Targeting arginase and nitric oxide metabolism in chronic airway diseases and their co-morbidities

Mariska PM van den Berg¹,², Herman Meurs¹,² and Reinoud Gosens¹,²

In the airways, arginase and NOS compete for the common substrate l-arginine. In chronic airway diseases, such as asthma and COPD, elevated arginase expression contributes to airway contractility, hyperresponsiveness, inflammation and remodeling. The disrupted l-arginine homeostasis, through changes in arginase and NOS expression and activity, does not only play a central role in the development of various airways diseases such as asthma or COPD. It possibly also affects l-arginine homeostasis throughout the body contributing to the emergence of co-morbidities. This review focusses on the role of arginase, NOS and ADMA in co-morbidities of asthma and COPD and speculates on their possible connection.

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Introduction
Arginase catalyzes the reaction in which l-arginine is converted to l-ornithine and urea. In humans, two arginase isoenzymes have been identified, arginase 1 and arginase 2, that differ in cellular location and tissue distribution [1]. Both arginase enzymes are constitutively expressed in the airways. The cytosolic arginase 1 and mitochondrial arginase 2 can particularly be found in airway endothelial cells, epithelial cells, fibroblasts and macrophages [2]. Furthermore, the expression of both enzymes can be induced in airway smooth muscle cells [3,4].

Downstream metabolism of l-ornithine leads to the formation of polyamines and l-proline, which are involved in cell proliferation and differentiation, and collagen production, respectively [1,5]. Next to the effects of metabolic products of arginases, many biological effects of the enzymes are related to their competition with nitric oxide synthases (NOS) for the common substrate l-arginine. Three distinct NOS enzymes are expressed in mammals; endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). As eNOS and nNOS are constitutively expressed in the airway epithelium, in inhibitory nonadrenergic noncholinergic neurons (nNOS) and airway vascular endothelial cells (eNOS), they are also referred to as constitutive NOS (cNOS). All NOS iso-enzymes use l-arginine for the formation of nitric oxide (NO) and l-citrulline. Increases in intracellular calcium concentrations, through the action of agonists or membrane depolarization, trigger cNOS to produce relatively low amounts of NO. iNOS is particularly expressed in epithelial cells and macrophages during inflammation. In contrast to cNOS, iNOS produces large amounts of NO and enzyme activation is dependent on changes gene expression, among others induced by proinflammatory cytokines [6]. Furthermore, when l-arginine levels are low, for example due to elevated arginase activity, NOS is uncoupled and superoxide is formed. Superoxide rapidly reacts with NO to form peroxynitrite, often leading to detrimental effects in the tissue by nitration of tyrosine residues [7].

The arginase and NOS pathways may interact at different levels (Figure 1). This could be through competition for l-arginine, inhibition of arginase by the intermediate NOs metabolite N0-hydroxy-l-arginine and through l-ornithine that causes feedback inhibition of arginase and inhibition of l-arginine uptake by cells producing NO. Next to arginase, NOS and their metabolic products, also methylated arginines such as the arginine derivatives asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) can greatly influence l-arginine homeostasis [8]. ADMA and its inactive stereoisomer SDMA are primarily formed as byproducts during the degradation of methylated arginine containing residues. Furthermore, small amounts of ADMA may be produced from free arginine directly [9]. Whereas ADMA serves as an endogenous competitive inhibitor of NOS, SDMA influences NO synthesis by competing with arginine and other methylated arginines for cellular transport [8].
We and others previously showed that an increased arginase activity in the airway contributes to airway obstruction and hyperresponsiveness, by reducing the available substrate for cNOS and iNOS [10]. As a result, production of bronchodilatory NO is decreased and superoxides are formed, which react with NO to form peroxynitrite, thereby enhancing airway contraction and inflammation. Furthermore, elevated airway arginase activity leads to increased L-ornithine production. Which potentially contributes to airway remodeling by increased cell proliferation and collagen formation [10,11]. The disrupted L-arginine homeostasis, through changes in arginase and NOS expression and activity, does not only play a central role in the development of various airways diseases such as asthma or COPD. It possibly also affects L-arginine homeostasis throughout the body contributing to the emergence of co-morbidities. This review focusses on the role of arginase and NOS in co-morbidities of asthma and COPD (Table 1) and speculates on their possible connection.

**Asthma**

The chronic airway inflammatory disease asthma is associated with enhanced levels of exhaled NO generated by iNOS in the airway epithelium [12]. In asthmatic patients local and systemic changes in iNOS, peroxynitrite, arginase, ADMA and arginine levels have been observed and are associated with i.a. lung function and asthma severity [5*,10,13]. In support, gene association studies in asthmatic patients [14] and different animal models of allergic asthma [15*,16] show a key role for arginase in different aspects of the disease.

Allergic rhinitis is a frequent co-morbidity of asthma [17]. Allergic rhinitis patients show increased nasal arginase and iNOS expression [18,19], and changes in nitrite/nitrate and nitrite serum levels during symptomatic periods [20,21]. Furthermore, peroxynitrite plays a likely role in nasal blockage after allergen encounter [22]. Interestingly, the role of arginase in allergic rhinitis has not much been studied. Treatment of allergic rhinitis patients with...
Table 1

<table>
<thead>
<tr>
<th>Co-morbidity</th>
<th>Changes in l-arginine regulation</th>
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<tbody>
<tr>
<td>Allergic dermatitis</td>
<td>iNOS and eNOS in dermal lesions [43,45]; iNOS induces α-MSH [44]; arginase activity in skin granulocytes and plasma [42].</td>
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<tr>
<td>Allergic rhinitis</td>
<td>In nasal mucosa: iNOS [10]; arginase 1 and 2 [18]. In serum: arginase, nitrite and nitrite/nitrate [20,21]. Role of ONOO− in nasal blockage [22].</td>
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<tr>
<td>Cardiovascular disease</td>
<td>Hypoxia induced arginase expression [51,52,54] leads to cardiac contractility and recovery and remodeling [39,53,55]. Arginase 1 enhances stability of atherosclerotic plaques [57].</td>
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<tr>
<td>Cerebrovascular disease</td>
<td>Arginase expression leads to vessel narrowing [57]; recovery after stroke [58]; AMDA expression leads to cerebral blood flow and chance of stroke [59-61].</td>
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<td>Lung cancer</td>
<td>Arginase 1 expression in myeloid cells and tumor samples [75], possibly leads to tumor proliferation [73,79] by polyamine production or NO; arginase as marker of T-cell induced tolerance [72].</td>
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<td>Metabolic syndrome</td>
<td>Cytokines and hypoxia induce: arginase, ADMA; and systemic NO [59].</td>
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<td>Muscle wasting</td>
<td>Ornithine production, possibly by arginase activity, leads to creatine production [80]. M2 macrophages promote muscle proliferation [81].</td>
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<td>Obesity</td>
<td>In blood and liver: arginase and NO [64,65,82]. In adipose tissue: p38/MAPK induces eNOS uncoupling [83]; arginase and l-arginine deficiency [66]; adipose tissue m1 macrophage infiltration and inflammation [69,84].</td>
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<td>Obstructive sleep apnea syndrome</td>
<td>In serum: arginase activity and NO levels [37,38]; plasma NO after treatment [37,41].</td>
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<td>Osteoporosis</td>
<td>Arginase expression and activity in bone and BMSCs [70].</td>
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<tr>
<td>Psychological diseases</td>
<td>Major depression: arginase serum activity [25]; platelet NOS activity and plasma NO metabolites [27]; ADMA concentration [26]. Chronic stress: iNOS and nNOS in neocortex and hippocampus [29].</td>
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<td>Respiratory infection</td>
<td>Arginase in lung myeloid cells leads to pathogen survival [31**,32]. iNOS shortly after infection by Th1 cells; NO, NO metabolites and ADMA [39-42]; conversion of l-citrulline by T-cells leads to l-arginine [31**].</td>
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<td>Type II diabetes</td>
<td>Insulin resistance correlated with ADMA and arginase [88]. High glucose induces arginase and NO production [70]. Changes in arginase 1 metabolism in macrophages [85].</td>
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</tbody>
</table>

ADMA, asymmetric dimethylarginine; BMSC, bone marrow stromal cell; eNOS, endothelial NOS; iNOS, inducible NOS; MAPK, mitogen-activated protein kinase; α-MSH, α-melanocyte-stimulating hormone; NO, nitric oxide; nNOS, neuronal nitric oxide synthase; ONOO−, peroxynitrite.

the leukotriene antagonist montelukast, leads to decreased arginase serum levels compared to the control group [23], indicating that in allergic rhinitis, arginase may be affected by mast-cell mediator release, leading to a reduced bioavailability of l-arginine for NOS.

Psychological disorders such as depression and anxiety are important co-morbidities of both asthma and COPD [17,24]. Major depressed patients show a positive correlation between arginase activity and disease severity [25] and elevated ADMA and decreased SDMA concentration [26]. A significant decrease in arginase levels, an increase in l-arginine/ADMA ratio and a trend for increased global arginine bioavailability is observed after first improvement at hospital discharge [26]. In addition, patients with major depression have lower levels of platelet NOS activity and NO metabolites in plasma [27]. Antidepressants have normalizing effects on plasma NO levels [28]. Several studies have looked into the effect of NOS isoenzymes and inhibition on various brain areas during stress, however these show contrasting results [29].

After viral and bacterial respiratory infection, common causes of asthma exacerbation [30], toll like receptor stimulation upregulates arginase in lung myeloid cells [31**,32]. In response, iNOS is upregulated in lung macrophages and polymorphonuclear leukocytes shortly after infection, leading to an increase in NO production, NO metabolites and ADMA levels [33-35] promoting inflammation. T-cells can replenish l-arginine levels through the conversion of l-citrulline [31**], thereby supporting the anti-viral or anti-bacterial response. Inhibition of arginase with 2(δ)-amino-6-borohexanoic acid (ABH), leads to a similar increase in l-arginine [35].

Nasal obstruction, an increased upper airway collapsibility and a decrease in pharyngeal cross-sectional area in asthma patients can promote symptoms of obstructive sleep apnea syndrome (OSAS) [36]. In OSAS patients, serum NO levels are reduced compared to control subjects [37,38]. In line, serum arginase activity is increased [38]. This might be induced by intermittent hypoxia that can also cause upregulation of pulmonary arginase and pulmonary arterial hypertension [39]. Moreover, concentrations of ADMA are found to be increased [40]. Two independent studies showed that, plasma NO levels can be increased by treatment with continuous positive airway pressure [37,41]. Whether OSAS treatment also decreases arginase activity has not been investigated.

In contrast, granulocytes and plasma of allergic dermatitis patients show a decreased arginase activity compared to controls [42]. iNOS expression is found to be upregulated in skin biopsies of allergic dermatitis patients [43].
finding is supported by two different mouse models of allergic dermatitis, where it is shown that iNOS possibly induces α-melanocyte-stimulating hormone, leading to exacerbation of symptoms [44] and an increase in protein-bound nitrosothine in eosinophils in skin lesions due to a disrupted NO-balance [45].

**COPD**

As in asthma, also in COPD, increased expression of arginase has been reported, and tobacco smoke may increase expression of arginase in human subjects [46,47]. Increased ADMA levels have also been reported in COPD, and both the increased expression of arginase and ADMA contribute to remodeling and inflammation, via both NO-dependent and NO-independent pathways [48,49,50*]. Accordingly, arginase inhibition protects against the development of COPD-like inflammation and remodeling in a guinea pig model of COPD [49].

Inhibition of NO production appears to not only regulate local airway inflammation, remodeling and reactivity but is a key regulatory mechanism in cardiovascular changes in COPD as well. Local hypoxia in tissues promotes arginase activity, and represses vasodilating NO production [51,52]. In the lung, this mechanism contributes to pulmonary hypertension and arginase inhibition protects against the development of pulmonary hypertension and right cardiac remodeling [39,49]. Likewise, hypoxia in left heart failure drives arginase expression by endothelial cells, which plays a clear role in repressing cardiac contractility and recovery from ischemia [53,54]. Accordingly, inhibition of arginase promotes cardiac contractility and improves cardioprotection after injury [55].

Such a mechanism may also contribute to cerebrovascular disease, which is often found as a co-morbidity in patients with COPD [24]. NO is critical in blood flow regulation in the brain [56]. Therefore, increased arginase expression may lead to vasoconstriction, increasing susceptibility to stroke [57]. In support, arginase 2 deficient mice have improved cerebral blood flow after brain injury [58]. In addition, ADMA is considered a prime biomarker and driver of impaired cerebral blood flow and stroke [59–61]. Though not directly related to arginase, ADMA expression is increased in smokers and shunts arginine to the arginase pathway [50*], providing a clear mechanistic link between increases in ADMA and arginase activity. Surprisingly little data is available on pharmacological arginase inhibition and its impact on cerebrovascular disease, although clearly such studies would be of considerable interest.

In COPD and, as indicated above, also in asthma, several metabolic changes occur that impact on systemic co-morbidities in COPD such as muscle wasting, osteoporosis and type II diabetes [17,24]. It is not fully clear how changes in arginase expression in COPD contribute to each of these co-morbidities specifically, although general relationships between arginase and nitric oxide metabolism on the one hand and muscle wasting, osteoporosis and type II diabetes have been reported. Tumor necrosis factor (TNF) driven nuclear factor-κB activation underlies muscle wasting [62] and is inhibited by NO mediated S-nitrosylation of p65 and inhibitor of NF-κB kinase [63]. Increased arginase activity in COPD may suppress this, leading to enhanced p65 activation. De-repression of NO-mediated anti-inflammatory effects and endothelial cell function due to elevated arginase activity may also play a role in fatty acid driven changes in insulin sensitivity and obesity [64]. Thus, increased expression of arginase has been reported in obese subjects in comparison to normal weight subjects [51,65] and arginase overexpression has been shown to drive eNOS uncoupling in mice aortas in response to overweight [66*]. Furthermore, arginase inhibition restores endothelial dysfunction, hepatic abnormalities and adipose tissue inflammation (interleukin-6, TNF-α, M1 macrophage counts) [67,68*,69] in animal models. Arginase is also abundantly expressed in bone, and streptozocin-induced diabetes was associated with reductions in bone mass and bone mineral density, both of which could be prevented by the arginase inhibitor ABH [70*].

Lung cancer is a co-morbidity of COPD with a major role for arginase. Arginase expression is elevated in non-small cell lung cancer and drives proliferation by tumor cells and tumor associated fibroblasts, possibly via polyamine production or by lowering vasodilating NO, facilitating hypoxia that promotes cancer stem cell survival [71]. Arginase is also a marker of myeloid derived suppressor cells that are anti-inflammatory and repress cytotoxic T-cell responses [72]. In T-cell biology, arginase therefore promotes tolerance and arginase inhibition boosts antitumor T-cell activity [73*,74,75].

**Concluding remarks**

It is clear that a disordered l-arginine homeostasis by changes in arginase, NOS and ADMA activity and expression, is not only vital in the chronic airway diseases, asthma and COPD, but also seems to play an important role in many co-morbidities. Unknown, however, is whether l-arginine disbalance in the lung systemically affects other organs, thereby contributing to the development of co-morbidities. Moreover, it is not clear in how far altered l-arginine metabolism in systemic comorbidities may contribute to the severity of asthma and COPD. In the same line of reasoning, it is also largely unknown if restoring l-arginine-balance in the airways, for example by using arginase inhibitors, will alleviate symptoms of co-morbidities and vice versa. Remarkably, we recently found that the arginase inhibitor ABH inhibited airway inflammation and remodeling as well as right ventricular hypertrophy in a guinea pig model of COPD [49].
Currently, lung diseases are preferably treated locally by inhalation of nebulized drugs. In this way, low doses of drugs can be used and side-effects are reduced. In animal models of asthma and COPD, both systemic [76] and local treatment with arginase inhibitors lead to an increase in bioavailable $\omega$-arginine and reduced pulmonary symptoms [16,49,77]. However, most studies in this area have focused on the organ of interest instead of systemic effects of arginase inhibition. As with all treatments, caution should be paid to potential side-effects occurring during the use of arginase inhibitors, particularly with regard to ammonia detoxification in the liver. However, short-term, as well as long-term systemic treatment in animal models of hypertension and atherosclerosis did not show any toxic side-effects or induction of a compensatory enzyme upregulation [78]. Naturally, each co-morbidity requires a different approach in treatment. Nevertheless, it would be beneficial if possible treatment of asthma or COPD by arginase inhibitors, or other drugs interfering with $\omega$-arginine metabolism, could also lead to relief of symptoms in other organs.

Conflict of interest statement
MvdB declares no relevant conflict of interest. HM declares to have received grant support from Boehringer Ingelheim and is co-author on patent US12/515,866 on the use of arginase inhibitors in the treatment of asthma and allergic rhinitis. RG declares to have received grant support from Boehringer Ingelheim, Chiesi and Aquilo.

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References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

1. of special interest
2. of outstanding interest


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This is the first study showing that during mycobacterial infection, T-cells use L-citrulline in microenvironments with low bioavailable L-arginine to replenish L-arginine pools, thereby supporting anti-mycobacterial defense. These findings could have an impact on the development of new therapies and vaccines against respiratory infections.


In a cohort of COPD patients with evidence of a persistent low-grade systemic inflammation, the authors found a significant positive linear relationship between airflow limitation and ADMA. This indicates an influence of ADMA on the development and progression of COPD, and creates possibilities for new therapeutic approaches.


In a mouse model of high-fat diet induced obesity, it was demonstrated that L-arginine deficiency and eNOS uncoupling in perivascular adipose tissue causes a clear reduction in aorta vasodilator response to acet ylcholine, which could be reversed by use of L-arginine treatment. This study shows a possibility for treatment strategies for obesity-induced vascular disease.


This study provides a novel mechanistic and potential therapeutic view on insulin resistance in human obesity and an animal model of insulin resistance, by showing that up-regulation of arginase and increases in ADMA are important factors contributing alterations in the L-arginine/NO-pathway and endothelial dysfunction.


By using both in vitro and in vivo models, the authors show the role of disrupted L-arginine homeostasis, and the effect of arginase inhibition, in bone-related complications of diabetes. These findings could impact novel therapeutic approaches for musculoskeletal related diseases such as osteoporosis.


The authors demonstrated for the first time that a recombinant human enzyme could induce caspase-dependent apoptosis and cytotoxicity in NSCLC cells. Thereby providing a novel insight in the role of arginase in autophagy and providing a novel approach in NSCLS treatment.


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