Metabolism of asymmetric dimethylarginine in hypoxia: from bench to bedside

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Abstract

Acute hypoxia and chronic hypoxia induce pulmonary vasoconstriction. While hypoxic pulmonary vasoconstriction is a physiological response if parts of the lung are affected, global exposure to hypoxic conditions may lead to clinical conditions like high-altitude pulmonary hypertension. Nitric oxide is the major vasodilator released from the vascular endothelium. Nitric oxide-dependent vasodilation is impaired in hypoxic conditions. Inhibition of nitric oxide synthesis is the most rapid and easily reversible molecular mechanism to regulate nitric oxide-dependent vascular function in response to physiological and pathophysiological stimuli. Asymmetric dimethylarginine is an endogenous, competitive inhibitor of nitric oxide synthase and a risk marker for major cardiovascular events and mortality. Elevated asymmetric dimethylarginine has been observed in animal models of hypoxia as well as in human cohorts under chronic and chronic intermittent hypoxia at high altitude. In lowlanders, asymmetric dimethylarginine is high in patients with pulmonary hypertension. We have recently shown that high asymmetric dimethylarginine at sea level is a predictor for high-altitude pulmonary hypertension. Asymmetric dimethylarginine is a highly regulated molecule, both by its biosynthesis and metabolism. Methylation of L-arginine by protein arginine methyltransferases was shown to be increased in hypoxia. Furthermore, the metabolism of asymmetric dimethylarginine by dimethylarginine dimethylaminohydrolases (DDAH1 and DDAH2) is decreased in animal models of hypoxia. Whether these changes are caused by transcriptional or posttranslational modifications remains to be elucidated. Current data suggest a major role of asymmetric dimethylarginine in regulating pulmonary arterial nitric oxide production in hypoxia. Further studies are needed to decipher the molecular mechanisms regulating asymmetric dimethylarginine in hypoxia and to understand their clinical significance.

Both acute hypoxia and chronic hypoxia (CH) induce pulmonary vasoconstriction. While regional hypoxic pulmonary vasoconstriction (HPV) is a physiological response if parts of the bronchial tree are obstructed, global exposure to hypoxic conditions may lead to severe clinical conditions like high-altitude pulmonary hypertension (HAPH). Being the major endothelium-derived vasodilator, nitric oxide (NO) counterbalances the impact of vasoconstrictor stimuli like endothelin-1, angiotensin II, and others.[1,2] NO formation is enhanced by hypoxia in the systemic vasculature, where hypoxia causes compensatory vasodilation and enhanced blood flow.[3] The role of NO in modulating HPV has remained much less clear. For example, inhibition of NO formation in porcine pulmonary arterioles resulted in enhanced hypoxic

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vasoconstriction,[4] but acute exposure to high-altitude-associated hypobaric hypoxia was paralleled by increased generation of NO in healthy mountaineers.[5] Thus, it appears that NO release in the pulmonary circulation modulates the pulmonary vasoconstrictor response to hypoxia, but it does not completely prevent it. Asymmetric dimethylarginine (ADMA) is the major endogenous inhibitor of NO synthase (NOS); it has been shown to be increased in animal models of hypoxia and in human studies. This review aims to summarize our current understanding of the role of the endothelial L-arginine–ADMA–NO pathway in the responses of the systemic and pulmonary circulation to hypoxia, in HAPH, and in pulmonary arterial hypertension (PAH) in general.

Biology of the dimethylarginines

Dimethylarginines are formed during the methylation of L-arginine residues within specific proteins, a process that is catalyzed by a family of enzymes named protein arginine methyltransferases (PRMTs). The complex process of arginine methylation is a mechanism of posttranslational modification of proteins which affects protein function and has been extensively been reviewed elsewhere.[6] When methylated proteins are cleaved during physiological protein turnover, monomethyl-L-arginine (NMMA) as well as ADMA and symmetric dimethylarginine (SDMA) are released. ADMA and NMMA are competitive inhibitors of NO synthesis, while SDMA does not directly inhibit NOS activity (for an extensive review, cf., Böger[7]). ADMA has been shown to be associated with numerous cardiovascular and metabolic diseases, cardiovascular risk factors, and it has been characterized as a prospective marker of major adverse cardiovascular events and mortality.[8] Furthermore, ADMA and SDMA interfere with the cellular uptake of L-arginine by the y + carrier for basic amino acids,[9] thereby potentially reducing the bioavailability of L-arginine as substrate for NOS activity.

ADMA, but not SDMA, is a substrate for dimethylarginine dimethylaminohydrolase (DDAH), an enzyme that occurs in at least two isoforms with distinct tissue distribution and regulation.[10] DDAH degrades ADMA to L-citrulline and dimethylamine. An alternative metabolic pathway for both dimethylarginines has recently been identified: Alanine glyoxylate aminotransferase-2 (AGXT2) can utilize both, ADMA and SDMA, as alternative substrates, degrading them to dimethylguanidinovaleric acid.[11,12] Fig. 1 depicts the complex pathways involved in the regulation of endogenous methylarginines.

ADMA has been shown to be a risk marker of major cardiovascular events and mortality in numerous studies including populations with a broad range of cardiovascular risk.[13]

ADMA is increased in hypoxia and in PAH: Observational human studies

Multiple observational studies in human cohorts that included a broad range of different populations as well as patients with chronic lung diseases reported elevated ADMA concentrations. Table 1 gives an overview of published observational studies of ADMA in human cohorts.

Acute or chronic exposure to high-altitude results in hypobaric hypoxia, a condition enabling us to dissect the effects of hypoxia in its purest manner if primarily healthy human subjects are being studied. Our group longitudinally followed a group of 72 primarily healthy male army draftees during three months of military services at high altitude in the Andean mountains (five days at 3550 m, followed by two days at sea level per week).[26] We compared these individuals, who were exposed to chronic-intermittent hypobaric hypoxia (CIH), with a group of 16 male Andean natives who had lived at an altitude above 3500 m for most of their lives (CH). In this study, we were the first to report a continuous increase of ADMA but not of SDMA in CIH; Andean natives had significantly higher ADMA levels than the CIH cohort after three months of exposure (Fig. 2a and b). We recently validated these results in another cohort of 100 primarily healthy male Chilean subjects who had never been exposed to altitude before and were followed for up to six months of CIH exposure.[15] In this cohort, ADMA continued to increase throughout the six months' observational period, while SDMA levels significantly dropped during the same time period (Fig. 2c and d). In another study, we measured ADMA and SDMA in a cohort of 120 Chilean mining workers who had been exposed to CIH for a mean of 14 ± 0.5 years.[16] As compared to healthy reference populations, ADMA was elevated in this group, while SDMA was within published reference ranges.

Both chronic and chronic intermittent hypoxia exposure may eventually result in PAH and right ventricular hypertrophy.[16] The cut-off level of mean pulmonary arterial pressure (mPAP) to diagnose PAH in lowlanders is 25 mm Hg. For individuals exposed to high altitude, this threshold has been set to 30 mm Hg to account for the fact that mild to moderate increases in mPAP occur even in healthy individuals.[27] In our recent study in Chilean mining workers, individuals who had been diagnosed with HAPH (mPAP > 30 mm Hg) had significantly higher ADMA concentrations ($1.01 \pm 0.15 \,\mu mol/l$) than those without HAPH ($0.81 \pm 0.18 \,\mu mol/l$; $p < 0.001$).[16] Although it cannot be excluded that possible confounders contributed to this difference, this remarkable result may help to identify people at risk at an early time point. Further clinical validation is urgently required for this purpose.

Another condition that may arise from high-altitude exposure is high-altitude pulmonary edema (HAPE)—a condition associated with leakage of the pulmonary endothelium and fluid accumulation in the lung interstitium. Although it is still unclear whether a change in endothelial NO formation is involved in the development of HAPE, NO clearly is a mediator that affects endothelial integrity.[28–30] Ali et al. studied a group of 400 primarily healthy sojourners at high altitude (3500 m), of whom 200 had developed
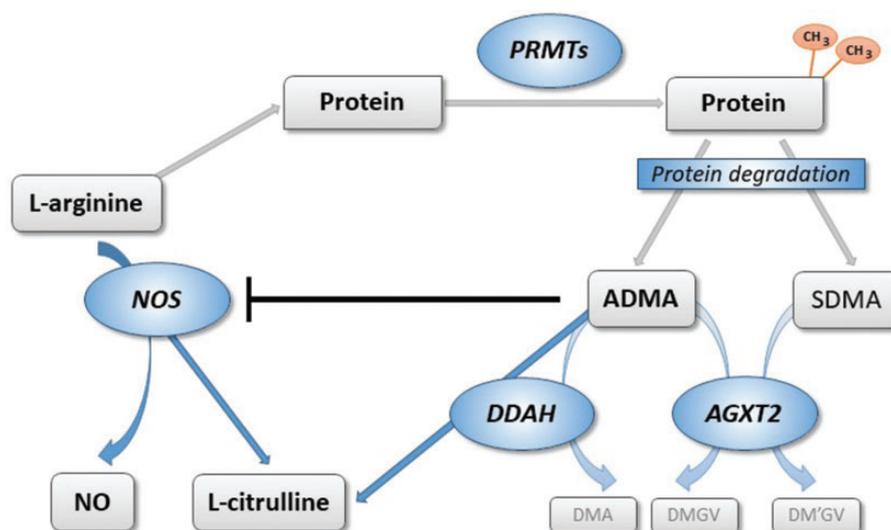
Fig. 1. Biosynthesis and metabolism of ADMA and SDMA. L-arginine residues within specific proteins are subject to methylation by PRMTs. Degradation of di-methylated proteins during physiological protein turnover results in the release of ADMA and SDMA. ADMA is a competitive inhibitor of NOS, while SDMA does not directly impair NOS activity. ADMA, but not SDMA, is enzymatically degraded by DDAH into L-citrulline and DMA. DDAH exists in two distinct isoforms with different regulation and tissue distribution. Both dimethylarginines may be cleaved by an alternative pathway through the activity of AGXT2, resulting in the formation of symmetric or asymmetric dimethylguanidinovaleric acid (DMGV and DM'GV). AGXT2: alanine glyoxylate aminotransferase 2; ADMA: asymmetric dimethylarginine; PRMT: protein arginine N-methyltransferase; NO: nitric oxide; NOS: NO synthase; SDMA: symmetric dimethylarginine; DDAH: dimethylarginine dimethylaminohydrolase; DMA: dimethylamine.

HAPE and 200 had remained healthy, and compared them with high-altitude residents of Tibeto-Burman ethnicity.[17] They found elevated levels of the vasoconstrictor mediator, endothelin-1, and increased activity of the renin-angiotensin-aldosterone system, but decreased levels of NO, in altitude sojourners as compared to altitude residents. These changes were significantly more prominent in HAPE patients than in HAPE-free individuals; HAPE patients also had a significantly elevated mean ADMA concentration and lower levels of superoxide dismutase as compared to both, HAPE-free sojourners and altitude residents. These data, however, are difficult to interpret because another study yielded opposite results: Tannheimer et al. studied 12 healthy human volunteers who stayed at hypobaric hypoxia in a hypobaric chamber for 24 h.[18] Five of the investigated individuals (42%) developed acute mountain sickness (AMS, Lake Louise score (LLS) > 5), had a massive increase in mPAP above 40 mm Hg, and showed a decrease in plasma ADMA concentration (−36.2%), while four individuals with mild AMS (LLS 0–3) and less increase in mPAP (<40 mm Hg) had a significant increase in ADMA (+36.3 %); the remaining three study participants had values in-between. On the other hand, we observed a significant positive association of ADMA with acclimatization status to high altitude,[26] where poor acclimatization was defined as the presence of AMS plus arterial oxygen saturation below 89%). Thus, the association of ADMA with AMS and HAPE remains to be further elucidated.

Besides exposure to high altitude, chronic or chronic intermittent hypoxia may also occur in lung diseases like sleep apnea syndrome and the advanced stages of chronic obstructive lung diseases. A group of Turkish investigators compared 40 patients with obstructive sleep apnea syndrome (OSAS) to 20 healthy controls.[19] They observed higher ADMA but lower L-arginine serum concentrations in OSAS patients than in healthy controls. These differences were independent of the presence of traditional cardiovascular risk factors, as the subgroup of OSAS patients without risk factors showed similar differences to controls. In another study, Telo et al.[20] compared patients with chronic-obstructive lung disease (COPD) who had developed PAH (defined as systolic PAP (sPAP) > 35 mm Hg) to COPD patients without elevated sPAP and to healthy controls. They found a significantly elevated mean ADMA concentration in COPD patients with PAH as compared to both other groups. In this study, ADMA was negatively correlated with arterial oxygen saturation and positively correlated with sPAP.

In adult patients with congenital heart disease, plasma ADMA levels were significantly higher in patients with PAH than in those without PAH.[31]

Another series of studies analyzed ADMA concentration in various subtypes of PAH according to the current Nice classification of the World Health Organization.[32] Kielstein et al. reported significantly higher ADMA plasma concentrations in patients with idiopathic PAH than in healthy controls (0.53 ± 0.15 µmol/l; controls, 0.36 ± 0.05 µmol/l; $p < 0.001$).[21] ADMA is also elevated in PAH associated with connective tissue disease: Dimitroulas et al. studied 66 patients with systemic sclerosis who had developed

Table 1. Observational studies of ADMA in chronic hypoxia and pulmonary arterial hypertension in humans.

Clinical condition	Study design	Observation	Reference
High altitude			
CIH	72 healthy Chilean lowlanders exposed to CIH during three months; 16 Andean highlander natives	ADMA ↑ by x% in CIH; no change in SDMA in CIH; highest ADMA in highland natives	Lüneburg et al.[14]
CIH	100 healthy Chilean lowlanders exposed to CIH during six months; echocardiography at six months	ADMA ↑ by x% in CIH; SDMA ↓ by x% in CIH	Siques et al.[15]
CIH	120 Chilean mining workers after exposure to CIH for a mean 14 ± 0.5 years	ADMA, but not SDMA, ↑ as compared to reference levels; higher ADMA in workers with HAPH (mPAP > 30 mm Hg) than in those without	Brito et al.[16]
HAPE	200 HAPE patients, 200 HAPE-free altitude sojourners, and 450 healthy highlanders	ADMA significantly ↑ in HAPE-patients and in highlanders than in HAPE-free sojourners	Ali et al.[17]
Acute hypobaric hypoxia (hypobaric chamber)	12 healthy humans during a 24 h stay in a hypobaric chamber	N = 5 developed AMS, high mPAP, and *decreased* ADMA; N = 4 had mild AMS, mildly elevated mPAP, and *elevated* ADMA	Tannheimer et al.[18]
Lung diseases			
OSAS	40 OSAS patients, 20 healthy controls	ADMA ↑ in OSAS vs. controls	In et al.[19]
COPD	COPD patients with or without PAH (sPAP > 35 mm Hg), healthy controls	ADMA ↑ in COPD with PAH vs. both other groups	Telo et al.[20]
PAH			
IPAH	Patients with IPAH, healthy controls	ADMA ↑ in IPAH	Kielstein et al.[21]
PAH in systemic sclerosis	66 European patients with systemic sclerosis (24 with PAH, 42 without PAH), 30 age-matched healthy controls	ADMA ↑ in systemic sclerosis with PAH, not in systemic sclerosis without PAH	Dimitroulas et al.[22]
PAH in connective tissue disease	88 Chinese patients with connective tissue diseases (43 with PAH, 45 without PAH), and 40 healthy controls	ADMA ↑ in connective tissue diseases with PAH, not in connective tissue diseases without PAH	Liu et al.[23]
HIV-associated PAH	214 HIV patients, of whom 85 underwent right heart catheterization for suspected PAH	ADMA ↑ in HIV patients with PAH than in those without; mPAP 14.2% higher per each 0.1 µmol/l increase in ADMA	Parikh et al.[24]
CTEPH	135 CTEPH patients, 40 healthy controls	ADMA ↑ in CTEPH patients than in controls	Skoro-Sajer et al.[25]

AMS: acute mountain sickness; CIH: chronic-intermittent hypobaric hypoxia; COPD: chronic obstructive lung disease; CTEPH: chronic thromboembolic pulmonary hypertension; HAPE: high-altitude pulmonary edema; HAPH: high-altitude pulmonary hypertension; HIV: human immunodeficiency virus; IPAH: idiopathic PAH; mPAP: mean pulmonary arterial pressure; OSAS: obstructive sleep apnea syndrome; PAH: pulmonary arterial hypertension; sPAP: systolic pulmonary arterial pressure; ADMA: asymmetric dimethylarginine; SDMA: symmetric dimethylarginine.

PAH (N = 24) or not (N = 42) and 30 age-matched healthy controls.[22] ADMA was significantly elevated in patients with PAH as compared to patients without PAH. In another study, 88 Chinese patients with connective tissue diseases, among whom 43 had developed PAH, were compared with 40 healthy controls.[23] ADMA concentration was significantly elevated in patients with connective tissue disease and PAH but not in those without PAH. PAH may also result from human immunodeficiency virus (HIV) infection. Parikh et al.[24] studied ADMA concentration in 214 HIV-infected individuals, among whom 85 underwent right heart catheterization for suspected PAH. ADMA was significantly associated with the presence of PAH;

mPAP was 14.2% higher per each increase in ADMA by 0.1 µmol/l.

Finally, in chronic thromboembolic pulmonary hypertension (CTEPH), ADMA was demonstrated to be significantly elevated as compared to healthy controls:[25] mean plasma ADMA concentration was 0.62 [0.51–0.73] vs. 0.51 [0.45–0.6] µmol/l in 135 CTEPH patients vs. 40 healthy controls (p < 0.0002). CTEPH patients who underwent surgery had lower ADMA concentration at baseline than inoperable patients, suggesting a correlation of ADMA with the extent and severity of CTEPH.

In summary, these observational studies suggest that PAH, irrespectively of its pathogenesis, is associated
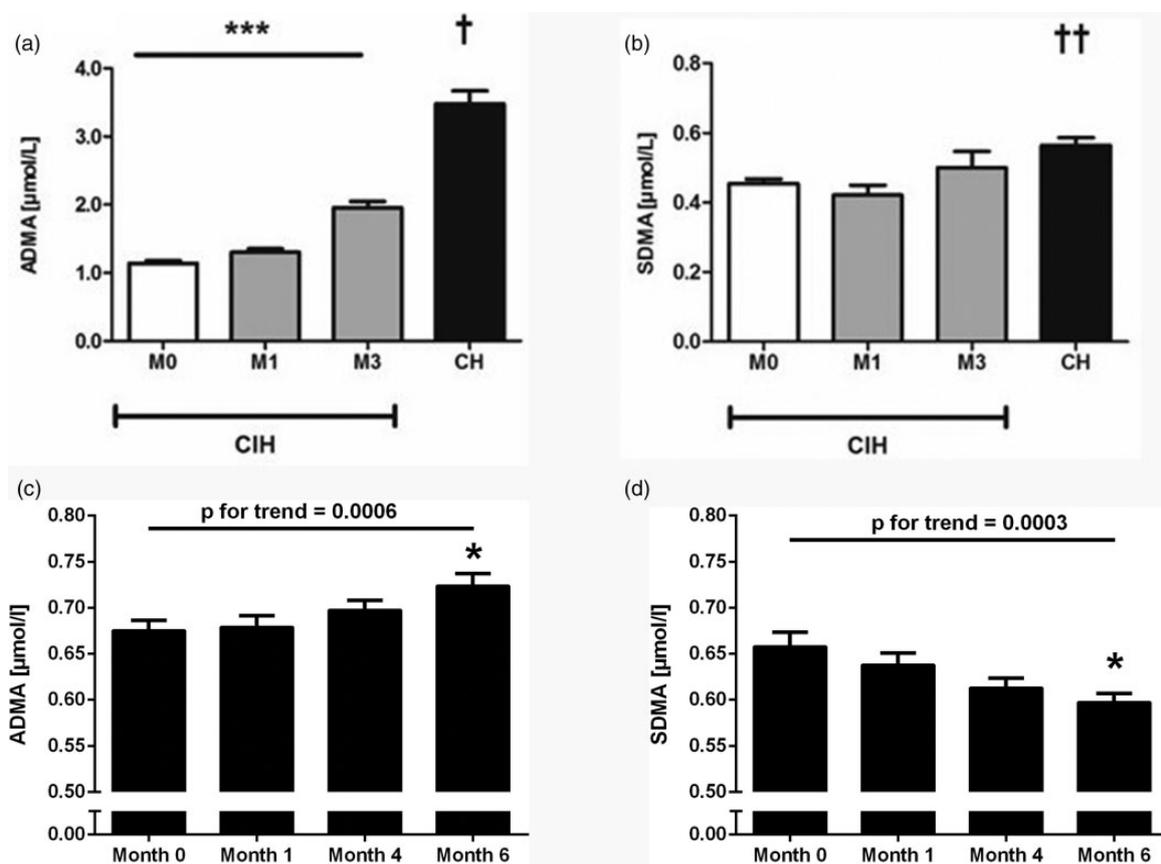
Fig. 2. Time courses of ADMA and SDMA during long-term exposure to CIH in humans. (a and b) The plasma concentrations of ADMA, but not SDMA, increase continuously in a cohort of 72 healthy young males during three months of CIH residents native to the Andean plateau have significantly more elevated plasma levels of both, ADMA and SDMA. Figure reproduced with permission from Lüneburg et al.[26]. ***p<0.001 for trend during 3 months of follow-up; *p<0.001 for differences between CH and M0, M1, and M3; **p<0.01 for differences between CH and M0, M1. In a prospective cohort of 100 healthy male individuals who were exposed to CIH during six months, there was a continuous, significant increase in ADMA (c) and a significant decrease in SDMA plasma concentrations (d). Figure reproduced with permission from Siques et al.[15] ADMA: asymmetric dimethylarginine; SDMA: symmetric dimethylarginine; CIH: chronic-intermittent hypobaric hypoxia; CH: chronic hypoxia. *p<0.01 vs. Month 0.

with elevated circulating ADMA concentration, as is any regimen of CH or chronic-intermittent hypoxia. Derangements in the biosynthesis and/or metabolism od ADMA are therefore likely to be involved in the pathophysiology of PAH, even if the observational studies cited above do not prove any causal relationship.

ADMA as a prognostic and predictive biomarker of PAH

Several clinical studies analyzed the prospective association of ADMA with physical performance and health status in individuals exposed to hypoxia or in patients with PAH: Dimitroulas et al. reported elevated ADMA concentrations in patients with systemic sclerosis who had developed PAH as compared to patients without PAH.[22] Within the group of patients with PAH, ADMA showed a significant inverse correlation with the results of the 6-min' walk test as a measure of physical performance. In another study, ADMA concentration showed a significant inverse correlation with

mixed-venous oxygen saturation and a weak but significant positive correlation with right atrial pressure in 57 patients with idiopathic PAH.[21] In the latter study, survival of patients with an ADMA concentration above the median (0.51 μmol/l) was significantly reduced as compared to patients with ADMA below the median.

Skoro-Sajer et al. calculated an ADMA concentration of 0.64 μmol/l to be the optimal cut-off value to predict mortality with a sensitivity of 81.1% and a specificity of 79.3% in patients with chronic thromboembolic PAH.[25] They also showed that ADMA plasma concentrations above that level predicted increased mortality in patients who underwent surgical thrombectomy as well as in patients who were inoperable.

In a clinical study of patients with COPD who had developed PAH or not, a significant negative correlation was determined between serum ADMA levels and arterial oxygen saturation, and a significant positive correlation was found between ADMA and systolic pulmonary artery pressure.[20] Another group of investigators reported that

ADMA plasma concentration was positively correlated with the severity of bronchial obstruction in COPD patients.[33] These investigators confirmed that there was a trend toward higher pulmonary arterial pressure with higher ADMA concentrations.

While all of these studies addressed the prognostic association of ADMA with disease outcome and/or physical performance in patients with established disease, we recently reported the first data on the predictive value of ADMA for PAH in primarily healthy individuals:[15] Out of a group of 100 young healthy male Chileans who were for the first time exposed to chronic-intermittent hypoxia at high altitude for a period of six months, we had echocardiographic estimations of mPAP available for 43 individuals. Nine of these subjects had developed HAPH after six months of CIH (i.e., they had mPAP levels >30 mm Hg). Baseline ADMA concentration, which was measured in a blood sample taken at sea level before the first exposure to high altitude, was a highly significant predictor for the development of HAPH, with an optimal cut-off level of 0.665 µmol/l (sensitivity, 100%, specificity, 63.6%). Individuals with ADMA concentration below this cut-off had significantly lower mPAP than those with ADMA above the cut-off level (Fig. 3). Table 2 summarizes the results of prospective studies of ADMA.

The latter study suggests that not only is there an increase of ADMA in hypoxia, but the pre-existence of high ADMA concentration predisposes to maladaptation of the pulmonary circulation to hypoxia. Therefore, we believe that it might be worthwhile studying single-nucleotide
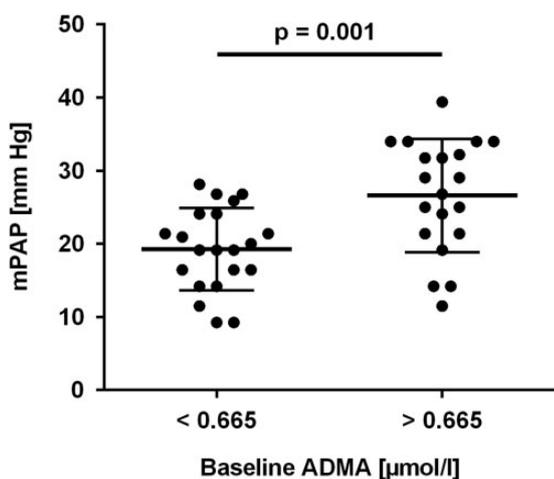

Fig. 3. Baseline ADMA concentration at sea level predicts the elevation of pulmonary arterial pressure during chronic intermittent hypobaric hypoxia. In a prospective study, baseline ADMA concentration at sea level was significantly associated with mPAP after six months of exposure to chronic intermittent hypobaric hypoxia. A cut-off ADMA level of 0.665 µmol/l was optimal to predict the development of high-altitude pulmonary arterial hypertension (i.e., mPAP >30 mm Hg). Figure reproduced with permission from Siques et al.[15] ADMA: asymmetric dimethylarginine; mPAP: mean pulmonary arterial pressure.

polymorphisms (SNPs) in genes involved in the regulation of the L-arginine–ADMA–NO pathway, such as eNOS, PRMTs, DDAHs, and/or AGXT2, to better understand the genetic basis of genetic susceptibility for maladaptation to CH. Accordingly, evidence is given in a study by Trittmann et al. showing an association of DDAH1 SNP rs480414 with a lower risk of PAH in children with bronchopulmonary dysplasia.[34]

Mechanisms of regulation of the L-arginine–ADMA–NO pathway in hypoxia: Evidence from animal studies

Experimental studies in various animal models and focusing on the different enzymes in this complex pathway have been performed to unravel the potential mechanisms of ADMA regulation and its pathophysiological consequences.

Bulau et al. determined whether enzymes contributing to ADMA biosynthesis and metabolism are present in the lung.[35] They showed expression of PRMT1-6 but not PRMT7 in the lung of which PRMT2 was almost exclusively found in the lung. This corresponded to high levels of protein-bound ADMA in lung tissue homogenates, compared to liver, kidneys, and heart tissues. The lungs also contained DDAH1 and DDAH2 proteins and DDAH enzymatic activity, suggesting that the complete pathway of ADMA biosynthesis and metabolism is present in the lungs.

Yildirim et al. investigated changes in PRMT expression during exposure to three weeks of normobaric hypoxia (10% oxygen) in adult BALB/c mice.[36] This study showed that among all investigated PRMT isoforms (PRMT1-6), only expression of PRMT2, which was barely detectable in normoxia, increased at the mRNA and protein levels under hypoxic conditions, accompanied by an increase in ADMA concentration. These results suggest that hypoxia might influence ADMA metabolism in the lung by upregulating L-arginine methylation by PRMT2.

We recently analyzed mRNA expression of PRMT1, PRMT2, PRMT3, and PRMT5 in C57/bl6 mice subjected to 21 days of normobaric hypoxia (10% oxygen) vs. normoxic controls by quantitative real-time polymerase chain reaction. We found small but significant increases in PRMT2 and PRMT5 mRNA expression after three weeks of CH, while PRMT1 and PRMT3 remained unchanged (own, unpublished data; Fig. 4a). All PRMTs analyzed by us showed relatively small absolute changes in mRNA expression, raising the question whether upregulation of PRMTs is the major molecular mechanism behind the increase in ADMA during hypoxia.

Several animal studies investigated the metabolism of ADMA by the DDAH enzymes in hypoxia. The reported findings are controversial. Millatt et al.[37] reported a 37% decrease in DDAH1 protein expression in the lungs of adult male rats exposed to one week of hypoxia (10% oxygen) as compared to rats kept under normoxic conditions. This was accompanied by 37% decrease in lung DDAH activity and a

Table 2. Prospective clinical studies of ADMA in chronic hypoxia and pulmonary arterial hypertension in humans.

Clinical condition	Study design	Prospective observation	Reference
IPAH	57 patients with IPAH, follow-up for up to 40 months	Survival significantly reduced in patients with ADMA > median (i.e., 0.51 μmol/l)	Kielstein et al.[21]
CTEPH	135 CTEPH patients	ADMA cut-off 0.64 μmol/l differentiates high vs. low mortality (sensitivity, 81.1%, specificity, 79.3%)	Skoro-Sajer et al.[25]
PAH in systemic sclerosis	66 patients with systemic sclerosis (24 with PAH, 42 without PAH)	ADMA inversely associated with performance in the 6-min walk test	Dimitroulas et al.[22]
COPD	COPD patients with or without PAH (sPAP > 35 mm Hg)	ADMA correlated positively with sPAP and inversely with arterial oxygen saturation	Telo et al.[20]
COPD	42 COPD patients	ADMA positively correlated with severity of bronchial obstruction; trend toward higher PAP with higher ADMA	Parmaksiz et al.[33]
CIH	100 healthy Chilean lowlanders exposed to CIH during six months; 43 participants with echocardiography at six months	Baseline ADMA at sea level predicts HAPH after six months of CIH; ADMA cut-off 0.665 μmol/l (sensitivity, 100%, specificity, 63.6%)	Siques et al.[15]

CIH: chronic-intermittent hypobaric hypoxia; COPD: chronic obstructive lung disease; HAPH: high-altitude pulmonary hypertension; PAH: pulmonary arterial hypertension; sPAP: systolic pulmonary arterial pressure; IPAH: idiopathic PAH; ADMA: asymmetric dimethylarginine; CTEPH: chronic thromboembolic pulmonary hypertension.

2.3-fold elevation of ADMA concentration in lung tissue. Despite an enhanced eNOS protein expression in hypoxic lungs, NO metabolite levels in lung homogenates were reduced by 22% in this study. These results suggest that eNOS expression was upregulated in hypoxia, but its catalytic activity is impaired by increased ADMA secondary to its impaired degradation by DDAH1.

More recently, Sasaki et al. investigated changes in the L-arginine–ADMA–NO pathway in monocrotaline-induced PAH in rats.[38] They observed significantly reduced eNOS protein expression and NOS activity and increased arginase activity in PAH. In addition, they found a 41% decrease in DDAH activity which was mainly caused by a nearly complete loss of DDAH1 protein expression, while DDAH2 protein levels remained unchanged. This study therefore suggests that NO production in pulmonary hypertension is impaired by reduced eNOS expression, enhanced competition for L-arginine as a substrate by arginase, and accumulation of endogenous NOS inhibitors caused by downregulation of DDAH1.

Incubation of pulmonary arterial endothelial cells in hypoxic conditions resulted in downregulation of DDAH1 and DDAH2 mRNA and protein expression, DDAH enzymatic activity, and increased ADMA levels.[39] In this study, mRNA 21 was suggested to mediate the hypoxia-induced downregulation of DDAH1.

By contrast, DDAH2 was the main isoform changed by hypoxia in four other studies: Arrigoni et al.[40] studied the effects of three days of hypoxia on newborn piglets. They reported an about 90% reduction of DDAH2 protein but not mRNA, which was paralleled by an about 70% reduction in DDAH activity and no change in DDAH1 protein or mRNA.

A marked increase in plasma ADMA and SDMA levels was observed in chronic pulmonary hypertensive rats and patients with idiopathic PAH by Pullamsetti et al.[41] In this study, expression of DDAH2 was reduced at mRNA and protein levels with no significant changes in DDAH1 expression.

Hypoxia-reoxygenation caused an increase in DDAH2 protein expression in murine peritoneal macrophages in vitro, which was associated with 24% lower ADMA concentration and enhanced NO production by these cells.[42] DDAH2-deficient murine monocytes demonstrated no increase in NO production after hypoxia-reoxygenation challenge. However, the results of this study are not comparable to the other studies cited here, as measurements performed after several hours of reoxygenation constitute an experimental model different from acute hypoxia or CH as such, and DDAH2 is the only DDAH isoform found in immune cells.

Our group recently reported the effects of 30 days of exposure to either chronic or CIH on the L-arginine–ADMA–NO pathway in adult Wistar rats.[14] Similar to the findings of Millatt et al.,[37] we found a strong increase in eNOS expression but no change in NOS catalytic activity. In our study, this was accompanied by decreased arginase and DDAH activities and elevated concentrations of ADMA in lung tissue homogenates. These effects were observed in both, chronic and chronic-intermittent hypoxia, but they were stronger in CH. These results are in line with our observations in humans studied under the conditions of chronic or chronic-intermittent hypoxia (see above; Lüneburg et al.[26]).

While our recent data from C57/bl6 mice exposed to normobaric hypoxia for 21 days confirmed significant upregulation of eNOS and iNOS mRNA levels in the lung (own,
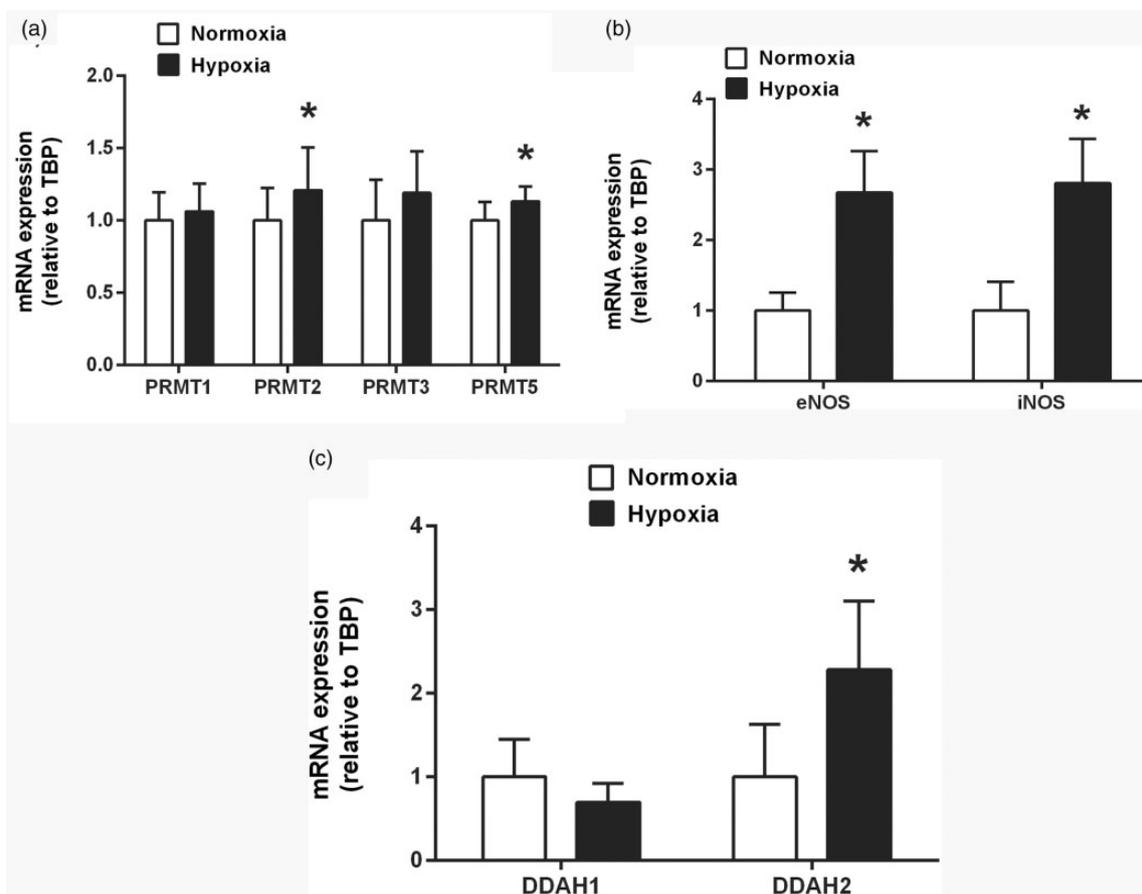
Fig. 4. Expression of genes involved in the regulation of ADMA concentration during chronic hypoxia in mice. Lung tissue was collected and homogenized after 21 days of exposure to hypoxia (10% O_2), total RNA was extracted, and mRNA expression was determined by real-time quantitative polymerase chain reaction. There were moderate changes in PRMT expression, with a slight, but significant upregulation of PRMT2 and PRMT5 mRNAs (a). NOS isoenzymes II and III (iNOS and eNOS) were consistently and strongly upregulated (b), and DDAH2 was also significantly upregulated, while DDAH1 showed no differential expression in chronic hypoxia (c). PRMT: protein arginine methyltransferase; NOS: nitric oxide synthase; DDAH: dimethylarginine dimethylaminohydrolase; TBP: TATA-box binding protein. *$p < 0.05$ vs. normoxia.

unpublished data; Fig. 4b), we observed upregulation of DDAH2 mRNA, while DDAH1 mRNA levels were not significantly changed (own, unpublished data; Fig. 4c).

Overexpression of DDAH1 in mice resulted in reduced ADMA concentration and increased NO metabolites in isolated lung perfusates during hypoxic ventilation.[43] This was paralleled by reduced pulmonary arterial pressure in response to chronic, but not to acute hypoxia.

Taken together, available data on the effects of hypoxia on PRMT and DDAH isoforms suggest complex mechanisms of regulation, which appear to be highly variable between animal strains and species, experimental models, and age of the investigated animals.

Species differences were also observed by Mizuno et al.[44] in comparing components of the L-arginine–ADMA–NO pathway between yak and cow. Yaks, a species with a genetically determined high level of adaptation to life at high altitude, displayed higher expression levels of eNOS, DDAH1, and DDAH2 protein, significantly higher DDAH activity, and significantly lower mean ADMA plasma concentration. These results may explain previously reported differences in the pulmonary endothelial response to acetylcholine between yak and domestic cow, suggesting that pulmonary endothelial function and structure critically determine adaptation to life at high altitude.[45]

In another study, Lopez et al.[46] studied expression and activity of components of the L-arginine–ADMA–NO pathway between newborn llamas and sheep at high and low altitude. Newborn sheep had significantly elevated ADMA plasma concentration and decreased DDAH2 mRNA expression in lung tissue as compared to sheep born in lowland. By comparison, llamas had extremely low ADMA concentrations and likewise low DDAH2 expression irrespectively of the altitude where they were born. Unfortunately, this study did not report expression levels for DDAH1. While downregulation of DDAH2 expression in sheep born at high altitude may explain high ADMA levels in these animals, the extremely low expression levels of DDAH2 in llamas cannot explain why this species had more than 10 times lower ADMA levels.
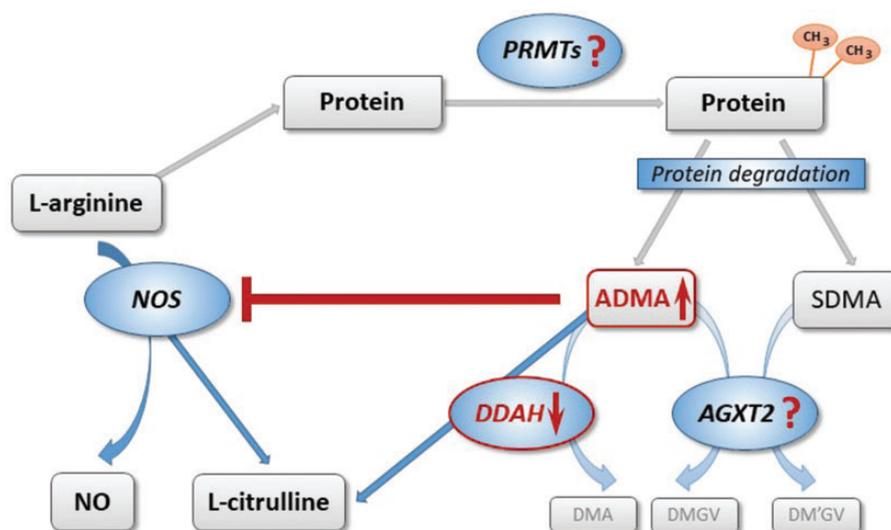
Fig. 5. Effects of chronic hypoxia on enzymatic pathways involved in the biosynthesis and metabolism of asymmetric and symmetric dimethy-larginine. There are consistent data showing upregulation of ADMA concentrations, both in plasma and tissues, while SDMA levels are either unchanged or decreased in chronic hypoxia. The majority of studies suggest downregulation of DDAH expression and/or activity, with some controversy whether DDAH1 or DDAH2 is more affected. There is uncertainty about the effects of chronic hypoxia on the expression and activities of PRMTs and AGXT2 (marked by "?" in the chart), which is due to sparsity of available published data. AGXT2: alanine glyoxylate aminotransferase 2; ADMA: asymmetric dimethylarginine; PRMT: protein arginine N-methyltransferase; NO: nitric oxide; NOS: NO synthase; SDMA: symmetric dimethylarginine; DDAH: dimethylarginine dimethylaminohydrolase; DMA: dimethylamine; DMGV and DM'GV: symmetric or asymmetric dimethylguanidinovaleric acid.

Conclusions and future perspectives

By contrast to the vasodilator response to hypoxia in the systemic circulation, the pulmonary vascular bed shows vasoconstriction in hypoxia. There is plenty of evidence that inhibition of endothelial NO production by endogenous methylarginines, among which ADMA is the most abundant, contributes to this regulation. The concentration of ADMA is highly regulated by multiple enzymatic pathways. Recent data from observational and prospective clinical studies suggest that not only is there upregulation of ADMA in hypoxia, but individuals with high ADMA at baseline have an exacerbated response of pulmonary arterial pressure to chronic hypoxic exposure. Fig. 5 summarizes our current understanding of the enzymatic pathways affecting ADMA that have been shown to be altered in hypoxia.

Genetic selection during hundreds of years at high altitude may have led to changes in expression and activity of enzymes involved in the L-arginine–ADMA–NO pathway, which may have contributed to adaptation to life at high altitude in these animal species. Whether similar findings can be made in human highland populations and how the genes involved have been altered during evolution remains to be elucidated in future studies. Such studies may also shed light on SNPs and/or epigenetic modifications in the relevant genes which convey sensitivity toward HAPH and/or AMS.

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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