Water vapour and carbon dioxide decrease nitric oxide readings

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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 CO₂ were prepared by adding 0–10% CO₂ 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 CO₂ we found a decrease in NO reading of 1.04±0.07% per volume CO₂ (%).

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 CO₂ concentrations in the normal respiratory range is negligible. With high expiratory CO₂ levels as applied in permissive hypercapnia, the effects may be substantial.
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, Eco-physics, 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 NOx 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 CO₂ were prepared by adding 0–10% CO₂ 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 0.86±0.007 (p<0.001, 95% confidence interval (95% CI) 0.838–0.882), at a partial pressure of water vapour (P_H₂O) of 2.81 kPa (water temperature 23°C). At a P_H₂O of 6.63 kPa (temperature 38°C), a decrease of 24.5±1.5% was observed, whereas no decrease was apparent at a P_H₂O 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_H₂O) was 0.038±0.004 kPa⁻¹.

From the experiments with CO₂, we found a decrease in NO reading of 1.04±0.07% per volume CO₂ (%) (fig. 4) (p<0.005). The value of b for CO₂ (b_CO₂) (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.
During the experiments, the readings in the NO\textsubscript{2} channel were continuously monitored. All readings were concordant with the values obtained from the NO channel, indicating that no additional NO\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{2}O and bCO\textsubscript{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\textsubscript{2}O and CO\textsubscript{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\textsubscript{2}O and CO\textsubscript{2} found in the present study, is in good agreement with values reported previously [3, 4].

The influence of CO\textsubscript{2} is limited. A variation of 2% CO\textsubscript{2} in the expired air (e.g. the difference between mixed expired and end-tidal CO\textsubscript{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\textsubscript{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\textsubscript{2} concentration of 10% would give a reading of 1,522 ppb, as calculated from Equation 1, using the observed values of bH\textsubscript{2}O and bCO\textsubscript{2}. In these cases it would be advantageous to remove both water and CO\textsubscript{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] (P\textsubscript{H\textsubscript{2}O}=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\textsubscript{2}O 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 (P\textsubscript{H\textsubscript{2}O}= 2.81 kPa) and 34°C (P\textsubscript{H\textsubscript{2}O}=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.

**References**


