. Miriel, T. Brown, P.M. Balanson, S. Ryoo, M. Anderson, K. Irani, F. Khanday, L. Di Costanzo, D. Nyhan, J.M. Hare, D.W. Christianson, R. Rivers, A. Shoukas, D.E. Berkowitz, Inducible NO synthase dependent S-nitrosylation and activation of arginase 1 contribute to age-related endothelial dysfunction, *Circ. Res.* 101 (2007) 692–702.
- [83] G. Hao, L. Xie, S.S. Gross, Argininosuccinate synthetase is reversibly inactivated by S-nitrosylation in vitro and in vivo, *J. Biol. Chem.* 279 (2004) 36192–36200.
- [84] J. Leiper, J. Murray-Rust, N. McDonald, P. Vallance, S-nitrosylation of dimethylarginine dimethylaminohydrolase regulates enzyme activity: further interactions between nitric oxide synthase and dimethylarginine dimethylaminohydrolase, *Proc. Natl. Acad. Sci. U.S.A.* 99 (2002) 13527–13532.
- [85] P. Bulau, D. Zakrzewicz, K. Kitowska, J. Leiper, A. Gunther, F. Grimminger, O. Eickelberg, Analysis of methylarginine metabolism in the cardiovascular system identifies the lung as a major source of ADMA, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 292 (2007) L18–24.
- [86] E. Klein, J. Weigel, M.C. Buford, A. Holian, S.M. Wells, Asymmetric dimethylarginine potentiates lung inflammation in a mouse model of allergic asthma, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 299 (2010) L816–825.
- [87] R.A. Dweik, R. Sorkness, S. Wenzel, J. Hammel, D. Curran-Everett, S.A. Comhair, E. Bleecker, W. Busse, W. Calhoun, M. Castro, K.F. Chung, E. Israel, N. Jarjour, W. Moore, S. Peters, W.G. Teague, B. Gaston, S.C. Erzurum, Use of exhaled nitric oxide measurement to identify a reactive, at-risk phenotype among patients with asthma, *Am. J. Respir. Crit. Care Med.* 181 (2010) 1033–1041.
- [88] A. Snyder, M.E. McPherson, J.F. Hunt, M. Johnson, J.S. Stamler, B. Gaston, Acute effects of aerosolized S-nitrosoglutathione in cystic fibrosis, *Am. J. Respir. Crit. Care Med.* 165 (2002) 922–926.
- [89] S. Kazani, M.E. Wechsler, E. Israel, The role of pharmacogenomics in improving the management of asthma, *J. Allergy Clin. Immunol.* 125 (2) (2010) 295–302.
- [90] P.E. Moore, Influence of gene–gene interactions on response to albuterol therapy, *Pharmacogenomics* 12 (1) (2011) 1–3.