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Exhaled nitric oxide in cystic fibrosis: relationships with airway and lung vascular impairments

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ABSTRACT: **A reduction of exhaled nitric oxide (NO) fraction and endothelial-mediated dysfunction have been reported in cystic fibrosis (CF). The aims of the present study were to search for relationships between flow-independent NO exchange parameters (bronchial NO flux ($J'_{aw,NO}$) and alveolar NO concentration ($C_{A,NO}$)) and lung function tests characterising airflow limitation and pulmonary vascular bed (capillary blood volume and physiological dead space/tidal volume (V_D/V_T) ratio on exercise).**

In total, 34 patients (16 children, 18 adults) with CF, without resting pulmonary hypertension, underwent spirometry, exhaled NO measurement (multiple constant flow analytical method), gas transfer assessment (carbon monoxide and NO, allowing the calculation of capillary volume and membrane conductance) and a graded exercise test with oxygen uptake (V'_{O_2}), carbon dioxide production (V'_{CO_2}) and arterial blood gas evaluations.

Both $J'_{aw,NO}$ and $C_{A,NO}$ correlated positively with airflow limitation. $C_{A,NO}$ correlated positively with capillary/alveolar volume. During exercise, criteria of mild pulmonary vascular disease were evidenced in some patients that participated in exercise limitation (negative correlation between physiological V_D/V_T and peak V'_{O_2}). $C_{A,NO}$ at rest correlated positively with these parameters of wasted ventilation during exercise (physiological V_D/V_T, minute ventilation (V'_E)/V'_{CO_2} at ventilatory threshold and V'_E/V'_{CO_2} slope).

Flow-independent exhaled NO parameters are linked to airway and early vascular diseases in patients with CF.

KEYWORDS: **Capillary blood volume, cystic fibrosis, exercise test, exhaled nitric oxide**

New measures are needed that detect subtle changes before overt decline in lung function in patients with cystic fibrosis (CF) [1]. There are several lines of evidence suggesting that nitric oxide (NO) could constitute a key mediator of CF pathophysiology due to its broad spectrum bactericidal properties, its role in modulating epithelial ion transports, and its bronchovasomotor and anti-proliferative functions [2]. Interestingly, NO can be measured in exhaled gas, but its sources (cellular and enzymatic), roles and relationships with disease markers remain debated. Due to the broad spectrum of activities of this messenger one may hypothesise that its measure could reflect or could be linked to various aspects of CF lung disease.

Along this line, NO fraction has been found to be decreased in upper (nasal) airways in CF patients, whereas exhaled NO fraction (F_{ENO}) had a nil to moderate decrease from infancy to adulthood [3–7]. This variable decrease in exhaled NO has been related to a defective expression of type 2 inducible NO synthase, which may participate to the susceptibility to *Pseudomonas aeruginosa* infection (bactericidal function) [8]. Reduced exhaled NO has also been related to impaired nasal potential difference in patients with CF (epithelial ion transport) [9]. Finally, only one study has described a negative correlation between exhaled NO and airflow limitation in CF patients (loss of anti-proliferative function linked to remodelling) [4] while other studies did not find such a relationship [5–7].

Therefore, our aim was to assess whether exhaled NO measurement is associated with lung function parameters obtained at rest and during exercise reflecting both airway and vascular impairments due to CF disease. To this end, a detailed analysis of exhaled NO was performed,

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which gives flow-independent parameters of NO exchange dynamics related to its physiology. Exhaled NO measurement at a single expiratory flow rate is a global assessment since exhaled NO output is the sum of alveolar and conducting airway NO outputs [10]. We hypothesised that the two origins of exhaled NO may be linked to different pulmonary function parameters, reflecting different aspects of CF pathophysiology. Consequently, the aim of the current prospective observational study was to describe exhaled NO using partitioning of its origins (from alveoli and conducting airways) to further assess the relationships between flow-independent NO exchange parameters (alveolar NO concentration ($C_{A,NO}$) and maximum conducting airway NO flux ($J'_{aw,NO}$), based on a two-compartment model of NO exchange) and lung function parameters. It was hypothesised that exhaled NO exchange parameters of conducting airways may be linked to airflow limitation, whereas $C_{A,NO}$ may be linked to tests describing the alveolar-capillary transfer of gas at rest and during exercise. Alveolar NO fraction has been shown to be either decreased or increased in CF patients [6, 7]. The endothelial origin of $C_{A,NO}$ remains a subject of controversy; nevertheless, a frequent impairment of endothelium-mediated vasodilation has been shown *ex vivo* on pulmonary arterial rings of CF patients with end-stage lung disease, which may occur before obvious pulmonary hypertension [11]. An NO-dependent impairment in flow-mediated dilation may impair the ability of the pulmonary vascular bed to dilate on exercise [12], resulting in an increased physiological dead space volume (V_D)/tidal volume (V_T) ratio. Therefore, one aim was to assess whether $C_{A,NO}$ could be linked to criteria of wasted ventilation on exercise. To this end, only patients without severe airflow limitation and pulmonary hypertension were enrolled in a prospective observational study to evaluate whether exhaled NO parameters could constitute markers of bronchial and lung vascular impairments.

METHODS

Patients

Children or adults with CF were recruited from three CF centres (Cochin, Trousseau and Necker Hospitals, all Assistance Publique – Hôpitaux de Paris, Paris, France). Ethical approval for the study protocol was received from a research ethics committee (Comité de Protection des Personnes IDF IV, Paris) and informed consent was obtained.

Inclusion criteria were a diagnosis of CF confirmed by sweat tests (chloride concentrations exceeding 60 $mmol \cdot L^{-1}$) and/or by two mutations of the CF transmembrane conductance regulator (CFTR) gene, a stable clinical condition, the absence of hepatic cirrhosis or asthma, an immunoglobulin E level <200 $UI \cdot L^{-1}$, no pulmonary arterial hypertension at rest on echocardiography and an age of $\geqslant$10 yrs. All functional tests were obtained within a week.

Exhaled NO

All exhaled NO and gas transfer measurements were performed in Georges Pompidou European Hospital (Paris). As discordant results concerning alveolar NO measurements have previously been described using different analytical methods based on the same model of NO exchange dynamics [6, 7], we used the two analytical approaches in our patients that have previously been described and compared in detail [13].

The dependency of the F_{eNO} on exhalation flow rate can be explained by a simple two-compartment model of the lung that has been used by several research groups. This two-compartment model describes exhaled NO arising from two compartments, the airways and the alveolar region, using three flow-independent exchange parameters: one describing the alveolar region (the steady-state $C_{A,NO}$), and two describing the airway region (airway NO diffusing capacity and either the maximum $J'_{aw,NO}$ or the airway wall NO concentration).

Two analytical approaches have been described in the literature to estimate flow-independent NO parameters: multiple constant flow (MCF) and dynamically changing flow methods. Their methodologies are briefly described.

MCF method

MCF $J'_{aw,NO}$ and $C_{A,NO}$ were calculated by a chemiluminescent analyser (ENDONO 8000; Seres, Aix-en-Provence, France) after obtaining several exhaled NO measurements at different expiratory flow rates (four to six expiratory flows between 50 and 200 $mL \cdot s^{-1}$), using the previously described linear approach [10]. The criteria used for each exhaled NO measurement were those recommended in international guidelines [14]. We used a validity criterion of this linear approach ($r^2 \geqslant 0.64$), as previously described [13]. Since F_{eNO} was measured at 50 $mL \cdot s^{-1}$ during the multiple exhalation method, results of F_{eNO} measured at 0.05 $L \cdot s^{-1}$ ($F_{eNO,0.05}$) were provided.

Dynamically changing flow method

The method consisted of an inspiration to total lung capacity, a 10-s breath hold followed by a slow (5–8 s) exhalation to functional residual capacity against a 7.5 cmH_2O positive expiratory pressure valve. The analog signals of NO and flow were digitised; mathematical estimation of the parameters (dynamically changing flow (DCF) $C_{A,NO}$, airway NO diffusing capacity and DCF $J'_{aw,NO}$) has previously been described in detail [13].

CO and NO transfers

Transfer factors of the lung for NO and CO ($T_{L,NO}$ and $T_{L,CO}$, respectively) were obtained from two separate measurements. The single, rapid, maximal exhalation at constant flow rate method described by PERILLO *et al.* [15] was used for measuring $T_{L,NO}$. For measurement of $T_{L,CO}$, the single-breath determination of CO uptake in the lung was determined according to recent international guidelines with automated equipment (MasterScreen Body; Jaeger, Wurzburg, Germany) [16].

Membrane conductance and pulmonary capillary blood volume (V_c) available for gas exchange were calculated according to the equation of ROUGHTON and FORSTER [17] and values of the different constant factors were selected according to GLENET *et al.* [18].

Spirometry

Spirometry was obtained in each centre. Measurement and theoretical values followed recent international guidelines [19].

Exercise test

Two investigators (F. Aubourg and C. Delclaux) performed the symptom limited incremental exercise tests. All tests consisted of 5 min of rest, 3 min of warm-up (20 W), the incremental work-rate period, and 3-min resting recovery. A ramp protocol was used with an incremental rate of 5–15 $W \cdot min^{-1}$ judged by the operator. Exercise tests were terminated at the point of symptom limitation. Arterial oxygen saturation measured by pulse oximetry, electrocardiographic monitoring of the heart rate and blood pressure by indirect sphygmomanometry were monitored. Breath by breath data were collected while subjects breathed through a mouth piece (with a nose clip). Computer software (SensorMedics, Yorba Linda, CA, USA) calculated minute ventilation (V'_E), oxygen uptake (V'_{O_2}), carbon dioxide production (V'_{CO_2}), end-tidal CO_2 tension, V_T and breathing frequency. Slopes of V'_{O_2}/power W, heart rate/V'_{O_2}, V'_E/V'_{O_2}, V'_E/V'_{CO_2} and anaerobic threshold (ventilatory threshold, using the V-slope method) were calculated. At rest and immediately before the end of exercise arterial sampling was performed (blood gas and lactate analyses) allowing the calculation of arterial minus end-tidal CO_2 tension ($P_{(a\text{-}ET)CO_2}$) and physiological V_D/V_T ratio (a value of V_D/V_T ratio >0.25 may be considered as abnormal in these young subjects [20]). Subjects were asked to score their sense of breathlessness and muscle effort/fatigue using Borg scales during the test. Predicted values of V'_{O_2} were calculated according to reference equations obtained in children and adults [21]. Ventilation was expressed as a percentage of predicted maximal voluntary ventilation (MVV):

$$((MVV - V'_E)/MVV)$$

The predicted MVV was calculated as 40 × forced expiratory volume in 1 s (FEV_1) [21].

Statistical analysis

We included 34 patients because using this sample size HO *et al*. [4] demonstrated a significant relationship between exhaled NO and airflow limitation. All results are expressed as median (25th–75th percentile). Since the shape of the relationship between parameters cannot be inferred, only Spearman correlation coefficients were determined (rho (ρ) values are given). Qualitative variables were compared using the Mann–Whitney U-test or Kruskall–Wallis test as appropriate. Statistical significance was defined by $p \leqslant 0.05$.

RESULTS

In total, 34 patients were prospectively included; their clinical characteristics are described in table 1 while their functional characteristics are described in table 2 (resting function) and table 3 (exercise function). In relation to table 2, SURI *et al*. [7] observed the following exhaled NO median (range) values in 22 children with cystic fibrosis (CF) using the MCF method: $C_{A,NO}$ 2.2 (0.6–5.6) ppb and $J'_{aw,NO}$ 27 (4–75) $nL \cdot min^{-1}$. SHIN *et al*. [6] observed the following exhaled NO values in nine children with CF using the DCF method: $C_{A,NO}$ 2.0±1.2 ppb, $J'_{aw,NO}$ 36±39 $nL.min^{-1}$, $D'_{aw,NO}$ 1.06±0.73 $nL.min^{-1}.ppb^{-1}$ and $C'_{aw,NO}$ 38±25 ppb.

Exhaled NO

The MCF method demonstrated that expiratory flow rate and exhaled NO output were linearly related in all patients (see r^2 value, table 2) suggesting that the two-compartment model adequately describes exhaled NO output in CF patients. The results of both analytical methods were linearly correlated (r^2=0.63 and r^2=0.47 for $C_{A,NO}$ and $J'_{aw,NO}$, respectively; $p<0.0001$ for both comparisons). It has to be noted that the MCF method gave higher values of $C_{A,NO}$ and lower values of $J'_{aw,NO}$ as compared with the DCF method ($p<0.01$ for both comparisons). Since the two analytical methods gave quite similar results (table 2), correlations obtained with the MCF method are further reported for simplicity (since this method is widely used), and only additional results obtained with the DCF method are given.

Exhaled NO values ($C_{A,NO}$ and $J'_{aw,NO}$) were not significantly modified by pancreatic insufficiency ($F_{eNO,0.05}$ 7.6 (5.2–15.9) ppb *versus* without insufficiency 17.7 (10.7–23.5) ppb; p=0.20), diabetes, inhaled corticosteroid ($F_{eNO,0.05}$ 12.1 (6.0–18.2) ppb *versus* without steroid 7.7 (5.9–13.6) ppb; p=0.52) or inhaled β_2-agonist treatment ($F_{eNO,0.05}$ 7.1 (5.0–16.9) ppb *versus* without 9.1 (6.8–15.3) ppb; p=0.69), bacterial colonisation or by the mutation group.

Lung capillary blood volume and membrane conductance

On average, $T_{L,CO}$ was preserved in our CF patients (table 2). As NO can react with bacteria, we assessed whether lung bacterial colonisation modifies $T_{L,NO}$, demonstrating similar values in patients with or without airway bacterial colonisation (data not shown).

Exercise test

Overall, a mild impairment in exercise capacity was evidenced (table 3). Peak V'_{O_2} % predicted was related to FEV_1 % pred (ρ=0.37, p=0.034). Physiological dead space on peak exercise was not related to pulmonary function tests (FEV_1 or $T_{L,CO}$, data not shown). The V_D/V_T ratio seemed to participate in exercise performance impairment as anaerobic threshold and peak V'_{O_2} (trend for oxygen pulse ρ= -0.37, p=0.066) were negatively correlated with physiological V_D/V_T ratio (fig. 1). Ventilation/perfusion ratio (V'/Q') inequalities were evidenced in some patients and there was a negative relationship between arterial oxygen tension (P_{a,O_2}) and $P_{(a\text{-}ET)CO_2}$ at peak exercise (ρ= -0.45, p=0.024).

Overall, 10 out of 26 patients had an increased physiological V_D/V_T ratio at peak exercise >0.25 (four out of 26 >0.30).

Relationships between flow-independent exhaled NO exchange parameters and functional tests obtained at rest and during exercise

Parameters characterising conducting airways

MCF $J'_{aw,NO}$ and $F_{eNO,0.05}$ correlated positively with airflow limitation (FEV_1 and FEV_1/forced vital capacity (FVC); fig. 2). These relationships were still significant (ρ=0.62, p=0.013 for both comparisons) in patients without inhaled corticosteroid (n=18), while the statistical significance was lost in patients (n=16) receiving inhaled corticosteroid.

A relationship was evidenced between $J'_{aw,NO}$ and baseline P_{a,O_2} (ρ=0.55, p=0.005). The DCF method additionally demonstrated that the airway wall NO concentration (but not airway NO diffusing capacity) also correlated with airflow limitation (ρ=0.51, p=0.011).

TABLE 1 Clinical characteristics

Subjects	34
Age yrs	19 (15–25)
Children/adults	16/18
Female/male	11/23
Weight kg	54 (46–63)
Height cm	163 (157–171)
BMI kg·m^{-2}	20.3 (17.9–21.7)
Mutations	
F508del/F508del	12
F508del/other	17
No F508del mutation	5
Pancreatic insufficiency	29
Diabetes	2
Bacterial airway colonisation	29
Staphylococcus aureus	26
Pseudomonas aeruginosa	15
Inhaled corticosteroid	16
Inhaled long-acting β-agonist	17
MRC score	1 (1–2)
BDI score	9 (7–10)

Data are presented as n or median (25th–75th percentile). BMI: body mass index; MRC: Medical Research Council dyspnoea scale (1–5, with 5 being the most severe score); BDI: Baseline Dyspnoea Index (1–12, with 12 being the least severe score). The MRC and BDI scores were significantly negatively correlated (data not shown).

TABLE 2 Results of resting pulmonary function tests

Characteristic	Result
Spirometry	34
FEV_1 % pred	71 (54–94)
FEV_1/FVC %	76 (70–84)
$T_{L,CO}$	32
V_A L	3.89 (3.17–4.37)
$T_{L,CO}/V_A$ mmol·min^{-1}·kPa^{-1}·L^{-1}	1.76 (1.63–1.99)
$T_{L,CO}$ mmol·min^{-1}·kPa^{-1}	6.77 (5.88–7.86)
$T_{L,CO}$ % pred	74 (67–85)
$T_{L,NO}$	18
$T_{L,NO}$ mmol·min^{-1}·kPa^{-1}	24.55 (19.90–27.40)
$D_{M,CO}$ mmol·min^{-1}·kPa^{-1}	12.45 (10.10–13.90)
V_C mL	75 (69–104)
$T_{L,NO}/T_{L,CO}$ ratio	3.35 (3.20–3.82)
Exhaled NO$^{\#}$	
MCF method	30
$r^{2\P}$	0.97 (0.93–0.98)
$C_{A,NO}$ ppb	3.3 (2.4–6.4)
$J'_{aw,NO}$ nL·min^{-1}	17 (9–30)
$F_{eNO,0.05}$ ppb	8.4 (6.2–16.2)
DCF method	26
$C_{A,NO}$ ppb	2.2 (1.2–5.0)
$J'_{aw,NO}$ nL·min^{-1}	29 (18–60)
$D'_{aw,NO}$ nL·min^{-1}·ppb^{-1}	0.24 (0.21–0.31)
$C'_{aw,NO}$ ppb	100 (61–218)

Data are presented as n or median (25th–75th percentile). FEV_1: forced expiratory volume in 1 s; % pred: % predicted; FVC: forced vital capacity; $T_{L,CO}$: transfer factor of the lung for carbon monoxide; V_A: alveolar volume; $T_{L,NO}$: transfer factor of the lung for nitric oxide (NO); $D_{M,CO}$: membrane diffusion capacity of carbon monoxide; V_C: pulmonary capillary blood volume; MCF: multiple constant flow; $C_{A,NO}$: alveolar NO concentration; $J'_{aw,NO}$: airway NO flux; $F_{eNO,0.05}$: exhaled NO fraction measured at 0.05 L·s^{-1}; DCF: dynamically changing flow; $D'_{aw,NO}$: airway NO diffusing capacity; $C'_{aw,NO}$: airway wall NO concentration. Normal values (nonatopic subjects) for $C_{A,NO}$, $J'_{aw,NO}$, $D'_{aw,NO}$ and $C'_{aw,NO}$ using the DCF method are 1.9±0.8 ppb, 28±16 nL·min^{-1}, 0.31±0.01 nL·min^{-1}.ppb^{-1} and 90±52 ppb, respectively [13]. $^{\#}$: four patients were unable to perform both measures of exhaled NO and four additional patients were unable to perform the DCF method; ¶: coefficient (linear regression) describing the linearity of the relationship between expiratory flow rate and NO output (linearity is an underlying assumption of the two-compartment model that needs to be verified) [13].

$C_{A,NO}$

A statistically significant relationship was observed between $C_{A,NO}$ and FEV_1/FVC at rest (fig. 2). This relationship was still significant ($\rho=0.55$, $p=0.027$) in patients without inhaled corticosteroids (n=18), while the statistical significance was lost in patients (n=16) receiving inhaled corticosteroids. A positive relationship was observed between $C_{A,NO}$ and V_C/alveolar volume (V_A; $\rho=0.55$, $p=0.027$).

During exercise, significant relationships were evidenced between $C_{A,NO}$ and parameters of wasted ventilation: physiological V_D/V_T ratio $\rho=0.44$, $p=0.046$; V'_E/V'_{CO_2} at anaerobic threshold $\rho=0.49$, $p=0.009$; and V'_E/V'_{CO_2} slope (fig. 3).

DISCUSSION

The first result of the present physiological study shows that flow-independent NO exchange parameters are related to airflow limitation in young patients with CF. The second finding suggests that defective lung vascular recruitment/dilation is present in CF, independently of the severity of airflow limitation, and that the resulting wasted ventilation may mildly impair exercise capacity. The third result shows that $C_{A,NO}$ at rest is linked to this wasted ventilation during exercise.

Partitioning of exhaled NO

The two-compartment model of NO exchange dynamics can be considered as valid when a linear relationship between expiratory flow and NO output is evidenced (agreement with the theoretical model) [22], such linearity was evidenced in all CF patients. Our values of $F_{eNO,0.05}$ are in agreement with previous reports, suggesting a nil or mild decrease as compared with healthy subjects [5–7]. Two analytical approaches of exhaled NO data were used, based on the same two-compartment model. The results obtained from the two methods were correlated but not equivalent as previously shown [13]. The higher values of $C_{A,NO}$ in the multiple flow approach may be related to the higher influence of axial diffusion of NO in this method [23]. Our results are at variance with those of SHIN *et al.* [6] (DCF method), obtained from nine children with CF and are in agreement with those of SURI *et al.* [7] (MCF method). The former group of investigators demonstrated that airway NO diffusing capacity was elevated and both airway wall NO concentration and $C_{A,NO}$ were reduced

TABLE 3 Results of exercise test

Characteristic	Result
Baseline arterial blood gas	31
P_{a,O_2} mmHg	93 (85–98)
P_{a,CO_2} mmHg	38.0 (36.4–41.0)
S_{a,O_2} %	98 (97–98)
Baseline physiological V_D/V_T ratio	0.37 (0.33–0.41)
Peak V'_{O_2}	
mL·min^{-1}·kg^{-1}	31.2 (26.5–35.2)
% pred	71 (65–80)
Peak respiratory rate cycles·min^{-1}	40 (34–49)
Peak V'_E L·min^{-1}	58.6 (49.5–71.3)
Ventilatory reserve %	34 (20–48)
Anaerobic threshold % peak V'_{O_2} pred	42 (35–51)
V'_E/V'_{CO_2} at anaerobic threshold	32 (28–36)
Peak HR % pred	82 (77–88)
Peak oxygen pulse % pred	90 (78–102)
Peak physiological V_D/V_T ratio	0.24 (0.19–0.29)
Peak $P_{(a\text{-}ET)CO_2}$ mmHg	1.1 (-0.7–3.2)
Peak arterial blood gas	26
P_{a,O_2} mmHg	92 (85–100)
$P_{A\text{-}a,O_2}$ mmHg	18 (11–28)
P_{a,CO_2} mmHg	39.0 (34.9–43.0)
S_{a,O_2} %	97 (95–98)
Lactates mmol·L^{-1}	7.1 (7.0–10.1)
Peak Borg dyspnoea score	5 (3–7)
Peak Borg fatigue score	4 (3–5)
Calculated slopes	
V'_{O_2}/power W	10.6 (9.7–12.4)
HR/V'_{O_2}	3.0 (2.4–3.4)
V'_E/V'_{CO_2}	27.5 (24.5–29.7)
V'_E/V'_{O_2}	25.8 (20.8–27.7)

Data are expressed as n or median (25th – 75th percentile). Three out of the 34 patients declined sampling of both baseline and peak arterial blood gas and we failed to sample arterial blood gas on peak exercise in five additional patients. P_{a,O_2}: arterial oxygen tension; P_{a,CO_2}: arterial carbon dioxide tension; S_{a,O_2}: arterial oxygen saturation; V_D: dead space volume; V_T: tidal volume; V'_{O_2}: oxygen uptake; % pred: % predicted; V'_E: minute ventilation; V'_{CO_2}: carbon dioxide production; HR: heart rate; $P_{(a\text{-}ET)CO_2}$: arterial minus end-tidal carbon dioxide tension; $P_{A\text{-}a,O_2}$: alveolar-arterial oxygen tension difference.

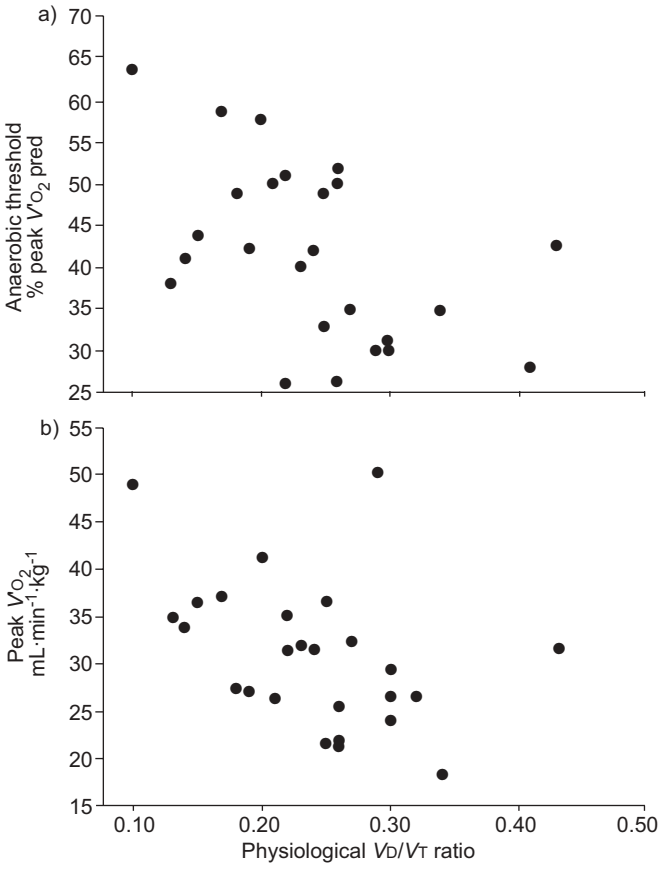

FIGURE 1. Wasted ventilation impairs exercise performance. The relationships between peak physiological dead space volume (V_D)/tidal volume (V_T) ratio and both a) anaerobic threshold (ρ= -0.47, p=0.025) and b) exercise performance (ρ= -0.50, p=0.012) are described. V'_{O_2}: peak oxygen uptake; pred: predicted.

compared with healthy subjects, giving normal F_{ENO} values using the DCF method. Of note, all their subjects with CF had atopy, were receiving albuterol and seven out of nine patients had a reactive airway disease and were receiving inhaled steroids, which may have impacted their results [6]. The study by SURI *et al.* [7], used a multiple flow rate measurement of exhaled NO and showed an increase in alveolar NO in CF patients. The enzymatic and cellular sources of exhaled NO remain largely unknown in both healthy and CF subjects. Nevertheless, NO synthases 2 (NOS2) and airway epithelial cells seem to be the main contributors for the bronchial origin of exhaled NO, and a reduction of NOS2 expression in bronchial epithelium has been demonstrated in CF [8]. The sources of alveolar NO, the concentration of which is near nil in healthy subjects, are undetermined; but there are arguments for an epithelial rather endothelial origin in the healthy condition. $C_{A,NO}$ increases in inflammatory settings at least due to macrophage and/or endothelial/epithelial stimulation [10].

The reduction in exhaled NO is linked to airflow limitation

Our study shows that NO exchange parameters characterising conducting airways are related to the degree of airflow limitation. In our study, $F_{ENO,0.05}$ was also linked to bronchial obstruction, as previously suggested by a single study [4]. Other investigators did not find such a relationship, which may be related to bronchial participation to F_{ENO}, this depends on the value of the expiratory flow rate chosen. Furthermore, we eliminated some potential confounders (atopy, asthma and cirrhosis) that may have favoured this relationship. We also showed that the statistical significance of the relationship was lost in patients receiving inhaled corticosteroids. Our results suggest that NO deficiency in conducting airways may participate in bronchial obstruction since GRASEMANN *et al.* [24] have shown that nebulised L-arginine not only significantly increased exhaled NO concentration but also resulted in a sustained improvement of FEV1 in patients with CF. Interestingly, in this latter study, oxygen saturation also increased significantly after the inhalation of L-arginine, which suggests an effect of NO on V'/Q' matching (we found a relationship between P_{a,O_2} and $J'_{aw,NO}$) [24]. We did not find

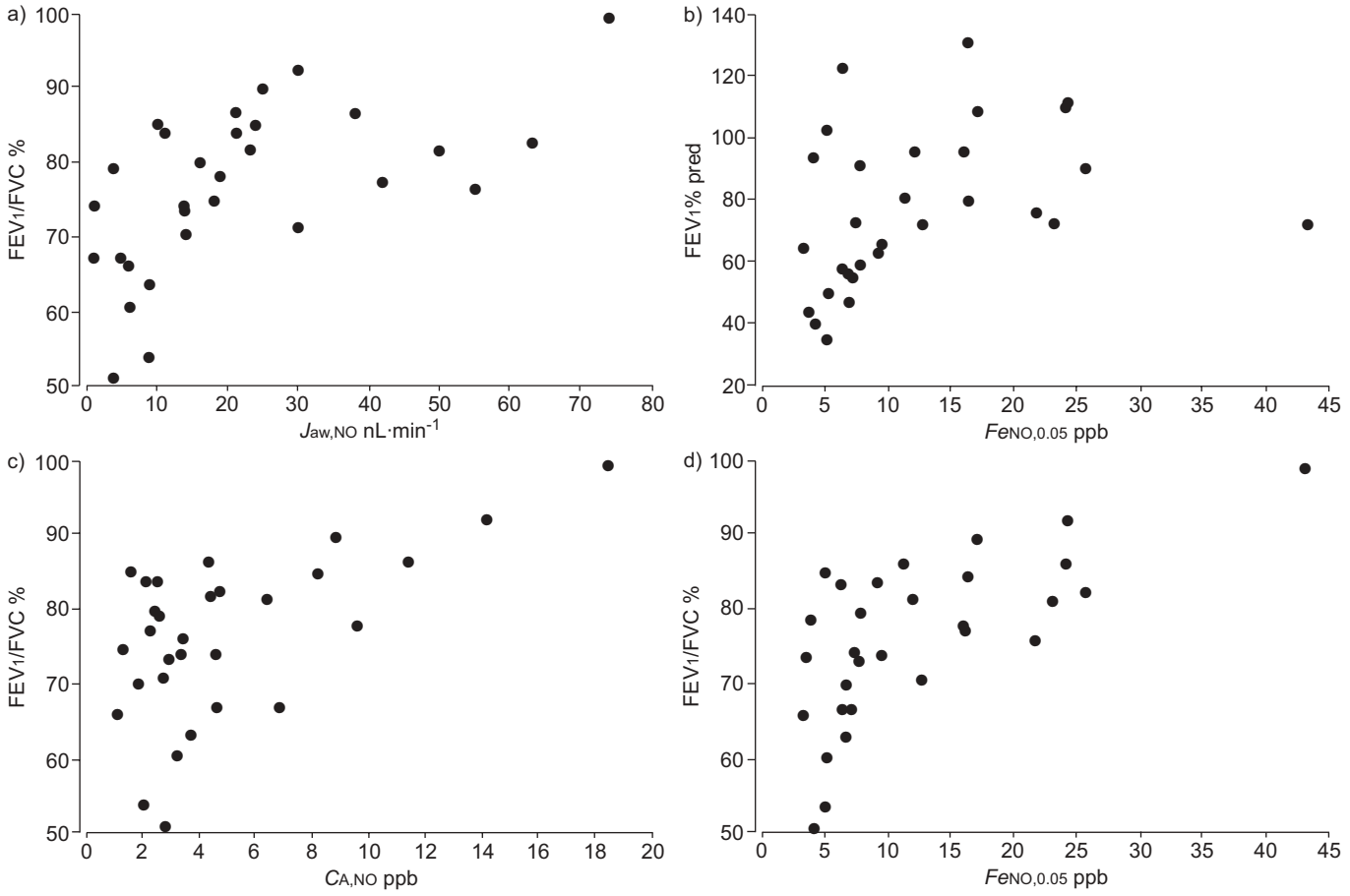

FIGURE 2. Reduction of exhaled nitric oxide (NO) is associated with airflow limitation. The relationships between airflow limitation (forced expiratory volume in 1 s (FEV_1) and FEV_1/forced vital capacity (FVC)) and a) bronchial maximum airway NO flux ($J'_{aw,NO}$; $\rho=0.64$, $p=0.0006$), b and d) global exhaled NO fraction ($F_{ENO,0.05}$; b) $\rho=0.51$, $p=0.006$; d) $\rho=0.63$, $p=0.0007$), and c) alveolar NO concentration ($C_{A,NO}$; $\rho=0.43$, $p=0.21$) are described. Exhaled NO parameters were obtained using the multiple constant flow method. FEV_1 % pred also correlated with $J'_{aw,NO}$ ($\rho=0.52$, $p=0.005$). % pred: % predicted.

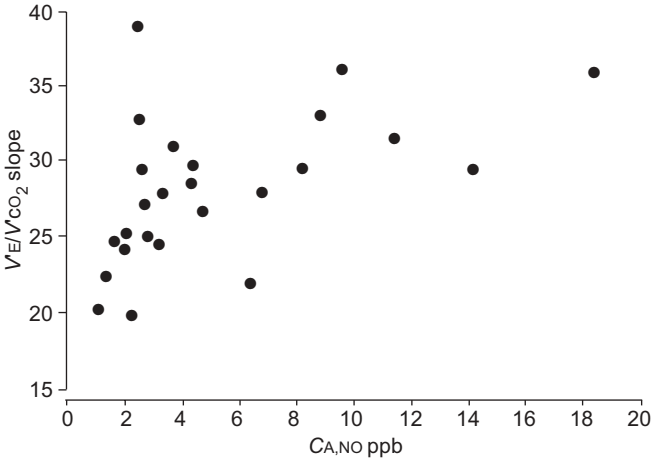

FIGURE 3. Increased alveolar nitric oxide concentration ($C_{A,NO}$) at rest is associated with increased wasted ventilation during exercise. The relationship between $C_{A,NO}$ and minute ventilation (V'_E)/carbon dioxide production (V'_{CO_2}) slope is shown. $\rho=0.61$, $p=0.002$.

that bacterial colonisation was associated with lower levels of $F_{ENO,0.05}$ or flow-independent NO exchange parameters, which is at variance with the results obtained by KEEN *et al*. [5]. GIRGIS *et al*. [25] have observed a decrease in F_{ENO} in patients with pulmonary arterial hypertension. Interestingly, they observed that bosentan reversed this defect, suggesting that suppression of NO may have been caused by endothelin. Along this line, we have recently shown that endothelial dysfunction in CF seems to be mediated by activation of the endothelin pathway [11].

Transfer of gas in CF lung

The diffusion capacity in children with CF is often preserved, despite ongoing airflow limitation [26]. Only one recent study to our best knowledge has reported values of V_c available for gas exchange and $T_{L,NO}/T_{L,CO}$ ratio in CF patients [27]. Our results are in agreement with the DRESSEL *et al*. [27] study results, showing a preserved V_c and a slightly decreased ratio, which may suggest an increased thickness of the alveolar blood barrier [18]. At rest, $C_{A,NO}$ correlated positively with V_c/V_A, which may argue for both NO-related vasodilation or vascular release of NO [12].

Vascular impact of CF disease suggested by exercise test results

CF subjects have a reduced peak exercise capacity that seems partly related to nonpulmonary factors in patients with mild to moderate disease [28]. In more severe patients (FEV1 <40%), ventilatory limitation seems to become a main limiting factor to exercise [28]. To our knowledge, few data are available in CF patients of indirect assessment of lung vascular recruitment/dilation (physiological V_D/V_T ratio, $P_{(a\text{-}ET)CO_2}$, alveolar-arterial oxygen tension difference). Usually, CF is not considered as a disease with an important pulmonary vascular impact. Nevertheless, pulmonary hypertension can occur in CF with a dramatically negative effect on survival [29]. Before obvious vascular remodelling leading to increased vascular resistance and hypertension, endothelial dysfunction of pulmonary arteries may occur. Along this line, MAUREY *et al.* [11] recently demonstrated that this endothelial dysfunction is common in end stage CF disease, and can be present despite the absence of resting pulmonary hypertension. Moreover, the vasodilator property of CFTR in pulmonary arteries has also been shown [30]. We therefore hypothesised that endothelial dysfunction may be associated with defective vasodilation of pulmonary vessels during exercise. Our results suggest that defective dilation on exercise exists since a significant alveolar V_D can be measured at peak exercise in some CF patients (four to 10 out of 26). Furthermore, this defective vasodilation tends to impair oxygen pulse (suggesting a reduction of stroke volume on exercise), is associated with a decreased anaerobic threshold and impaired performance (peak V'_{O_2}), and contributes to dyspnoea (increased ventilatory demand). Consequently, this vascular impairment seems to be of clinical significance. Our results are in agreement with other investigators who have demonstrated that, on exercise, pulmonary hypertension and reduction of stroke volume may occur in CF patients in the absence of obvious pulmonary hypertension [31]. On exercise, parameters reflecting alveolar dead space ventilation were related to $C_{A,NO}$ at rest. One could hypothesise that an increase in $C_{A,NO}$ may occur because of a distal lung inflammatory process and/or thickening of the alveolar-capillary barrier. This increased concentration may increase capillary blood volume available for gas exchange (preserved V_c in spite of reduced alveolar volume) at rest, but would be associated with defective vasodilation (increased physiological V_D/V_T ratio) on exercise due to the inability to further augment NO release (endothelial dysfunction). Further longitudinal studies are warranted to assess the prospective ability of $C_{A,NO}$ to detect early vascular disease in CF patients.

Limitations of the study

Given the small number of patients studied here, our results must be considered as preliminary. All pulmonary function tests were not obtained in the whole group due to technical limitations in our youngest and most severely affected patients. We did not evidence dynamic hyperinflation during the exercise test in patients with mild to moderate airflow limitation, but inspiratory capacity on exercise was only measured in one centre (George Pompidou, data not shown). Consequently, abnormal dynamic ventilatory mechanics cannot be ruled out in all CF patients and may have participated, to some extent, to their functional limitation. Some patients were receiving inhaled treatment (corticosteroid and long-acting β_2-agonist) which may have modified exhaled NO. Nevertheless, the reduction of exhaled NO in patients treated with inhaled corticosteroid is modest in the setting of CF [32]. Our analytical methods did not take into account the trumpet-like morphology of conducting airways or axial diffusion from bronchial source to alveoli. This latter effect is probably of minimal importance in the setting of CF in which conducting $J'_{aw,NO}$ is not elevated. Whether our $C_{A,NO}$ truly reflects alveoli NO fraction is beyond the scope of this clinical study. The imperfection of the parameters describing NO exchange dynamics is balanced by their ability to describe useful clinical end-points.

In conclusion, our study shows that flow-independent NO exchange parameters are related to both bronchial and lung vascular impairments in CF, namely the degree of airflow limitation (epithelial NO concentration of the conducting airway) and the capillary blood volume related to alveolar volume ($C_{A,NO}$).

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STATEMENT OF INTEREST

A statement of interest for C. Delclaux can be found at www.erj.ersjournals.com/misc/statements.dtl

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REFERENCES

1 Corey M. Power considerations for studies of lung function in cystic fibrosis. *Proc Am Thorac Soc* 2007; 4: 334–337.
2 Ricciardolo FL, Sterk PJ, Gaston B, *et al.* Nitric oxide in health and disease of the respiratory system. *Physiol Rev* 2004; 84: 731–765.
3 Franklin PJ, Hall GL, Moeller A, *et al.* Exhaled nitric oxide is not reduced in infants with cystic fibrosis. *Eur Respir J* 2006; 27: 350–353.
4 Ho LP, Wood FT, Robson A, *et al.* The current single exhalation method of measuring exhales nitric oxide is affected by airway calibre. *Eur Respir J* 2000; 15: 1009–1013.
5 Keen C, Olin AC, Edentoft A, *et al.* Airway nitric oxide in patients with cystic fibrosis is associated with pancreatic function, Pseudomonas infection, and polyunsaturated fatty acids. *Chest* 2007; 131: 1857–1864.
6 Shin HW, Rose-Gottron CM, Sufi RS, *et al.* Flow-independent nitric oxide exchange parameters in cystic fibrosis. *Am J Respir Crit Care Med* 2002; 165: 349–357.
7 Suri R, Paraskakis E, Bush A. Alveolar, but not bronchial nitric oxide production is elevated in cystic fibrosis. *Pediatr Pulmonol* 2007; 42: 1215–1221.
8 Kelley TJ, Drumm ML. Inducible nitric oxide synthase expression is reduced in cystic fibrosis murine and human airway epithelial cells. *J Clin Invest* 1998; 102: 1200–1207.
9 Texereau J, Fajac I, Hubert D, *et al.* Reduced exhaled NO is related to impaired nasal potential difference in patients with cystic fibrosis. *Vascul Pharmacol* 2005; 43: 385–389.
10 Delclaux C, Mahut B, Zerah-Lancner F, *et al.* Increased nitric oxide output from alveolar origin during liver cirrhosis *versus* bronchial source during asthma. *Am J Respir Crit Care Med* 2002; 165: 332–337.
11 Maurey C, Henno P, Delclaux C, *et al.* Role of endothelin-1 in endothelial dysfunction in cystic fibrosis. *Eur Respir J* 2007; 30: Suppl. 51, 30s.
12 Ide H, Nakano H, Ogasa T, *et al.* Regulation of pulmonary circulation by alveolar oxygen tension *via* airway nitric oxide. *J Appl Physiol* 1999; 87: 1629–1636.
13 Mahut B, Louis B, Zerah-Lancner F, *et al.* Validity criteria and comparison of analytical methods of flow-independent exhaled NO parameters. *Respir Physiol Neurobiol* 2006; 153: 148–156.
14 American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005; 171: 912–930.
15 Perillo IB, Hyde RW, Olszowka AJ, *et al.* Chemiluminescent measurements of nitric oxide pulmonary diffusing capacity and alveolar production in humans. *J Appl Physiol* 2001; 91: 1931–1940.
16 Macintyre N, Crapo RO, Viegi G, *et al.* Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J* 2005; 26: 720–735.
17 Roughton F, Forster R. Relative importance of diffusion and chemical reaction rates in determining rate of the exchange of gases in human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. *J Appl Physiol* 1957; 11: 290–302.
18 Glenet SN, De Bisschop C, Vargas F, *et al.* Deciphering the nitric oxide to carbon monoxide lung transfer ratio: physiological implications. *J Physiol* 2007; 582: 767–775.
19 Pellegrino R, Viegi G, Brusasco V, *et al.* Interpretative strategies for lung function tests. *Eur Respir J* 2005; 26: 948–968.
20 Wasserman K, Van Kessel AL, Burton GG. Interaction of physiological mechanisms during exercise. *J Appl Physiol* 1967; 22: 71–85.
21 Wasserman K, Hansen JE, Sue DY, *et al.* Normal values. *In*: Wilkins LW, ed. Principles of exercise testing and interpretation. Including pathophysiology and clinical applications. 4th Edn. Philadelphia, Lippincott Williams & Wilkins, 2005; pp. 160–182.
22 George SC, Hogman M, Permutt S, *et al.* Modeling pulmonary nitric oxide exchange. *J Appl Physiol* 2004; 96: 831–839.
23 Kerckx Y, Michils A, Van Muylem A. Airway contribution to alveolar nitric oxide in healthy subjects and stable asthma patients. *J Appl Physiol* 2008; 104: 918–924.
24 Grasemann H, Grasemann C, Kurtz F, *et al.* Oral L-arginine supplementation in cystic fibrosis patients: a placebo-controlled study. *Eur Respir J* 2005; 25: 62–68.
25 Girgis RE, Champion HC, Diette GB, *et al.* Decreased exhaled nitric oxide in pulmonary arterial hypertension: response to bosentan therapy. *Am J Respir Crit Care Med* 2005; 172: 352–357.
26 Merkus PJ, Govaere ES, Hop WH, *et al.* Preserved diffusion capacity in children with cystic fibrosis. *Pediatr Pulmonol* 2004; 37: 56–60.
27 Dressel H, Filser L, Fischer R, *et al.* Lung diffusing capacity for nitric oxide and carbon monoxide: dependence on breath-hold time. *Chest* 2008; 133: 1149–1154.
28 Moorcroft AJ, Dodd ME, Morris J, *et al.* Symptoms, lactate and exercise limitation at peak cycle ergometry in adults with cystic fibrosis. *Eur Respir J* 2005; 25: 1050–1056.
29 Fauroux B, Hart N, Belfar S, *et al. Burkholderia cepacia* is associated with pulmonary hypertension and increased mortality among cystic fibrosis patients. *J Clin Microbiol* 2004; 42: 5537–5541.
30 Robert R, Savineau JP, Norez C, *et al.* Expression and function of cystic fibrosis transmembrane conductance regulator in rat intrapulmonary arteries. *Eur Respir J* 2007; 30: 857–864.
31 Ionescu AA, Payne N, Obieta-Fresnedo I, *et al.* Subclinical right ventricular dysfunction in cystic fibrosis. A study using tissue Doppler echocardiography. *Am J Respir Crit Care Med* 2001; 163: 1212–1218.
32 Linnane SJ, Thin AG, Keatings VM, *et al.* Glucocorticoid treatment reduces exhaled nitric oxide in cystic fibrosis patients. *Eur Respir J* 2001; 17: 1267–1270.