Water vapour and carbon dioxide decrease nitric oxide readings

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Water vapour and carbon dioxide decrease nitric oxide readings. Th.W. van der Mark, E. Kort, R.J. Meijer, D.S. Postma, G.H. Koëter. ©ERS Journals Ltd 1997.
ABSTRACT: Measurement of nitric oxide levels in exhaled air is commonly performed using a chemiluminescence detector. However, water vapour and carbon dioxide affect the chemiluminescence process. The influence of these gases at the concentrations present in exhaled air, has not yet been studied.

For this *in vitro* study, mixtures of 50, 100 and 200 parts per billion (ppb) NO in air were prepared and fed into the NO analyser either directly or bubbled through water. Mixtures with CO_2 were prepared by adding 0–10% CO_2 to the diluent air.

We found a significant decrease in NO readings in the water-saturated samples compared to the dry gas (p<0.001), strongly dependent on the partial pressure of water. NO levels in exhaled air (mean 10±2 ppb) showed a decrease of 17±3% when waer vapour was not absorbed. From the experiments with CO_2 we found a decrease in NO reading of 1.04±0.07% per volume CO_2 (%).

Presence of water vapour, thus, leads to a systematic underestimation of NO levels. Insertion of a water absorber might, therefore, be advantageous. The influence of CO_2 concentrations in the normal respiratory range is negligible. With high expiratory CO_2 levels as applied in permissive hypercapnia, the effects may be substantial.
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Measurement of nitric oxide levels in exhaled air is commonly performed by a chemiluminescence detector, in which NO reacts with O_3 to form high energetic NO_2^*. Only a small fraction (typically less than 1%) of the energy carried by the NO_2^* molecules is dissipated by emission of photons. The major part is dissipated by interaction with other molecules in the reaction chamber. The latter process is called third body quenching, and is dependent on sample composition and reaction chamber pressure. The effect of variations in sample composition and reaction chamber pressure follows the well-known Stern-Volmer relation [1, 2]:

$$F = \frac{P_{NO_2}}{B\ (1 + \Sigma\ a_i P_i)} \qquad (1)$$

where F is the emission signal, B is a proportionality constant which is instrument dependent, P_{NO_2} is the partial pressure of the NO_2 formed (proportional to the NO concentration in the sample gas), P_i is the partial pressure of the i-th component in the sample gas, and a_i is the corresponding quenching constant. The relative quenching (R) can then be defined as the ratio of the NO signal in the presence of the quenching gas and the NO signal without that gas. From Equation 1 it follows that the relative quenching for a single gas can be given as [3]:

$$R = \frac{1}{1 + b.P} \qquad (2)$$

in which the constant b comprises both instrumental properties and the quenching constant of the gas at the partial pressure (P) in question.

Thus, gas composition influences the chemiluminescence signal. Water vapour and CO_2 have quenching constants considerably exceeding those of N_2 and O_2 [3], and may therefore decrease the NO signal. This is a well-known phenomenon in the analysis of combustion engine exhaust gases [2–4], where NO detector efficiency may decrease to 60% [3]. However, the composition of exhaust gases is quite different from exhaled air. The present study was undertaken to investigate whether water vapour and CO_2, at the concentrations present in exhaled air, have a measurable influence on NO readings.

Methods

Mixtures of 50, 100 and 200 parts per billion (ppb) NO in air were prepared by diluting a 100 parts per million (ppm) NO in N_2 mixture from a certified bottle with NO-free air (<2 ppb NO) by precision mass flow controllers (accuracy 0.1%, Bronkhorst Hi-Tec BV, Ruurlo, The Netherlands). Tubing, fittings and valves were made from polytetrafluoroethylene (PTFE) and stainless steel. The diluted gas was fed into a 50 L plastic bag. The whole system (bag and tubing) was tested for leaks and absorption of NO by filling the bag with a 100 ppb NO
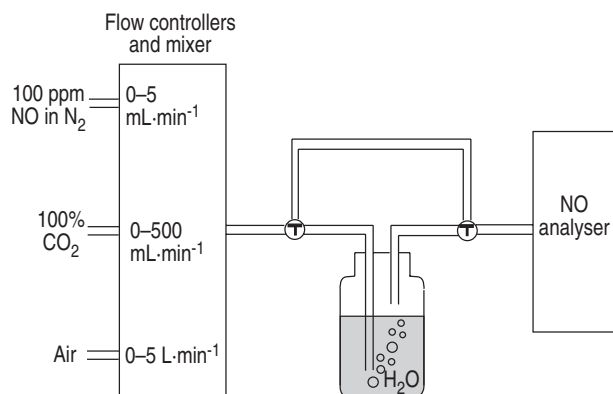
Fig. 1. – Schematic drawing of the connections of the flow controllers and mixer to the NO analyser. Each flow controller can be adjusted in steps of 1% of its entire range. ppm: parts per million.

mixture and analysing it after 1 h. We found no measurable change in NO concentration.

Samples from the mixing bag were passed into the measuring port of the NO analyser (CLD 700AL, Ecophysics, Basel, Switzerland), either directly or bubbled through a flask of distilled water of 23°C (fig. 1). The lower detectable limit of the analyser was 1 ppb with a resolution of ±1 ppb. The sampling flow was 600 mL·min⁻¹ and the response time, including lag and rise time was <7 s. The linearity was ±1% full scale. The analyser was equipped with a separate channel for NO_x measurements (NO plus higher oxides of nitrogen).

Consecutive measurements were performed with dry and wet gas in triplicate for each of the three NO concentrations. The gas from the diluting system was also connected directly to the separate calibration port present in the analyser, in order to perform calibration procedures according to the instructions of the manufacturer. Calibration was checked in this way before and after each measurement. A second set of measurements was made using an input concentration of 200 ppb and water temperatures of 23 and 38°C. Thereafter, the wet gas was passed through a cold trap, cooled by a Peltier element to -7°C. Mixtures with CO_2 were prepared by adding 0–10% CO_2 to the diluent air and fed into the analyser, bypassing the water flask.

Measurements in normal subjects were made using the tidal breathing method described by SCHILLING *et al.* [5]. Briefly, subjects breathed air containing <2 ppb NO *via* a two-way breathing valve. Expired air was collected in a bag for 5 min and subsequently analysed.

Results

We found a significant decrease in NO readings of 14.0±0.7% in the water-saturated samples compared to the dry gas (fig. 2), the slope of the calibration line for wet gas being 0.860±0.007 (p<0.001, 95% confidence interval (95% CI) 0.838–0.882), at a partial pressure of water vapour (PH_2O) of 2.81 kPa (water temperature 23°C). At a PH_2O of 6.63 kPa (temperature 38°C), a decrease of 24.5±1.5% was observed, whereas no decrease was apparent at a PH_2O of 0.34 kPa (temperature -7°C). The raw values must be corrected for the volume of the water vapour introduced by the bubbling through water, the corrected values being 12.0±0.7% (23°C) and 19.2±

1.5% (38°C). The relative quenching (R) for water was then calculated by fitting Equation 2 to the data points (fig. 3). The value of b for water (b_{H_2O}) was 0.038±0.004 kPa⁻¹.

From the experiments with CO_2, we found a decrease in NO reading of 1.04±0.07% per volume CO_2 (%) (fig. 4) (p<0.005). The value of b for CO_2 (b_{CO_2}) (Equation 2) was calculated as 0.011±0.001 kPa⁻¹, taking into account the barometric pressure (102.5 kPa) at the time of the measurements.
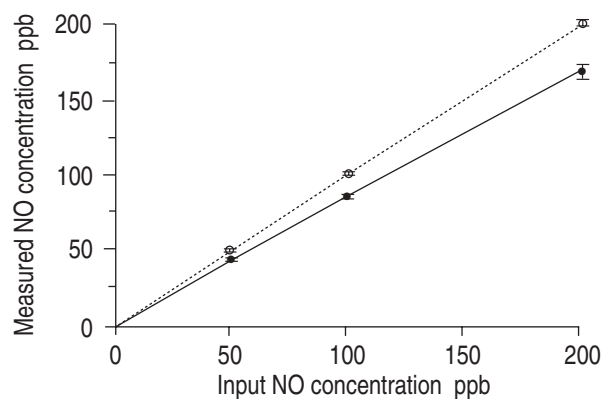

Fig. 2. – Calibration lines for dry and wet gas (saturated with water vapour at 23°C). ·············: dry gas; ————: wet gas. The line for dry gas is the same as the line of identity.
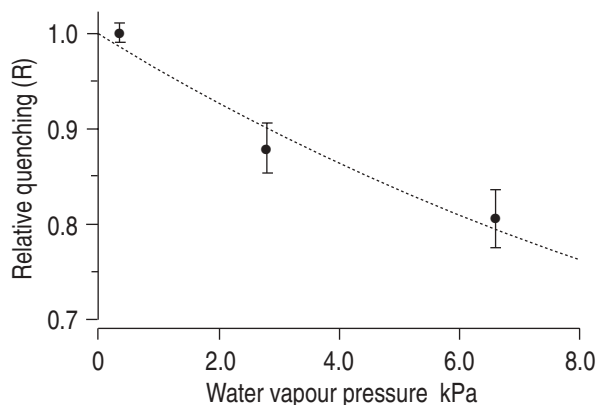

Fig. 3. – Relative quenching as function of the partial pressure of water vapour. The line is the best fit of equation 2 (see text) to the data points.
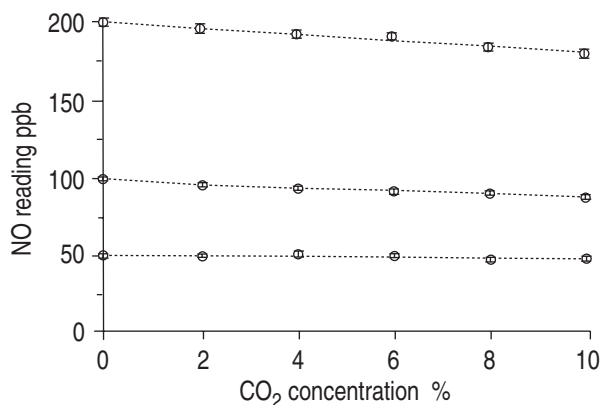

Fig. 4. – Decrease of NO readings with increasing CO_2 concentration at fixed NO concentrations of 50, 100 and 200 parts per billion (ppb).

During the experiments, the readings in the NO_x channel were continuously monitored. All readings were concordant with the values obtained from the NO channel, indicating that no additional NO_2 was formed.

In addition, we measured NO levels in exhaled air of three normal subjects (mean 10±2 ppb) with and without a conventional $CaCl_2$ water absorber in the tubing to the analyser. The NO reading without absorber was 8.3±2 ppb, 17±3% lower than with the water absorber.

In the course of the experiments we observed that prolonged sampling of wet gas resulted in an increased time for the analyser to reach the initial stable calibration values (from 5 min initially to 20 min after performing all the experiments).

Discussion

We found a distinct decrease in NO readings due to the presence of water vapour and CO_2, the former having a substantial influence when measuring NO concentration in exhaled air.

According to the equations given above, the partial pressures of the components of the sample gas determine the amount of quenching. By use of Equation 2 and the values found for the constants bH_2O and bCO_2, it is possible to calculate the relative quenching, and thereby the decrease in NO signal, for every partial pressure of these gases. The result is independent of the applied NO concentrations, since chemiluminescence detectors are known to be linear over several orders of magnitude [6].

It can be argued that the decrease of NO signal is partly caused by the reaction of NO with water. However, the reactions producing nitrous acid at these NO concentrations are very slow, as is the reaction with dissolved oxygen [7]. Moreover, we observed complete recovery of the NO signal after leading a wet gas sample through a cold trap. TIDONA *et al.* [3] performed similar experiments at a much higher NO concentration (92 ppm) and found no measurable absorption by water.

The values of relative quenching efficiency, in molar fraction, for H_2O and CO_2 reported elsewhere [3], were about half the values found in the present study. These investigators used an analyser operating with a reaction chamber pressure of 1.3 kPa, while our analyser had a reaction chamber pressure of 5.2 kPa. Since low pressure tends to reduce quenching, we would expect the differences between the values reported in the present study and that of TIDONA *et al.* [3] to result from the difference in analyser. The ratio of the quenching coefficients of H_2O and CO_2 found in the present study, is in good agreement with values reported previously [3, 4].

The influence of CO_2 is limited. A variation of 2% CO_2 in the expired air (*e.g.* the difference between mixed expired and end-tidal CO_2 concentration) would lead to a variation of 0.4 ppb at an average NO concentration of 20 ppb. This is below the detection limit. However, when using much higher NO concentrations, as applied in administering exogenous NO to patients with adult respiratory distress syndrome (ARDS), the effect of CO_2 could be substantial. This is even more so during ventilation of patients with permissive hypercapnia [8, 9]. For instance, at 2,000 ppb NO, a sample wet gas of 34°C and a CO_2 concentration of 10% would give a reading of 1,522 ppb, as calculated from Equation 1, using the observed values of bH_2O and bCO_2. In these cases it would be advantageous to remove both water and CO_2 prior to measurement.

The results obtained when measuring NO levels in exhaled air are in concordance with our *in vitro* data. Assuming a temperature of 34°C in the exhaled air [10] (PH_2O=5.32 kPa), the corresponding decrease in NO reading was calculated as 16.8%, compared to the experimental value of 17.0±3%.

Water vapour may have another effect on analyser performance. Due to the use of stainless steel capillary tubes inside the analyser and the strong polar character of the water molecule, water can be adsorbed at the inside surface of the tubing. When switching back to dry gas, some time will be needed to remove all the adsorbed water.

The decrease in NO readings in water-saturated samples may be an important source of variation. Since the partial pressure of saturated water vapour increases considerably with temperature in the physiological range (more than twofold [11]), and the partial H_2O pressure is the main determinant of the quenching effect, the temperature of the exhaled air determines the decrease in NO reading. This temperature depends on the means of sampling the exhaled air: directly at the mouth, or from a collection bag. Also, diameter and length of tubing are important. When the exhaled air temperature varies between 23°C (PH_2O= 2.81 kPa) and 34°C (PH_2O=5.32 kPa) this would lead to decreases in NO readings of 14–27%, *i.e.* 2.8–5.3 ppb, at an average NO concentration of 20 ppb. These figures are of the same order of magnitude as, for instance, the differences in NO concentration between mild asthmatics and normal subjects [12].

It is therefore advisable to use a water absorber in the tubing leading to the NO analyser. Our observation that prolonged sampling of wet gas may reduce analyser performance is an additional reason for such an absorber.

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