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# EXHALED NO IN CYSTIC FIBROSIS:

# RELATIONSHIPS WITH AIRWAY AND LUNG VASCULAR IMPAIRMENTS

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**ABSTRACT**

**Question**: A reduction of exhaled NO fraction and endothelial-mediated dysfunction have been reported in cystic fibrosis (CF). The aims of this study were to search for relationships between flow-independent NO exchange parameters (bronchial NO flux, J'$aw_{NO}$; alveolar NO concentration, $Calv_{NO}$) and lung function tests characterizing airflow limitation and lung vascular bed (capillary blood volume and physiological dead space/tidal volume [physVD/VT] ratio on exercise).

**Methods and Measurements**: Thirty-four patients with CF (16 children, 18 adults), without resting pulmonary hypertension, underwent spirometry, exhaled NO measurement (multiple constant flow analytical method), gas transfer assessment (CO and NO, allowing the calculation of capillary volume and membrane conductance) and a graded exercise test with V'$O_2$, V'$CO_2$ and arterial blood gas evaluations.

**Main results**: Both J'$aw_{NO}$ and $Calv_{NO}$ correlated positively with airflow limitation. $Calv_{NO}$ correlated positively with capillary volume / alveolar volume. On exercise, criteria of mild pulmonary vascular disease were evidenced in some patients that participated in exercise limitation (negative correlation between physVD/VT and peak V'$O_2$). $Calv_{NO}$ at rest correlated positively with these parameters of wasted ventilation on exercise (physVD/VT; V'E/V'$CO_2$ at ventilatory threshold and V'E/V'$CO_2$ slope).

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

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**Key words**: capillary blood volume, cystic fibrosis, exercise test, exhaled NO

## INTRODUCTION

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 broncho- vasomotor 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 activities of this messenger one may hypothesize 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 frankly decreased in upper (nasal) airways in CF patients, whereas exhaled NO fraction depicted 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 in 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 on exercise reflecting both airway and vascular impairments due to CF disease. To this end, a detailed analysis of exhaled NO was performed, 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 hypothesized 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 this 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 and maximum conducting airway NO flux, based on a two-compartment model of NO exchange) and lung function parameters. We hypothesized that exhaled NO exchange parameters of conducting airways may be linked to airflow limitation, whereas alveolar NO concentration may be linked to tests describing the alveolar-capillary transfer of gas at rest and on exercise. Alveolar NO fraction has been shown to be either decreased or increased in CF patients [6, 7]. The endothelial origin of alveolar NO concentration remains a subject of controversies; 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]. A NO-dependent impairment in flow mediated dilation may impair the ability of pulmonary vascular bed to dilate on exercise [12], resulting in an increased physiological dead space volume/tidal volume (VD/VT) ratio. One aim was therefore to assess whether alveolar NO concentration 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. Ethical approval for the study protocol was received from a research ethics committee and informed consent was obtained.

Inclusion criteria were a diagnosis of CF confirmed by sweat tests (chloride concentrations exceeding 60 mmol/L) and/or by two mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, a stable clinical condition, the absence of hepatic cirrhosis or asthma, an

IgE level < 200 UI/L, no pulmonary arterial hypertension at rest on echocardiography, and an age ≥ 10 years old. All functional tests (see below) were obtained within a week.

**Exhaled NO**

All exhaled NO and gas transfer measurements were performed in Georges Pompidou European hospital. 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, which have previously been described and compared in detail by our group [13].

The dependency of the exhaled NO fraction ($FE_{NO}$) 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 NO alveolar concentration [$Calv_{NO}$]), and two describing the airway region (airway NO diffusing capacity and either the maximum airway wall NO flux [$J'aw_{NO}$] or the airway wall NO concentration).

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

Multiple constant flow (MCF) method:

$J'aw_{NO,MCF}$ and $Calv_{NO,MCF}$ were calculated by the chemiluminescent analyser (ENDONO 8000, SERES, France) after obtaining several exhaled NO measurements at different expiratory flow rates (4 to 6 expiratory flows between 50 and 200 mL/s), 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 ($r^2 \geq 0.64$), as previously described [13]. Since $FE_{NO}$ was measured at 50 mL/s during the multiple exhalation method, $FENO_{0.05}$ results were provided.

Dynamically changing flow (DCF) method:

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

**CO and NO transfers**

NO (TLNO) and CO (TLCO) transfers were obtained from two separate measurements.

Single, rapid, maximal exhalation at constant flow rate for measuring TLNO:

We used the method described by Perillo and colleagues using a constant exhalation flow rate [15].

Measurement of TLCO:

The single-breath determination of CO uptake in the lung was determined according to recent international guidelines with automated equipment (MasterScreen Body, Jaeger, Germany) [16].

Calculation of membrane conductance ($D_m$) and capillary blood volume available for gas exchange ($V_c$):

These indexes were calculated according to the equation of Roughton and Forster [17] and values of the different constant factors were selected according to Glenet and colleagues [18].

**Spirometry**

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

**Exercise test**

Two investigators (FA and $CD_2$) performed the symptom limited incremental exercise tests. All tests consisted of a period of 5 min of rest, 3 min period of warm-up (20 Watts), the incremental work rate period, and a 3-min resting recovery period. A ramp protocol was used with an incremental rate of 5–15 $W.min^{-1}$ judged by the operator. Exercise tests were terminated at the point of symptom limitation. Oxygen saturation ($SpO_2$) by pulse oximetry, electrocardiographic monitoring of heart rate and blood pressure by indirect sphygmomanometry were monitored. Breath by breath data were collected while subjects breathed through a mouth piece (nose clip): computer software (Sensor Medics, Yorba Linda, CA) calculated minute ventilation (V'E), oxygen uptake ($V'O_2$), carbon dioxide production ($V'CO_2$), end-tidal $CO_2$ partial pressure ($P_{ET}$-$CO_2$), VT and breathing frequency. Slopes of $V'O_2$/Watts, $HR/V'O_2$, $V'E/V'O_2$, $V'E/V'CO_2$ 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 to calculate $P(a\text{-}ET)CO_2$ and physiological VD/VT ratio (a value of VD/VT ratio above 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'-V'E]/MVV'). The predicted MVV' was calculated as 40 x $FEV_1$ [21].

**Statistical analysis**

We included 34 patients as Ho and colleagues using this sample size demonstrated a significant relationship between exhaled NO and airflow limitation [4]. All results are expressed as median [$25^{th}$ – $75^{th}$ percentile]. Since the shape of the relationship between parameters cannot be inferred, only Spearman correlation coefficients were determined. Qualitative variables were compared using

Mann-Whitney U test or Kruskall Wallis test as appropriate. Statistical significance was defined by a P value $\leq 0.05$.

## RESULTS

Thirty-four 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).

### 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 ($r^2=0.63$, $r^2=0.47$, for $Calv_{NO}$ and $J'aw_{NO}$, respectively; $p<0.0001$ for both comparisons). It has to be noted that the MCF method gave higher values of $Calv_{NO}$ and lower values of $J'aw_{NO}$ as compared to 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 (and since this method is widely used), and only additional results obtained with the DCF method are given.

Exhaled NO values (and $Calv_{NO}$, $J'aw_{NO}$) were not significantly modified by pancreatic insufficiency ($FENO_{0.05}$: 7.6 ppb [5.2-15.9] versus without insufficiency 17.7 ppb [10.7-23.5]; p=0.20), diabetes, inhaled corticosteroid ($FENO_{0.05}$: 12.1 ppb [6.0-18.2] versus without steroid 7.7 ppb [5.9-13.6]; p=0.52) or inhaled β2-agonist treatment ($FENO_{0.05}$: 7.1 ppb [5.0-16.9] versus without 9.1 ppb [6.8-15.3]; p=0.69), bacterial colonization and of mutation group.

**Lung capillary blood volume and membrane conductance** (Table 2)

On average, TLCO was preserved in our CF patients. As NO can react with bacteria, we assessed whether lung bacterial colonization modifies TLNO, demonstrating similar values in patients with or without airway bacterial colonization (data not shown).

**Exercise test** (Table 3)

Overall, a mild impairment in exercise capacity was evidenced. Peak $V'O_2$ % predicted was related to $FEV_1$ % predicted (Rho=0.37, p=0.034). Physiological dead space on peak exercise was not related to pulmonary function tests (either $FEV_1$ or TLCO, data not shown). This VD/VT ratio seemed to participate in exercise performance impairment as anaerobic threshold and peak $V'O_2$ (trend for oxygen pulse: Rho=-0.37, p=0.066) were negatively correlated with physiological VD/VT ratio (figure 1). V'/Q' inequalities were evidenced in some patients and their was a negative relationship between $PaO_2$ and $P(a\text{-}ET)CO_2$ at peak exercise (Rho=-0.45, p=0.024).

Overall, 10/26 patients had an increased physiological VD/VT ratio at peak exercise above 0.25 (4/26 above 0.30).

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

Parameters characterizing conducting airways:

$J'aw_{NO,MCF}$ and $FE_{NO,0.05}$ correlated positively with airflow limitation ($FEV_1$ and $FEV_1/FVC$, figure 2). These relationships were still significant (Rho=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 $J'aw_{NO,MCF}$ and baseline $PaO_2$ (Rho=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 (Rho=0.51, p=0.011).

Alveolar NO concentration:

Resting condition: A statistically significant relationships was observed between $Calv_{NO,MCF}$ and $FEV_1/FVC$ (figure 2). This relationship was still significant (Rho=0.55, p=0.027) in patients without inhaled corticosteroid (n=18), while the statistical significance was lost in patients (n=16) receiving inhaled corticosteroid.

A positive relationship was observed between $Calv_{NO,MCF}$ and Vc/VA (Rho=0.55, p=0.027).

Exercise:

Significant relationships were evidenced between $Calv_{NO,MCF}$ and parameters of wasted ventilation: physiological VD/VT ratio, Rho=0.44, p=0.046; $V'E/V'CO_2$ at AT, Rho=0.49, p=0.009; $V'E/V'CO_2$ slope (figure 3).

**DISCUSSION**

The first result of this 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 alveolar NO concentration at rest is linked to this wasted ventilation on 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 $FENO_{0.05}$ are in agreement with previous reports, suggesting a nil or mild decrease as compared to 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 alveolar NO concentration 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 and colleagues (DCF method) obtained from 9 children with CF [6] and are in agreement with those of Suri and colleagues (MCF method) [7]. The former group of investigators demonstrated that $Daw_{NO,DCF}$ was elevated and both $Caw_{NO,DCF}$ and $Calv_{NO,DCF}$ were reduced as compared to healthy subjects, giving normal FENO values. Of note, all their subjects with CF had atopy, were receiving albuterol and 7/9 had a reactive airway disease and were receiving inhaled steroids, which may have impacted their results. Suri and colleagues, who used a multiple flow rate measurement of exhaled NO, showed an increase in alveolar NO in CF patients [7]. The enzymatic and cellular sources of exhaled NO remain largely unknown in both healthy and CF subjects. Nevertheless, 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, which concentration is near nil value in healthy subjects, are undetermined; but there are arguments for an epithelial rather endothelial origin in healthy condition. Alveolar NO concentration 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 characterizing conducting airways are related to the degree of airflow limitation. In our study, $FENO_{0.05}$ 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 FENO, which depends on the value of the expiratory flow rate chosen. Furthermore, we eliminated some potential confounders (atopy, asthma, cirrhosis) that may have favoured this relationship. We also show 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 and colleagues have shown that nebulized L-arginine not only significantly increased exhaled NO concentration but also resulted in a sustained improvement of $FEV_1$ in patients with CF [24]. Interestingly, in this latter study, oxygen saturation also increased significantly after the inhalation of L-arginine, which suggests an effect of NO on ventilation/perfusion matching (we find a relationship between $PaO_2$ and $J'aw_{NO}$). We did not find that bacterial colonization was associated with lower levels of $FENO_{0.05}$ or flow-independent NO exchange parameters, which is at variance with the results obtained by Keen and colleagues [5]. Girgis and colleagues have observed a decrease in FENO in patients with pulmonary arterial hypertension [25]. 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**

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 capillary blood volume available for gas exchange (Vc) and TLNO/TLCO ratio in CF patients [27]. Our results are in

agreement with their results, showing a preserved Vc and a slightly decreased ratio, which may suggest an increased thickness of the alveolar blood barrier [18]. At rest, alveolar NO concentration correlated positively with Vc/VA, 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 ($FEV_1$ < 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 VD/VT ratio, $P(a\text{-}ET)CO_2$, $P(A\text{-}a)O_2$). 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 and colleagues recently demonstrated that this endothelial dysfunction is common in end stage CF disease, and can be present despite the absence of resting pulmonary hypertension [11]. Moreover, the vasodilator property of CFTR in pulmonary arteries has also been shown [30]. We therefore hypothesized 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 dead space volume can be measured at peak exercise in some CF patients (4 to 10/26). 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'O2), and contributes to dyspnea (increased ventilatory demand). Consequently, this vascular impairment seems 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

absence of obvious pulmonary hypertension [31]. On exercise, parameters reflecting alveolar dead space ventilation were related to alveolar NO concentration at rest. One could hypothesize that an increase in alveolar NO concentration may occur because of a distal lung inflammatory process and/or thickening of alveolar-capillary barrier. This increased concentration may increase capillary blood volume available for gas exchange (preserved Vc in spite of reduced alveolar volume) at rest, but would be associated with defective vasodilation (increased physiological VD/VT 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 alveolar NO concentration 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 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 these patients with mild to moderate airflow limitation, but inspiratory capacity on exercise was measured in one centre only (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 β2-agonist) that 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 nor axial diffusion from bronchial source to alveoli. This latter effect is probably of minimal importance in the setting of CF in which conducting airway NO flux is not elevated. Whether our alveolar NO concentration 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 endpoints.

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 conducting airway) and the capillary blood volume related to alveolar volume (alveolar NO concentration).

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**Competing interests**

DH, FA, BF, LT, IS, GL, AC, ATDX, BL, BM: None declared.

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**FIGURE LEGENDS**

**Figure 1**: Wasted ventilation impairs exercise performance.

The relationships between physiological dead space volume / tidal volume (VD/VT) ratio and both anaerobic threshold (expressed as % peak $V'O_2$ predicted) (upper panel) and exercise performance ($V'O_2$, $mL.min^{-1}.kg^{-1}$) (lower panel) are described.

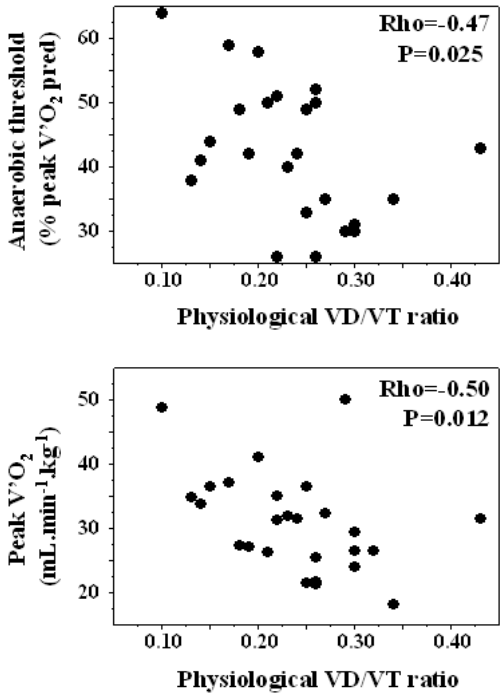


**Figure 2**: Reduction of exhaled NO is associated with airflow limitation.

The relationships between airflow limitation ($FEV_1$, $FEV_1$/FVC) and global ($FENO_{0.05}$), bronchial (maximum airway NO flux, $J'aw_{NO}$) and alveolar origin ($Calv_{NO}$) of exhaled NO are described. Exhaled NO parameters were obtained using the multiple constant flow method (see Methods). % pred denotes % of predicted value.

$FEV_1$ (% predicted) also correlated with $J'aw_{NO}$ (Rho=0.52, p=0.005).

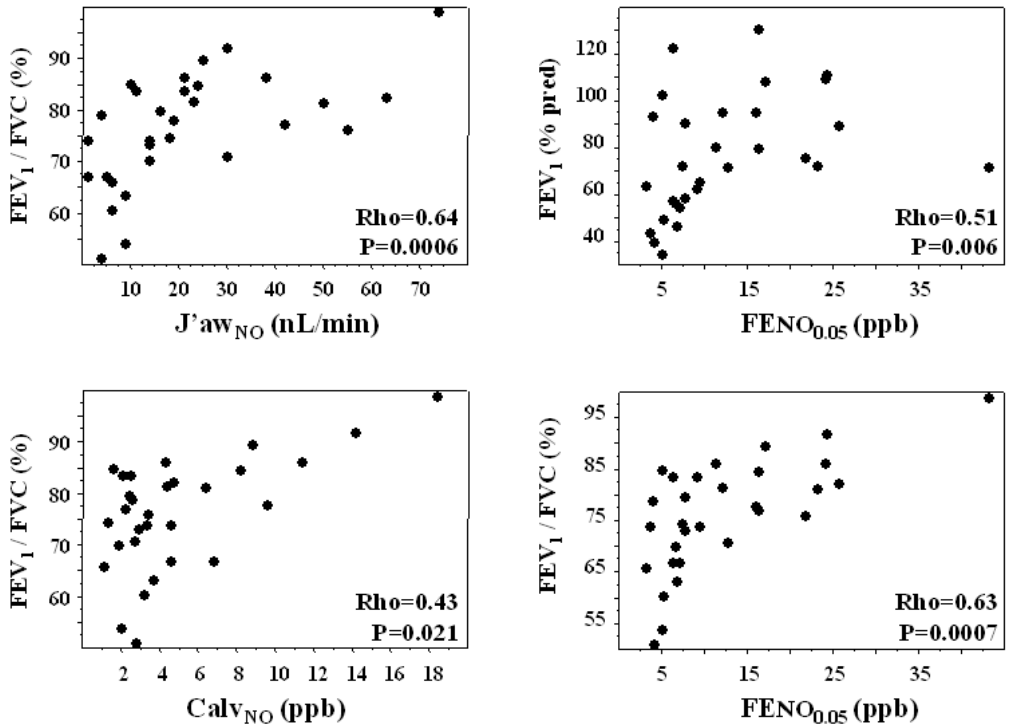


**Figure 3**: Increased alveolar NO concentration at rest is associated increased wasted ventilation on exercise.

The relationship between alveolar NO concentration ($Calv_{NO,MCF}$) and $V'E/V'CO_2$ slope is shown.

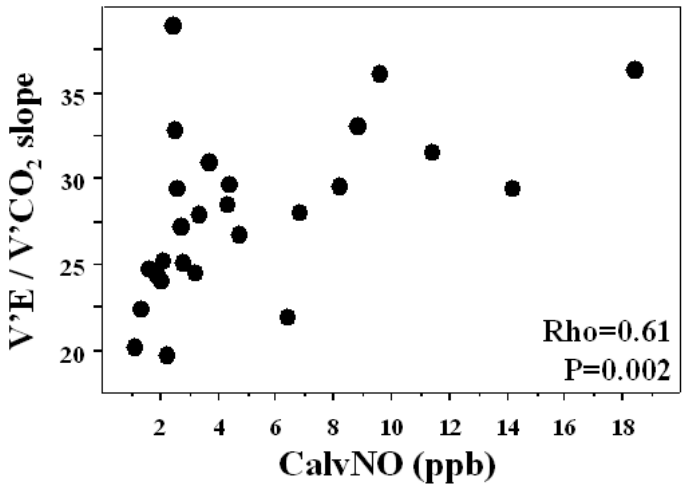
V'E / V'CO2 slope
35
30
25
20
2
4
6
8
10
12
14
16
18
CalvNO (ppb)
Rho=0.61
P=0.002

**Table 1:** Clinical characteristics

| Characteristic | 34 patients with cystic fibrosis |
|---|---|
| Age, yr | 19 [15-25] |
| Children/adults | 16/18 |
| Gender, 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, n | |
| F508del/F508del | 12 |
| F508del/other | 17 |
| no F508del mutation | 5 |
| Pancreatic insufficiency, n | 29 |
| Diabetes, n | 2 |
| Bacterial airway colonization, n | 29 |
| *Staphylococcus aureus*, n | 26 |
| *Pseudomonas aeruginosa*, n | 15 |
| Inhaled corticosteroid, n | 16 |
| Inhaled long acting β-agonist, n | 17 |
| MRC score | 1 [1-2] |
| BDI score | 9 [7-10] |

Results are expressed as median [$25^{th}$ – $75^{th}$ percentile]

MRC : Medical Research Council dyspnea scale (1 to 5, 5 being the more severe score)

BDI : Baseline Dyspnea Index (1 to 12, 12 being the less severe score). These two scores were significantly negatively correlated (data not shown).

**Table 2:** Results of resting pulmonary function tests

| Characteristic | |
|---|---|
| Spirometry, n | 34 |
| $FEV_1$, % predicted | 71 [54-94] |
| $FEV_1$/FVC, % | 76 [70-84] |
| TLCO, n | 32 |
| Alveolar volume (VA), L | 3.89 [3.17-4.37] |
| TLCO/VA, $mmol.min^{-1}.kPa^{-1}.L^{-1}$ | 1.76 [1.63-1.99] |
| TLCO, $mmol.min^{-1}.kPa^{-1}$ | 6.77 [5.88-7.86] |
| TLCO, % predicted | 74 [67-85] |
| TLNO, n | 18 |
| TLNO, $mmol.min^{-1}.kPa^{-1}$ | 24.55 [19.90-27.40] |
| DmCO, $mmol.min^{-1}.kPa^{-1}$ | 12.45 [10.10-13.90] |
| Vc, mL | 75 [69-104] |
| TLNO/TLCO ratio | 3.35 [3.20-3.82] |
| Exhaled NO # | |
| multiple constant flow (MCF) method, n | 30 |
| $r^2$ | 0.97 [0.93-0.98] |
| $Calv_{NO,MCF}$, ppb | 3.3 [2.4-6.4] |
| $J'aw_{NO,MCF}$, $nL.min^{-1}$ | 17 [9-30] |
| $FENO_{0.05}$, ppb | 8.4 [6.2-16.2] |
| dynamically changing flow (DCF) method, n | 26 |
| $Calv_{NO,DCF}$, ppb | 2.2 [1.2-5.0] |
| $J'aw_{NO,\ DCF}$, $nL.min^{-1}$ | 29 [18-60] |
| $Daw_{NO,DCF}$, $nL.min^{-1}.ppb^{-1}$ | 0.24 [0.21-0.31] |
| $Caw_{NO,DCF}$, ppb | 100 [61-218] |

Results are expressed as median [$25^{th}$ – $75^{th}$ percentile]

#: 4 patients were unable to perform both measures of exhaled NO and 4 additional patients were unable to perform the DCF method

$r^2$: 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]

Normal values (non atopic subjects) for $Calv_{NO,DCF}$, $J'aw_{NO,DCF}$, $Daw_{NO,DCF}$ and $C_{awNO,DCF}$ are (mean±SD) 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].

Suri and colleagues observed the following exhaled NO values [7], median (range), in 22 children with CF: $Calv_{NO,MCF}$ 2.2 (0.6–5.6) ppb and $J'aw_{NO,MCF}$ 27 (4–75) $nL.min^{-1}$. Shin and colleagues observed the following exhaled NO values (mean±SD) in 9 children with CF [6]: $Calv_{NO,DCF}$ 2.0±1.2 ppb, $J'aw_{NO,DCF}$ 36±39 $nL.min^{-1}$, $Daw_{NO,DCF}$ 1.06±0.73 $nL.min^{-1}.ppb^{-1}$ and $Caw_{NO,DCF}$ 38±25 ppb.

**Table 3:** Results of exercise test

| Characteristic | Results |
|---|---|
| Baseline arterial blood gas, n | 31 |
| $PaO_2$, mmHg | 93 [85-98] |
| $PaCO_2$, mmHg | 38.0 [36.4-41.0] |
| $SaO_2$, % | 98 [97-98] |
| Baseline physiological VD/VT ratio, % | 0.37 [0.33-0.41] |
| Peak $V'O_2$, $mL.min^{-1}.kg^{-1}$ | 31.2 [26.5-35.2] |
| Peak $V'O_2$, % predicted | 71 [65-80] |
| Peak respiratory rate, cpm | 40 [34-49] |
| Peak V'E, $L.min^{-1}$ | 58.6 [49.5-71.3] |
| Ventilatory reserve, % | 34 [20-48] |
| Anaerobic threshold, % peak $V'O_2$ predicted | 42 [35-51] |
| $V'E/V'CO_2$ at anaerobic threshold | 32 [28-36] |
| Peak heart rate, % predicted | 82 [77-88] |
| Peak oxygen pulse, % predicted | 90 [78-102] |
| Peak physiological VD/VT ratio, % | 0.24 [0.19-0.29] |
| Peak $P(a-ET)CO_2$, mmHg | 1.1 [-0.7 - +3.2] |
| Peak arterial blood gas, n | 26 |
| $PaO_2$, mmHg | 92 [85-100] |
| $P(A-a)O_2$, mmHg | 18 [11-28] |
| $PaCO_2$, mmHg | 39.0 [34.9-43.0] |
| $SaO_2$, % | 97 [95-98] |
| Lactates, $mmol.L^{-1}$ | 7.1 [7.0-10.1] |
| Peak Borg dyspnea score | 5 [3-7] |
| Peak Borg fatigue score | 4 [3-5] |
| Calculated slopes: | |
| $V'O_2$/Watts | 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] |

Results are expressed as median [$25^{th}$ – $75^{th}$ percentile]

Three of 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.