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Nitric Oxide in Primary Ciliary Dyskinesia

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Abstract

Nitric oxide is continually synthesized in the respiratory epithelium and is upregulated in response to infection or inflammation. Primary ciliary dyskinesia is characterized by recurrent sinopulmonary infections due to impaired mucociliary clearance. Despite chronic infections, nasal nitric oxide in such patients is markedly reduced and is used as a screening test for this condition. These low levels were first described over 15 years ago but the underlying mechanisms have yet to be fully elucidated. We review epithelial nitric oxide synthesis, release and measurement in the upper airways with particular reference to primary ciliary dyskinesia. The key hypotheses that have been proposed to explain the low levels in this condition are explored and the potential benefits of augmenting airway nitric oxide levels are considered. Further work in these patients clarifying both whether the respiratory epithelium is able to biosynthesise normal levels of nitric oxide and the role played by abnormalities in the anatomy of the paranasal sinuses is essential. While nitric oxide augmentation is unlikely to be beneficial in common primary ciliary dyskinesia phenotypes, it has potential in the treatment of secondary dyskinesias and may also improve treatment of bacterial infections, particularly where biofilms are implicated.

Keywords: Nasal nitric oxide, nitric oxide augmentation, nitric oxide synthase.
Introduction

Primary ciliary dyskinesia (PCD) is a rare autosomal recessive disorder, with considerable heterogenicity, characterized by a spectrum of corresponding defects in ciliary ultrastructure and/or ciliary function [1]. The impaired ciliary function impedes mucociliary clearance, which predisposes the patient to recurrent sinopulmonary infection [2]. Nitric oxide (NO) is a highly reactive gaseous molecule with numerous signalling roles within the airways. It is produced throughout the airways but is particularly abundant in the nasal sinuses [3]. NO biosynthesis is typically upregulated during infection, via increased inducible NO synthase (iNOS) transcription and activity [4]. Despite recurrent respiratory infection, nasal concentrations of NO are markedly reduced in the vast majority of PCD patients, compared to those without the disorder. Indeed nasal NO is now widely used as a screening test for PCD [5, 6]. Fractional exhaled NO (FeNO) from the lower airway is also low in PCD but is less specific at differentiating between PCD and healthy controls [3, 7, 8]. Whilst the association of low nasal NO in PCD has been recognized for over 15 years, the underlying mechanisms causing this phenomenon remain unclear.

Here we review studies of NO in the airway, focusing on epithelial-derived NO and the low levels found in PCD patients. We explore key hypotheses proposed to explain the lower NO and consider whether augmentation of NO would benefit these patients.
The biosynthesis and role of nitric oxide in the airway

Nitric oxide is an intra- and inter-cellular signalling molecule, involved in diverse physiological and pathophysiological processes [9] such as vascular homeostasis, immune cell activity and tumour progression [10]. NO has a short half-life and diffuses rapidly from its point of synthesis, interacting intracellularly as well as crossing the plasma membrane and leaving the cell, where it can act extracellularly [11, 12]. NO is synthesized via the oxidation of the amino acid L-arginine to L-citrulline, catalysed by three stereo-specific isoenzymes in the presence of nicotinamide adenine dinucleotide phosphate (NADP), oxygen and other cofactors (figure 1). The NOS isoenzymes neuronal and endothelial NOS (nNOS and eNOS) are expressed constitutively and require calmodulin binding and calcium activity to produce femtomole or picomole concentrations of NO [13]. In contrast, iNOS is permanently bound to calmodulin and is therefore independent of calcium activity. It may be transcriptionally induced by a number of cytokines, including interleukin 1β, tumour necrosis factor α and interferon γ [14], to produce nanomole concentrations of NO [4, 13]. The expression of iNOS may also be constitutive in airway epithelium [14]. NOS isoenzyme gene expression and protein localization have been demonstrated in the airway (table 1).

Within the airway NO is produced by numerous cell types, including epithelial cells, endothelial cells, fibroblasts, activated macrophages, nerve cells, airway and vascular smooth muscle cells. It has diverse roles as a modulator of ciliary function, neurotransmission, bronchodilatation, vasodilatation, platelet aggregation and
immune function [13]. Pertaining to ciliary function, in vitro studies of animal and human airway ciliated epithelium suggest that the induction of NOS and NO production increase ciliary beat frequency [15-21]. During oxidative stress conditions, the production of NO and reactive nitrogen species amplify inflammatory responses and hence may modulate chronic airway inflammatory disease [13]. Additionally, the production of NO within the airway is protective against infection and has bacteriostatic and bactericidal activity; sodium nitrite can kill *Pseudomonas aeruginosa, Staphylococcus aureus* and *Burkholderia cepacia* [22]. It can also modulate biofilm formation [23], and cause biofilm dispersal, making the bacteria more susceptible to antimicrobial treatment [24].

**Primary ciliary dyskinesia**

PCD is a heterogeneous disorder, usually inherited as an autosomal recessive trait [6], causing a range of ciliary ultra-structural and functional defects and resultant ciliary dyskinesia [25]. The prevalence of PCD is estimated to be between 1 in 15,000 – 30,000 live births [6, 26, 27] PCD is characterized by chronic upper and lower airway infection, and is associated with situs inversus and male infertility due to homology between respiratory cilia, embryonic nodal cilia and sperm.

The motile ciliary axoneme is formed in a “9+2” arrangement where nine peripheral microtubule doublets surround a central pair of single microtubules [28]. Over 250 other proteins maintain this structure including radial spokes and nexin links. The inner and outer arms of the axoneme generate the force required for ciliary beating
and are formed by dynein complexes, acting as mechano-chemical ATPases [29]. Radial spokes structurally coordinate the ciliary beat pattern (CBP), hence mutations affecting dynein arms or radial spokes render ciliary movement ineffective and dyskinetic [30]. Development of a genetic diagnosis for PCD has remained problematic due to the heterogeneity of the disease and in some cases candidate genes having large exomes. To date there are 11 published PCD causing gene mutations, which account for approximately 1/3rd of PCD cases [31, 32] and genetic diagnostic capabilities are being developed [6, 31].

Diagnosis

Diagnosis of PCD can be confirmed by analysis of ciliated bronchial or nasal epithelia. There is no ‘gold standard’ test that will diagnose all PCD phenotypes, and hence a diagnostic workup requires the rigorous assessment of both ciliary beat frequency (CBF) and ciliary beat pattern (CBP) by high-resolution, high-speed video microscopy and transmission electron microscopy (EM) of ciliary ultrastructure, as recommended by the European Respiratory Society Task Force consensus statement [6]. Assessment of CBP requires a high degree of experience and skill, but considering CBF in isolation risks mis-diagnosing PCD. There is also an increasing literature on a population of atypical patients with PCD and normal ciliary ultrastructure, associated with mutations in dynein axonemal heavy chain 11, that would be missed in centres where diagnosis depends on EM without access to high-speed video microscopy [33-35]. For difficult diagnostic cases the re-differentiation of basal epithelial cells at an air liquid interface in cell culture allows for reassessment
of ciliary function and ultrastructure that may differentiate primary from secondary dyskinesia [6, 36, 37].

**Screening – the role of nasal nitric oxide**

Only a small percentage of patients presenting with chronic upper and lower respiratory tract infections have PCD. PCD diagnostic investigation, outlined above, requires specialist skills, is time consuming, costly and only available in a small number of specialist centres. A reliable screening test is therefore desirable [36]. For many years the saccharin test was used, but it is difficult to perform and is unreliable in children [6, 38].

Nitric oxide detection in human exhaled breath was first described in 1991 [39]. Studies suggest that the majority of NO originates from the paranasal sinuses, with lower concentrations found in exhaled breath [40, 41]. Paranasal sinus biopsies taken from healthy patients demonstrated that the iNOS isoenzyme expression was most abundant in *in situ* hybridization and immunohistochemistry experiments, and notably more so than seen in matched nasal biopsies, suggesting iNOS as the predominant isoenzyme involved in NO biosynthesis within the paranasal sinuses [41, 42].

PCD patients have low nasal NO and FeNO compared to healthy controls (table 2) [3, 7, 8]. Nasal NO is sufficiently low in PCD to be used as a screening test for the condition [3, 7, 43-46]. One group reported a specificity of 88%, a sensitivity of
100%, and a positive predictive value of 89% for correctly diagnosing PCD when using a nasal NO cut-off level of <105 parts per billion (ppb) [45]. While initially undertaken as a research tool, nasal NO was quickly introduced as part of the clinical diagnostic pathway in many larger European PCD centres. It has been included in the British guidelines since 2007 [5] and European consensus guidelines since 2009 [6]. Nasal NO measurement is not yet approved for clinical use in United States where only centres with major research programs in PCD are routinely using it. The measurement is extremely helpful in guiding the diagnostic pathway, but major drawbacks are the cost of equipment and consumables. It is also important to note that there is no widely agreed cut off level used for the screening of PCD as levels vary significantly with age, due to the development of the paranasal sinuses over the first decade of life, and, to a lesser extent, with the device used to perform the measurement [41, 47]. Nasal NO has also been found to be useful in atypical phenotypes where normal ciliary ultrastructure makes diagnosis difficult [33, 46]. While the best validated technique for the measurement of nasal NO is performed by nasal aspiration during breath-holding [48] there is recent literature on levels recorded in PCD patients during both humming and tidal breathing. Tidal measurements allow levels to be measured in children as young as 6 months of age, although there is limited experience in this age younger age group [47, 49].

Contrary to the majority of published data [3, 7, 43-46], two groups [50, 51] have recently reported normal and raised levels of nasal NO in patients with PCD. A study of PCD positive patients, confirmed by live ciliary function analysis and scrutiny of ciliary axonemal ultra-structure [6], reported 5 patients with nasal NO within or above the normal range [51]. A further study demonstrated that 24% of an Italian PCD
clinic (n=41) had nasal NO of over 250 ppb, although the diagnostic evidence for PCD in these patients is unclear [50]. These studies highlight that patients with a history strongly suggestive of PCD should not be excluded from further diagnostic evaluation on the basis of nasal NO.

The value of using nasal nitric oxide as a screening tool for PCD is clear, but there are a number of other conditions in which reduced nasal NO levels occur, although usually not as low as in PCD, including cystic fibrosis [52], diffuse panbronchiolitis [53], nasal polyps [54] and chronic sinusitis [55]. It is also reduced in smokers [56]. The lack of specificity requires robust diagnostic evaluation to confirm PCD in patients with low nasal NO.

Measurement of alveolar and bronchial nitric oxide in health and in PCD

Exhaled lower airway NO as measured by FeNO is typically sampled at a set exhalation flow rate of 50 ml/s, denoted as FeNO_{50} [48]. Several two-compartment mathematical models have been described, allowing estimates of alveolar (C_{alv}) and conducting airway NO (J_{NO}) [57, 58]. The two-compartment model has been used to investigate whether low NO is confined to the upper airway in PCD, or whether it is low throughout upper and lower airways. Three published studies that have used the multiple-flow technique to estimate J_{NO} and C_{alv} in PCD patients have conflicting findings [59-61]. All found J_{NO} was reduced in PCD compared to controls but only one [59] found low C_{alv}. While further work is needed to consider this discrepancy
the model might suggest that the lower FeNO$_{50}$ seen in PCD compared to controls is principally due to a decreased bronchial biosynthesis of NO.

**Why are nitric oxide levels reduced in Primary Ciliary Dyskinesia?**

Several hypotheses have been put forward at both a cellular and anatomical level for the low airway NO in PCD; some extrapolated from the finding of reduced NO levels seen in cystic fibrosis (CF) [62-65]. At the epithelial level it has been suggested that there is reduced biosynthesis of NO [8, 64, 66] or increased breakdown, either within the cell, in a viscous mucus layer [62, 63] or by denitrifying bacteria [65]. At the anatomical level it has been suggested that NO is sequestered in the upper respiratory tract within blocked paranasal sinuses or alternatively nasal NO biosynthesis or NO storage capacity is limited due to agenesis of the sinuses [50, 67] (figure 2).

We review below four broad hypotheses that have been proposed to explain the low NO levels found in patients with PCD.

**Hypothesis 1: Increased breakdown of nitric oxide to metabolites**

As NO is highly reactive, it is rapidly broken down from the breath exhalate by its reaction with, among others, oxygen, superoxides and cysteine thiols producing reactive nitrogen species, including the potent oxidants peroxynitrite and nitrogen dioxide, and S-nitrosothiols [68-71].
The low NO levels seen in PCD may be associated with increased consumption of NO by superoxide anions to form reactive nitrogen species, which has been suggested as the cause of low NO in adult respiratory distress syndrome [72]. A study of 23 PCD patients and 11 healthy volunteers seemed to support this, demonstrating increased concentrations of the oxidative stress marker 8-isoprostane (8-IP) in the PCD group. However there were a number of PCD patients, with apparently normal 8-IP levels, inconsistent with oxidative stress associated rapid breakdown of NO in these patients [73]. Csoma & colleagues demonstrated no difference in the mean concentrations of three NO metabolites: nitrite, nitrite/nitrate or S-nitrosothiol in the exhaled breath condensates of 15 PCD patients, with markedly decreased nasal NO levels (table 2), compared to 14 healthy controls, suggesting that NO biosynthesis does occur in the PCD airway [66]. Given the variability in levels of oxidative stress seen in PCD patients it seems unlikely that this alone is responsible for the consistently low NO detection in PCD, however further experimental work is required.

* Nitric oxide is trapped and broken down in viscous sputum  
* Impaired mucociliary clearance in PCD causes mucus accumulation in the airways, potentially trapping NO at the periciliary level or in the mucus itself where it is then broken down into NO metabolites. In CF patients it has been suggested that the viscous mucus may trap the highly reactive NO, preventing it from being exhaled freely [62, 63]. Viscous mucus is a particular issue for CF patients but similar biophysical properties have been identified in sputum of patients with PCD [74]. The significance of this potential mechanism requires further evaluation. 
Consumption of nitric oxide by denitrifying bacteria. Increased breakdown of airway NO by bacterial nitric oxide reductase may contribute to low FeNO [75]. There is evidence in CF that chronic colonization of the airway with denitrifying organisms lowers FeNO and that antibiotic treatment for exacerbations increases NO concentrations [65]. PCD patients similarly suffer from recurrent infection and, to some degree colonization, but to our knowledge the effect of antibiotic treatment on FeNO has not been assessed. However denitrifying bacteria seem unlikely to be the cause of the low NO concentrations seen as CF patients are more chronically colonized with bacteria than patients with PCD but have significantly higher nasal NO concentrations.

Hypothesis 2: Reduced biosynthesis of nitric oxide

NO synthesis occurs in patients with PCD [66], however a number of reasons why synthesis may be reduced in PCD epithelia have been postulated. These include an absence or decreased expression of NOS isoenzymes, a reduction in NOS isoenzyme output directly related to loss of ciliary function [8] or limitations in the availability of the NOS substrate L-arginine, either due to its reduced availability or increased metabolism [64, 76-78].

Decreased expression of NOS isoenzymes It has been suggested that iNOS is the main contributor to FeNO, when looking at healthy, asthmatic and atopic children [79]. It has also been reported that patients with CF have reduced or absent iNOS expression in the airway epithelia [75, 80, 81]. A recent study comparing NOS2 and NOS3 mRNA expression (the genes for iNOS and eNOS respectively) in the nasal
mucosa of patients with PCD and secondary ciliary dyskinesia (SCD) found that there was no difference in NOS3 expression but lower levels of NOS2 expression in the PCD group. There was however significant overlap between the two groups making these results difficult to interpret. Since PCD is a polygenic disorder, its genetic linkage to NOS gene polymorphisms is highly unlikely. Furthermore, ‘next generation sequencing’ of exomes, targeted to identify PCD causing genetic mutations, to date, has not identified the NOS genes (NOS1, NOS2, NOS3) as candidate genes for PCD [31].

Loss of NOS activity via mechano-chemical uncoupling in PCD Narang et al proposed that normal NOS activity requires ciliary function via a mechano-chemical coupling to dynein ATPases, that would be uncoupled in patients with PCD [8]. This hypothesis was based on observations from patients with Duchenne Muscular Dystrophy (DMD) where mutations in the dystrophin gene result in an uncoupling of nNOS from the contractile apparatus, leading to the loss of contractile function seen in DMD causing low serum NO [83-85]. While further work is required to elucidate the mechanism regulating NOS activity an uncoupling of NOS from dynein ATPase seems unlikely as PCD phenotypes with hyper-frequent or motile dyskinetic cilia have low NO similar to patients with static cilia.

Limitations to availability of the NOS substrate L-arginine Arginase competes with NOS isoenzymes for their common substrate L-arginine. It has been suggested in CF that high levels of arginase may be present and compete with NOS isoenzymes for L-arginine, hence leading to reduced NO biosynthesis [64]. In CF, high levels of
arginase activity are seen in sputum even after 14 days of intravenous antibiotics which significantly lowers arginase activity, when compared with induced sputum from healthy control subjects \( (p=0.0001) \) [64]. While this has not been directly assessed in PCD patients there have been pilot studies that demonstrate increased nasal and exhaled NO levels in PCD patients following administration of both intravenous and nebulised L-arginine, discussed further below [78, 86]. Further work is needed to clarify the availability of this NOS substrate in PCD epithelial cells.

**Hypothesis 3: Nitric oxide is trapped in the obstructed paranasal sinuses**

In health the predominant source of exhaled NO is the upper airway [40]. Evidence from studies using manoeuvres such as humming suggest NO is sequestered within the nasal cavities [67, 87, 88]. The oscillation generated by the humming is thought to increase gas exchange across the osteomeatal complex [87, 89]. During tidal breathing it can take up to 30 minutes to completely clear sinus gases into the nasal cavity but with humming this occurs in one exhalation [90, 91]. In healthy volunteers humming leads to a 15-fold peak in nasal NO, compared to quiet exhalation [87]. This humming peak is absent in patients with chronic sinusitis and those with CF [88, 92]. There is evidence, in both these conditions, to suggest this is secondary to decreased expression of iNOS leading to decreased NO biosynthesis [75, 80, 81, 93]. Patients with PCD suffer from chronic sinusitis and, in a small study, the absence of a nasal NO humming peak has been demonstrated in PCD compared to healthy controls [67]. In this study 13 of the 14 PCD patients underwent computed tomography (CT) scan of the paranasal sinuses and no differences were seen in nasal NO humming peaks between those who had complete opacification of the paranasal sinuses and those with only partial opacification with patent osteomeatal
complexes, however the numbers were small [67].

A recent observational study reported five patients with PCD with normal nasal NO levels [51]. Notably none of these five patients suffered from chronic sinusitis [51], supporting the hypothesis that a patent osteomeatal complex permits normal nasal NO levels in PCD. However in the authors’ patient population some have PCD without sinusitis but very low nasal NO and there are very few reported cases of PCD patients with normal nasal NO levels raising the possibility that these five patients may have an unusual phenotype or have been incorrectly diagnosed with PCD.

So while it has been demonstrated that humming does not increase nasal NO in PCD patients, it remains unclear whether this is a consequence of the underlying inability of the airway epithelia to produce NO or that the manoeuvres did not overcoming the obstruction to osteomeatal complex.

**Hypothesis 4: Reduced production and storage capacity of nitric oxide in the paranasal sinuses**

Aplastic or hypoplastic nasal sinuses can occur in PCD and would lead respectively to both absent or reduced epithelial production and storage capacity of NO in the paranasal sinus. This might explain, particularly given the importance of the paranasal sinuses in NO production [40], why nasal NO concentrations are low and do not vary significantly with manoeuvres to improve sinus ventilation [50, 67, 94]. In a recent study, CT scans of the paranasal sinuses at the time of diagnostic investigation for PCD demonstrated that frontal and/or sphenoidal sinuses were either aplastic or hypoplastic in 30 (73%) of 41 PCD patients, compared to only 38% of those with a SCD [50]. There was a significant inverse correlation between the
score for aplasia/hypoplasia of each paranasal sinus and nasal NO values in PCD (p=0.008, r=-0.432) but not in SCD (p=0.07, r=-0.271) [50], supporting the hypothesis that smaller sinuses are associated with lower nasal NO levels. However, unusually, 24% of this PCD study population had normal nasal NO; if these patients were excluded no correlation between aplasia/hypoplasia score and nasal NO level would have been seen. There is again the possibility that these ‘outliers’ with normal NO may have an unusual PCD phenotype or have been incorrectly diagnosed.

Further evidence in support of this hypothesis is that the paranasal sinuses develop over the first 10 years of life and nasal NO levels are seen to increase with age over this time period [41]. It is also noteworthy that most conditions associated with lower nasal NO levels involve disease of the paranasal sinuses, CF [52, 95], nasal polyps [54], chronic sinusitis [55] and PCD, which may lead to either obstruction of the osteomeatal complex or reduced storage capacity of the paranasal sinuses, or combination of both.

**The value of augmentation of nitric oxide levels in PCD**

As the evidence that NO plays a functional role in the lung increases, research has focused on augmenting levels to observe effects. Most of the work in this field has been undertaken using the NOS substrate L-arginine, which increases FeNO levels in healthy subjects [77, 78, 96, 97].

Nasal NO and FeNO levels have been compared before and after infusion of intravenous L-arginine in PCD (n=7) and healthy controls (n=11). A 1.4-fold increase
in nasal NO occurred immediately after infusion and a 1.8-fold increase in FeNO 3 hours after infusion in PCD patients. However concentrations remained reduced compared to the baseline values of the control group [78]. Another group compared the use of nebulized L-arginine in 10 PCD patients with 10 healthy controls in a double-blinded placebo controlled trial. They found that L-arginine not only increased nasal NO levels but also increased CBF and decreased mucociliary clearance time [86]. However they did not define the functional or ultrastructural defects in their PCD group, and atypically, 7 of the 10 patients reported to have PCD had normal CBF, hence calling into question their diagnosis. The two PCD patients with the common phenotype of static or extremely slow cilia showed no change in CBF following treatment with L-arginine [86].

Another reason for augmenting NO is its antibacterial properties [22, 98, 99]. Increasing nasal NO levels potentially reduces nasopharyngeal carriage of pathogens thereby reducing the risk of lower respiratory tract infection. There is case literature of a healthy volunteer showing that nasal application of NOS inhibitors both reduces nasal NO and leads to sinus infection [93, 100].

NO has a role in the dispersal of bacterial biofilms. *Pseudomonas aeruginosa* is the best characterized bacteria to undergo the genetic adaptations from motile planktonic bacteria to a non-cytotoxic, non-motile mucoid phenotype caused by over-production of a surface polysaccharide known as alginate. Biofilm formation also occurs with *Haemophilus influenzae* and *Staphylococcus aureus*, common pathogens in the PCD population [101-103]. Bacteria embedded within biofilms can be 1000-fold more resistant to antibiotic treatments [104]. They undergo coordinated dispersal events,
in which the bacteria convert back to motile planktonic bacteria. Exposing in vitro 
Pseudomonas aeruginosa biofilms to sodium nitroprusside, an NO donor, has been 
shown to induce biofilm dispersal, making it more susceptible to antimicrobial 
treatment [24]. Furthermore recent studies have demonstrated that the slow release 
of nitric oxide from charged catheters inhibit Escherichia coli biofilm formation and 
also that sodium nitrite can kill Pseudomonas aeruginosa, Staphylococcus aureus 
and Burkholderia cepacia [22, 23].

While the evidence that augmenting NO can improve ciliary function in PCD is not 
compelling, the potential to promote host defence, by direct bactericidal properties 
and biofilm dispersal merits further investigation. However there is a potential risk 
that increasing NO levels may lead to increased “nitrosative stress”, in what is 
already a pro-inflammatory environment.

**Summary**

Whilst the association of low NO concentrations with PCD has been recognized for 
over 15 years, the underlying mechanisms remain unclear. We have reviewed the 
data that demonstrate low levels of nasal NO in PCD to a degree that renders this a 
useful screening test. The limited studies to date suggest that biosynthesis of the 
molecule occurs, and there is little to suggest that increased breakdown or 
metabolism is responsible for the low levels. There is some direct and circumstantial 
data to suggest the reduced size of the paranasal sinuses caused by aplasia or
opacification and the lack of patency of the osteomeatal complex might contribute. However this is supported mainly by patients, diagnosed with PCD, who have normal levels of nasal NO and, given that the vast majority of PCD patients have extremely low NO levels, it does raise questions over the reliability of their diagnosis or the possibility of them having an usual phenotype of PCD. In order to clarify the underlying cause of the low levels of NO seen it is essential to establish both whether the airway epithelium in PCD is able to biosynthesis normal levels of NO and the role played by abnormalities in the anatomy of the paranasal sinuses.

There has been speculation that NO augmentation may be beneficial in PCD and other diseases associated with low NO. It is unlikely that ciliary function will be normalized in common PCD phenotypes, although it has potential for treatment of patients with secondary dyskinesias. More plausible is the suggestion that NO augmentation will improve treatment of bacterial infections, particularly where biofilms are implicated. A number of small observational and in vitro studies have been conducted, but larger clinical trials are required before this can be considered a therapeutic option.
Clinical implications and future directions

- The majority of studies indicate that more than 95% of PCD patients have very low nasal NO, confirming its suitability as a screening test.

- The small number of PCD patients with reportedly normal nasal NO require further evaluation, firstly to confirm the diagnosis of PCD, and then to explain the atypical NO level in this group.

- Outstanding questions regarding NO in PCD:
  - Is NO low throughout the airway, or only upper respiratory tract?
  - What mechanisms underlie low NO levels?
  - Is there a mechanistic link between ciliary beating and NO biosynthesis?
  - What are the implications of low NO in PCD? (E.g. are there effects on innate immunity?)
  - Does augmenting airway NO benefit patients?
References


Figure Legends

Figure 1 – Schematic diagram to demonstrate nitric oxide (NO) biosynthesis by the conversion of L-arginine (NOS substrate) to L-citrulline via nitric oxide synthases (NOS) isoenzymes. During this process the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), is oxidised in the presence of oxygen (O₂) to form nicotinamide adenine dinucleotide phosphate (NADP⁺) and water (H₂O). Other cofactors are also required for NO biosynthesis (not shown).

Figure 2 – Schematic diagram to represent normal nitric oxide (NO) metabolism and release from the epithelium of healthy patients and hypotheses for the potential causes of low nasal NO concentrations in PCD, based on events at the epithelium: A - increased breakdown of highly reactive NO to NO metabolites (nitrite and nitrates); either within the epithelial cell, within extra viscous sputum or by denitrifying bacteria within the mucus, hence NO metabolites are predominantly released into the airway (Hypothesis 1); B - absence of, or reduced, NO biosynthesis within the epithelial cell (Hypothesis 2); C - normal NO biosynthesis within the epithelial cell however obstruction to the osteomeatal complex inhibits NO release from the paranasal sinuses into the nasal passage (Hypothesis 3), or hypoplasia or agenesis of the paranasal sinuses reduces NO production and storage capacity, hence low measurable nasal NO levels are seen (Hypothesis 4).
## Table 1 Expression and protein localization in the human airway of the nitric oxide synthase isoenzymes

<table>
<thead>
<tr>
<th>NOS isoenzyme</th>
<th>Protein</th>
<th>Gene</th>
<th>Chromosome</th>
<th>Localisation</th>
<th>Reference</th>
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<tr>
<td>Neuronal</td>
<td>nNOS</td>
<td>NOS1</td>
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<td>Airway neuronal cells</td>
<td>[105]</td>
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<td>Inducible</td>
<td>iNOS</td>
<td>NOS2</td>
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<td></td>
<td></td>
<td></td>
<td>Alveolar epithelial cells</td>
<td>[107]</td>
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<td></td>
<td></td>
<td></td>
<td>Paranasal epithelial cells</td>
<td>[41]</td>
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<tr>
<td>Endothelial</td>
<td>eNOS</td>
<td>NOS3</td>
<td>7</td>
<td>Airway epithelial &amp; endothelial cells</td>
<td>[108]</td>
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NOS - nitric oxide synthase
Table 2 – Main studies assessing FeNO and nasal NO in patients with PCD compared to healthy controls*

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<th>PCD (ppb)</th>
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<td>FeNO&lt;sub&gt;50&lt;/sub&gt;</td>
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<tr>
<td>2.1 (1.3-3.5)*</td>
<td>6.7 (2.6–11.9)*</td>
<td>0.001</td>
<td>[43]</td>
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<td>(n=14)</td>
<td>(n=37)</td>
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<tr>
<td>2.9 (1.9-4.6)**</td>
<td>6.4 (4.8-8.6)**</td>
<td>0.11 (NS)</td>
<td>[45]</td>
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<td>(n=17)</td>
<td>(n=24)</td>
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<td>8.1 (±1.3)**</td>
<td>12.5 (±1.6)**</td>
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<td>[60]</td>
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<td>(n=24)</td>
<td>(n=20)</td>
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<td>3.2 (±0.2)**</td>
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<td>p&lt;0.0001</td>
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<td>7.1 (5.7-8.8)**</td>
<td>13.9 (11.7-16.4)**</td>
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<td>759 (±145.8)$</td>
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<td>13.7 (6.8-27.8)**</td>
<td>223.7 (175.5-285.2)**</td>
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*median (range), **Mean (95% confidence interval), §mean (±standard deviation), @mean (±standard error), NS – non significant
Figure 1

L-Arginine

NADPH + O₂

NADP⁺ + H₂O

L-Citrulline  NO

NOS
Figure 2

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<td>Bacteria</td>
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<tr>
<td>Epithelia</td>
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