Supplementary material

Experimental design and Material and methods

We utilised the tidal measurement approach to investigate basal and GTN-induced exhaled nitric oxide (NO) in our patients. Unlike for spontaneously breathing patients, currently there is now international recommendation or recognised consensus on measurements of exhaled NO in mechanically ventilated patients [1]. However, an expert analysis by the ATS working group on different measurement techniques in ventilated patients summarised current approaches and relative advantages and disadvantages of currently used methods [2].

The approach of Tornberg et al is the closest to the current ATS/ERS recommendations for spontaneously breathing subjects and utilises multiple, single-breath exhalations at various flow rates to obtain flow independent parameters [3]. This requires disconnection from the ventilator circuit and performing a manual deep inspiration followed by a flow-controlled exhalation using an aspiration suction device. This method allows for repeated measurements at various controlled exhalation flow rates. Such approach would have been particularly interesting in our study as it would have provided opportunities for extended exhaled NO analysis and partitioning of exhaled NO to alveolar and conducting airways compartments. However, a major limitation of this method is that it is not applicable to patients with severe pulmonary dysfunction, as prolonged disconnection of the respiratory tubings to perform single-breath measurements may not be performed without risking alveolar collapse and deteriorating gas exchange, thereby harming the patient [3,2]. Considering the end stage CF disease, CO2 retention and ventilator dependency of these patients during the transplant operation, we decided to utilise the breath-to-breath (tidal) measurement method.

Breath-to-breath analysis allows resolution of the NO concentration profile within one respiratory cycle and results in a dynamically changing NO concentration versus time plot, reflecting NO production in the lower airways of intubated patients (Supplementary Figure 1 A). However, in this method the observed pattern is related to continuously changing expiratory flow, and other confounding factors such as ventilation parameters during controlled mechanical ventilation, different ventilation modes, composition of inhaled gases, changes in pulmonary blood flow and intrathoracic blood volume [2,4,5]. To control for these variables, we standardized ventilation for the 80 sec exhaled NO measurement period for inspired gas (100% O_2), tidal volume (5 ml/kg) respiratory rate (10/min) and inspiratory and expiratory ratio (1:3) [4,5]. To eliminate the influence of positive end expiratory pressure on gas phase NO, PEEP was set to zero [6]. Exhaled NO and CO_2 was sampled through a Teflon tube positioned at the distal end of the endotracheal tube. Inspired and expired samples for analysis of NO and CO_2 were continuously withdrawn at a flow rate of 150 ml/minute and monitored for 80 sec.

As reported before, and discussed in the ATS workshop document the NO versus time trace obtained by breath-to-breath analysis can be described in several ways, including the peak FE_{NO} concentration, mean FE_{NO}, the area under the concentration curve for an indicated time period, or NO output rate [2]. As in our previous works, we utilised peak NO concentrations and area under the curve determination over 30 sec representing generally 5 breath cycles [7-9].

Our experimental design involved obtaining baseline measurements to study endogenously derived exhaled NO followed by an investigation on dynamism of exhaled NO following intravenous administration of nitroglycerin (GTN). Following published animal studies, we and others have observed in patients a transient, proportionate, and dose-dependent increase in NO in exhaled air after administration of GTN (supplementary figure

1B) [10-14]. We proposed that this approach may be useful in monitoring metabolic function of the pulmonary microvascular endothelium, as well as investigating nitrate pharmacology and tolerance. Indeed, we have recently applied this tool to investigate pulmonary microvascular dysfunction in cardiac surgery and lung transplantation [7-9].

For the current studies we utilised 250 µg bolus of GTN while exhaled NO was continuously monitored. For data analysis we used the 5 breath cycle during GTN metabolism (starting with the second breath following GTN bolus, as previous studies indicated that the first increase in exhaled NO after GTN administration occurs approximately 10 seconds following injection) [7, 13]. The average peak exhaled NO and the area under curve of the 5 breath was calculated and compared to baseline values just prior to GTN administration. GTN data are reported as GTN-induced changes (delta values) from baseline.

Supplementary Figure 1 legend .

Representative traces of gas phase nitric oxide (NO) and carbon dioxide (CO_2) in patients before (Baseline) and after (GTN) intravenous administration of 250 µg bolus of nitroglycerin in patients presented for open heart surgery for coronary arterial bypass grafting (CABG) or for lung transplantation for end stage cystic fibrosis (CF). Note greatly reduced exhaled NO in CF under baseline, and comparable GTN-induced exhaled NO (from baseline) in CF and CABG patients. GTN traces start with the second breath following GTN administration. Note increased end tidal CO2 in the CF patient representing CO2 retention in end stage disease and permissive hypercapnia during the lung transplant operation.

Supplementary References

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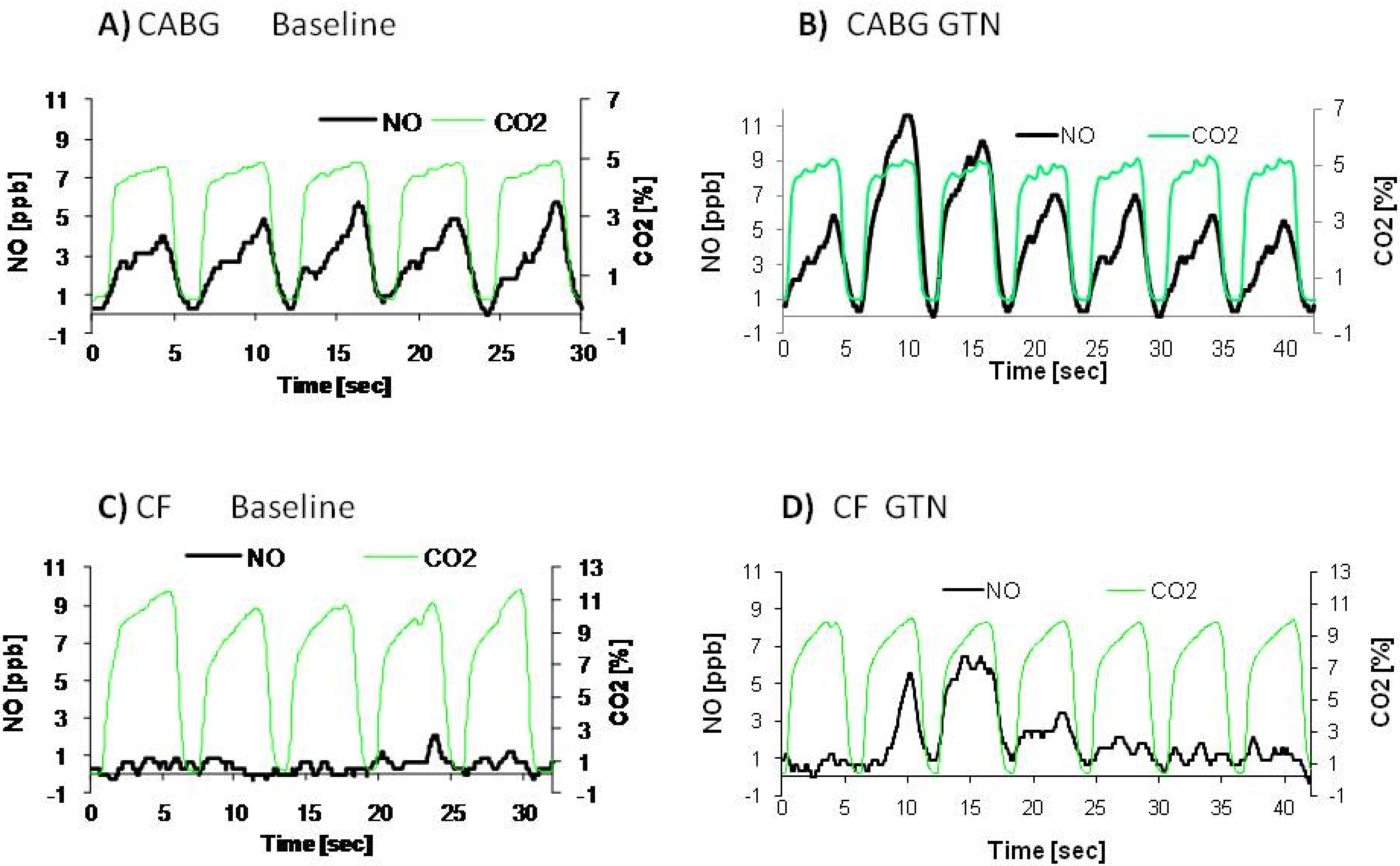
A) CABG Baseline
NO
CO2
NO [ppb]
CO2 [%]
Time [sec]
11
9
7
5
3
1
-1
7
5
3
1
-1
0 5 10 15 20 25 30
B) CABG GTN
NO
CO2
NO [ppb]
CO2 [%]
Time [sec]
11
9
7
5
3
1
-1
7
5
3
1
-1
0 5 10 15 20 25 30 35 40
C) CF Baseline
NO
CO2
NO [ppb]
CO2 [%]
Time [sec]
11
9
7
5
3
1
-1
13
11
9
7
5
3
1
-1
0 5 10 15 20 25 30
D) CF GTN
NO
CO2
NO [ppb]
CO2 [%]
Time [sec]
11
9
7
5
3
1
-1
13
11
9
7
5
3
1
-1
0 5 10 15 20 25 30 35 40