Impact of breath sampling on exhaled carbon monoxide

Ramin Ghorbani, Anders Blomberg and Florian M Schmidt

1 Department of Applied Physics and Electronics, Umeå University, Umeå SE-90187, Sweden
2 Department of Public Health and Clinical Medicine, Umeå University, Umeå SE-90187, Sweden
E-mail: florian.schmidt@umu.se

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Abstract
The influence of breath sampling on exhaled carbon monoxide (eCO) and related pulmonary gas exchange parameters is investigated in a study with 32 healthy non-smokers. Mid-infrared tunable diode laser absorption spectroscopy and well-controlled online sampling is used to precisely measure mouth- and nose-exhaled CO expirograms at exhalation flow rates (EFRs) of 250, 120 and 60 ml s⁻¹, and for 10 s of breath-holding followed by exhalation at 120 ml s⁻¹. A trumpet model with axial diffusion is employed to fit simulated exhalation profiles to the experimental expirograms, which provides equilibrium airway and alveolar CO concentrations and the average lung diffusing capacity in addition to end-tidal concentrations. For all breathing maneuvers, excellent agreement is found between mouth- and nose-exhaled end-tidal CO (ETCO), and the individual values for ETCO and alveolar diffusing capacity are consistent across maneuvers. The eCO parameters clearly show a dependence on EFR, where the lung diffusing capacity increases with EFR, while ETCO slightly decreases. End-tidal CO is largely independent of ambient air CO and alveolar diffusing capacity. While airway CO is slightly higher than, and correlates strongly with, ambient air CO, and there is a weak correlation with ETCO, the results point to negligible endogenous airway CO production in healthy subjects. An EFR of around 120 ml s⁻¹ can be recommended for clinical eCO measurements. The employed method provides means to measure variations in endogenous CO, which can improve the interpretation of exhaled CO concentrations and the diagnostic value of eCO tests in clinical studies.

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1. Introduction

Detection of carbon monoxide (CO) in exhaled breath is an established method to assess recent uptake of exogenous CO, such as from smoking [1, 2] or exposure to air pollution [3]. The measurement of endogenous CO production via exhaled breath is less common, but could have high diagnostic and clinical value, for example when it comes to non-invasive assessment of respiratory diseases [4, 5]. However, to successfully resolve endogenous CO concentrations, sensitive and precise analytical methods, as well as knowledge about the natural and breath sampling-induced variability of exhaled CO, are required.

A typical measurement of exhaled breath carbon monoxide (eCO) is today synonymous with acquiring a single experimental value corresponding to the end-tidal (ETCO) or mixed-breath CO concentration. The obtained eCO value is often assumed to be equal to the equilibrium alveolar CO concentration, which has been shown to strongly correlate with, and can be used to estimate, blood carboxyhemoglobin (HbCO) [6] and the red blood cell lifespan [7]. For the purpose of exposure evaluation, the baseline of ETCO has been rather well established [1, 8–11]. Depending on the CO concentration in the ambient air inhaled during the last day(s), the healthy non-smoker ETCO levels can vary between 1 and 6 parts per million (ppm), whereas smoking can easily give rise to tens of ppm.

Endogenous CO arises mainly from systemic heme oxygenase (HO-1) and accounts for 1–3 ppm of the healthy non-smoker background [3]. Being a neurotransmitter, the gas is involved in cell signalling...
and several physiological mechanisms connected to oxidative stress and inflammation [5, 12–15]. These mechanisms can also induce HO-1 activity locally in the respiratory tract, e.g. in the alveolar macrophages, the tracheobronchial region or the upper airways, which thus may give rise to elevated ETCO or an airway CO contribution. The heme catabolism pathway and different factors influencing exhaled CO are schematically illustrated in figure 1.

The main factor determining eCO in healthy non-smokers is the HbCO level. Exogenous CO taken up by the blood and eliminated via breath is a confounding factor for the determination of endogenous CO. It is commonly assumed that eCO is independent of the exhalation flow rate (EFR), since the water solubility of CO is low and there is little airway interaction. However, since pulmonary CO elimination is diffusion limited, i.e. dependent on alveolar residence time, and may be influenced by inhaled CO and local production in airways or nasal cavity, the breath sampling conditions are expected to be an important factor in the assessment of endogenous eCO levels [16, 17].

Commonly used electrochemical sensors for detection of mouth-exhaled ETCO may be sufficient for the assessment of external CO exposure, but are less suitable for detection of endogenous eCO in clinical studies for several reasons. First, the electrochemical sensors exhibit low sensitivity, precision and accuracy, leading to a poor agreement in ETCO between different sensor types [10, 11, 18], ambiguous results in clinical studies and a general debate about the suitability of eCO as biomarker for non-invasive disease assessment [19]. Second, the sensors do not offer standardized, well-controlled sampling procedures and the possibility to quantify ambient air CO. Third, mouth-exhaled ETCO reflects CO in the alveolar region and cannot be used to resolve potential contributions from other parts of the respiratory tract. In contrast, laser-based optical techniques enable highly accurate, precise and selective quantitative breath gas analysis of small biomarker molecules [20]. Most implementations are calibration-free, offer real-time detection and allow robust use in clinical applications. As a consequence, majority of the clinically approved breath tests employ optical sensors [20].

In an attempt to contribute to an improved assessment of endogenous eCO and to enhance the information gained from single-exhalations, we have recently introduced an extended breath CO analysis approach, where detection of real-time CO exhalation profiles with laser absorption spectroscopy [21, 22] is combined with pulmonary gas exchange modeling and least-squares fitting of the measured eCO profiles [23, 24]. This strategy has enabled determination of additional eCO parameters, such as equilibrium airway (tissue) and alveolar CO concentrations and the average lung diffusing capacity. However, only few subjects have so far been analyzed using this method, and little is known about the physiological relevance of the eCO parameters, as well as their dependence on the breath sampling procedure.

In this work, the extended eCO analysis approach is applied to investigate the influence of breath sampling on exhaled endogenous CO in a cohort of 32 healthy non-smokers. Single-exhalation eCO profiles are recorded at three different EFRs and...
following a 10 s breath-holding (BH) maneuver, and are analyzed using the pulmonary gas exchange model. The inter- and intra-individual variations of the obtained eCO parameters and their dependence on the breath sampling conditions, such as exhalation through mouth or nose, exhalation flow rate and volume, and ambient CO are scrutinized. Implications for CO physiology and eCO analysis in clinical settings with diseased cohorts are discussed.

2. Materials and methods

2.1. Laser-based CO sensor and online breath sampler

A compact optical sensor based on tunable diode laser absorption spectroscopy (TDLAS) was employed to measure CO concentrations in breath and ambient air in real-time [22]. The system utilized an interband cascade laser (Nanoplus) operating at 4.69 µm to access the strong fundamental vibrational-rotational absorption band of CO in the mid-infrared spectral range. Selective and sensitive CO quantification down to 9 parts per billion (ppb) at a precision of 5 ppb and an acquisition time of 0.1 s was ensured by using a low-volume (38 ml) multipass sample cell (IR Sweep, IRcell-4M) with an absorption path length of 4 m, and 2 f wavelength modulation spectroscopy for noise reduction.

Fast, online breath sampling was realized using a 15 cm long, low-volume (30 ml) buffer tube made of Teflon. Real-time data on respiratory parameters, such as inhalation flow rate (IFR), EFR, exhaled volume, mouth pressure and exhaled carbon dioxide (CO₂) were obtained using an inline flow meter (Phillips Respironics, FloTrak Elite Module) and a capnograph (Phillips Respironics, Capnostream 5) installed at the buffer tube inlet. An antibacterial filter and Teflon mouthpieces designed for either mouth- or nose-breathing were also mounted at the inlet. Audiovisual indicators helped subjects to keep a certain EFR and breathing frequency. Both inhalation and exhalation were performed through the sampler with the help of a 2-way-valve (Rudolph Inc.) installed at the buffer tube outlet. A detailed description of the breath sampler is given in [24].

A portion of the inhaled air or exhaled breath was continuously extracted from the center of the buffer tube at a flow rate of 50 ml s⁻¹ and led to the laser-based CO sensor. The breath gas was analyzed in the multipass cell at room temperature and a pressure of 100 Torr. The ambient CO concentration was measured during the inhalation preceding the analyzed expirogram. The true real-time detection capability of the combined system (breath sampler and TDLAS sensor) has previously been confirmed by comparison of CO₂ expirograms measured with TDLAS in the MPC and by capnography directly at the mouth [21].

2.2. Pulmonary gas exchange model

A one-dimensional trumpet model with axial diffusion (TMAD) adapted from Shin and George [25] was used to simulate the gas exchange dynamics of CO in the respiratory tract, and corresponding single-exhalation profiles, as established by Ghorbani et al. [23]. Following the extended breath CO analysis approach, simulated expirograms were fitted to the experimental eCO profiles using a weighted, nonlinear least-squares fitting algorithm [24]. The four independent TMAD fitting parameters, maximum airway CO flux (JawCO), airway diffusing capacity (DawCO), maximum alveolar CO flux (Jaco) and alveolar diffusing capacity (Daco), were free to vary in the ranges of 100–500 pl s⁻¹, 1.0–1.6 pl s⁻¹ ppb⁻¹, 5 × 10⁻⁶–2 × 10⁻⁵ pl s⁻¹, and 300–5 × 10⁵ pl s⁻¹ ppb⁻¹, respectively, around established initial values [23]. Other input data to the model included the actual IFRs and EFRs and exhaled volumes measured with the breath sampler, as well as the ambient air CO concentration. A summary of the parameter definitions and units is given in table 1.

2.3. Interpretation of extended eCO parameters

A certain end-tidal CO concentration can arise from different combinations of Jawco and Dawco, but the entire expirogram shape taken into account by the TMAD curve fit allows only a single, unique combination. The ratios Jawco/Dawco and Jaco/Daco then reflect the equilibrium airway (tissue) and alveolar CO concentrations, Caw and Caco, respectively. The maximum CO flux from blood or tissue to the gas in the respiratory tract is defined as the hypothetical flux that occurs when the CO concentration in the breath gas is zero. In general, as CO is mostly exchanged in the alveoli, the alveolar CO flux is much higher than the airway flux.

The diffusing capacity is a measure of the amount of CO that diffuses from the breath gas across the tissue membrane to blood per unit time and concentration gradient. In case of systemic elimination at inhaled CO lower than the back pressure from blood, the net alveolar flux and diffusion of CO will occur from blood towards breath until equilibrium is reached or the breath is exhaled. Since CO gas exchange is diffusion limited, it may take longer than a respiratory cycle to establish equilibrium in the airways and alveolar sacs, and ETCO may not be equal to alveolar equilibrium CO. In contrast to the conventional, clinically used diffusing capacity of the lung for CO (Dlco) [26], the alveolar diffusing capacity obtained here from normal breathing expirograms can be seen as an average over the entire exhalation process. Typical Daco values are higher than Dlco, not only due to the significantly different physiological test conditions, but also because morphological models, such as the TMAD, tend to overestimate the diffusing capacity [26]. The airway CO concentration
is anticipated to be equal to inhaled CO, or higher, if CO is produced in the airways.

2.4. Human subjects and study protocol

A total of 32 healthy non-smokers (22 male and 10 female) with mean age of 37 ± 10 years and mean body mass index (BMI) of 23.4 ± 2.4 kg m⁻² participated in the eCO baseline study. The sample size was chosen based on experience, taking into account the accuracy and precision observed in previous applications of the method [23, 24]. The study was approved by the Regional Ethical Review Board at Umeå University (2017/306-31). All subjects were recruited among the employees of Umeå University, Sweden, and gave their written informed consent to the participation. Details on the study population are presented in table 2.

The measurements were performed between November and March, with sessions either in the morning or in the afternoon. All subjects were asked to refrain from food and drink intake as well as exercise for at least 60 min prior to the start of the measurement. The subjects were then requested to sit in an upright position and perform ‘normal breathing’ maneuvers (close to tidal breathing at normoventilation) at IFRs and EFRs of 250, 120 and 60 ml s⁻¹, here referred to as EFR 250, EFR 120 and EFR 60, respectively. The EFR 250 and EFR 120 maneuvers comprised a sequence of 5–10 consecutive exhalations. A fourth maneuver included inhalation at 120 ml s⁻¹, and 10 s of BH followed by exhalation at 120 ml s⁻¹, which is referred to as BH 10. These four breathing maneuvers were conducted twice, first with exhalation through the mouth and then through the nose. The repeatability of the extended breath CO analysis approach, including the breath sampling, the optical eCO measurement and the subsequent fit of the physiological model to the measured expirograms, was previously found to be excellent, with an intra-individual variation in the alveolar parameters of less than 6% [24].

2.5. Data analysis

Correlations between the measured eCO parameters (ETCO, \(I_{ACO}\), \(D_{ACO}\), \(C_{ACO}\) and \(C_{tiss}\) for mouth and nose), the respiratory data (EFR, exhaled volume, ETCO₂ and ambient CO) and the subject data (e.g. gender, age and weight) were assessed using Spearman’s rank correlation coefficient \(r\) by means of the OriginPro 2018 software. A p-value of < 0.05 was considered statistically significant, and correlations with p-values lower than 0.001 are referred to as strong. When the data are shown as notched box-whisker plots, the box represents the interquartile range (IQR), the box waist shows the median, the notch shows the confidence interval around the median, the open marker indicates the mean, and the whiskers show 1.5 times the IQR to identify outliers.

3. Results

3.1. Real-time CO exhalation profiles

Typical measured single-exhalation profiles (open markers) from one of the subjects are presented as a function of exhaled volume in figure 2. Mouth-exhaled CO profiles at mean EFRs of 61, 113 and 246 ml s⁻¹ are shown in figure 2(a), whereas an expirogram for a mean EFRs of 117 ml s⁻¹ after 10 s of BH is displayed in figure 2(b). All experimental data are shown together with TMAD fits (solid lines), and the corresponding real-time EFR traces (lower panels).

The discrepancy between experiment and fit in exhalation phase II (steep CO increase) can be explained by differences between model-assumed and actual morphologic data (number and distribution of alveoli, airway and lung cross sectional areas, lung symmetry), as well as gas mixing mechanisms and

| Parameter | Definition | Unit |
|-----------|------------|------|
| \(C_{ACO}\) | Alveolar CO concentration at equilibrium | ppb |
| \(C_{tiss}\) | Airway tissue CO concentration at equilibrium | ppb |
| \(C_{amb}\) | Ambient air CO concentration | ppb |
| ETCO | End-tidal CO concentration | ppb |
| ETCO₂ | End-tidal CO₂ concentration | % |
| \(D_{mCO}\) | Total diffusing capacity of CO in the airways | pl s⁻¹ ppb⁻¹ |
| \(D_{ACO}\) | Total diffusing capacity of CO in the alveolar region | pl s⁻¹ ppb⁻¹ |
| \(I_{mCO}\) | Total maximum volumetric flux of CO from the airways | pl s⁻¹ |
| \(I_{ACO}\) | Total maximum volumetric flux of CO from the alveoli | pl s⁻¹ |
| IFR | Volumetric flow rate of air during inhalation | ml s⁻¹ |
| EFR | Volumetric flow rate of air during exhalation | ml s⁻¹ |
| V | Average exhaled volume | ml |

| Variable | Mean (n = 32) | SD | Median | Min | Max |
|----------|---------------|----|--------|-----|-----|
| Age (yrs) | 37 | 10 | 35 | 26 | 69 |
| Weight (kg) | 71.1 | 11.2 | 71 | 50 | 94 |
| Height (m) | 1.74 | 0.09 | 1.75 | 1.57 | 1.95 |
| BMI (kg/m²) | 23.4 | 2.4 | 23.5 | 18.4 | 28.1 |
Figure 2. Typical measured CO exhalation profiles (open markers) and TMAD fits (solid lines) at exhalation flow rates around 250, 120 and 60 ml s\(^{-1}\) (a) and after 10 s breath holding (120 ml s\(^{-1}\)) (b) from one of the study participants. The real-time EFR traces and average EFRs are shown in the lower panels. Note the decrease in eCO towards the end of exhalation at EFR 60.

Figure 3. End-tidal CO and TMAD-derived equilibrium alveolar CO shown by notched box-whisker plots (box is IQR, waist is median, notch is confidence interval, whiskers are ±1.5 IQR) for all 32 subjects and the four breathing maneuvers.

Figure 4. Maximum alveolar CO flux and the alveolar CO diffusing capacity shown by notched box-whisker plots (box is IQR, waist is median, notch is confidence interval, whiskers are ±1.5 IQR) for all 32 subjects and the four breathing maneuvers.

ventilation heterogeneity not accounted for in the TMAD \cite{23}. For most of the subjects, the EFR 60 profiles showed a noticeable decrease in the alveolar plateau concentration beyond an exhaled volume of 700 ml (figure 2(a)). These experimental data points were excluded from the fit, as the phenomenon
cannot be explained by the morphological model. For all exhalation profiles, the end-tidal CO concentration was equal to the measured value corresponding to the last point of the curve fit. For healthy non-smokers, the airway contribution to exhaled CO is small and normal expirograms (figure 2(a)) show a low sensitivity to the airway TMAD parameters owing to the short gas residence time in airways. Therefore, in this study, the airway TMAD values are first determined from fits to BH 10 profiles (figure 2(b)), and then fixed in the fits to the normal breathing profiles (figure 2(a)). Consequently, only one set of airway parameters (from 10 s BH followed by 120 ml s\(^{-1}\) exhalation) could be determined in this study.

### 3.2. Dependence on mouth/nose and EFR

The measured end-tidal CO\(\text{t}d\) and TMAD-derived equilibrium alveolar CO concentrations are presented as notched box-whisker plots in figure 3 for all 32 subjects (solid markers) and the four breathing maneuvers, i.e. for normal breathing at EFRs of 250, 120 and 60 ml s\(^{-1}\) and for 10 s BH with exhalation at 120 ml s\(^{-1}\). In figure 4, the maximum alveolar CO flux and alveolar CO diffusing capacity are displayed as notched box-whisker plots for all 32 subjects (solid markers) and the four breathing maneuvers. The actual mean EFRs were 238 ± 11, 119 ± 16, 68 ± 14 and 112 ± 9 ml s\(^{-1}\) for the mouth exhalations, and 240 ± 16, 120 ± 6, 69 ± 16 and 112 ± 8 ml s\(^{-1}\) for the nose exhalations, close to the intended values. The mean exhaled volume was 1054 ± 120 ml.

The different eCO and respiratory parameters for mouth- and nose-exhalations at EFR 120, including statistical analysis, are presented in detail in table 3. To illustrate the eCO dependence on the exhalation flow rate, table 4 provides selected mouth-exhaled CO parameters for the four breathing maneuvers.

As can be seen from figures 3 and 4 and tables 3 and 4, for each breathing maneuver, the eCO parameters exhibit a similar median value, range and spread within the inter-individual uncertainty for mouth- and nose-exhalations. Separate normality tests (data not shown) confirmed that the sample data have been drawn from a normally distributed population. The narrowest distributions are observed for the EFR 120 data, for which nose-exhaled eCO parameters show even lower uncertainties than mouth-exhaled values. Clearly, end-tidal and alveolar CO increase with decreasing EFR, and \(J_{ACO}\) and \(D_{ACO}\) decrease with decreasing EFR. The median alveolar CO is consistently higher than the median ETCO, but the difference decreases for lower EFRs and BH. Outliers in the eCO parameters are mostly related to outliers in EFR or exhalation volume. End-tidal CO\(_2\) levels around 6% for EFR 120 and EFR 60 indicate slight hypoventilation.

### 3.3. Airway tissue CO and dependence on ambient air

The TMAD-derived airway tissue CO concentrations determined from BH 10 maneuvers for mouth- and nose-exhalations are shown as notched box-whisker plots in figure 5(a) for all 32 subjects (solid markers). The corresponding measured ambient air CO concentrations are displayed for comparison. Outliers are related to unusually high ambient CO levels. Figure 5(b) presents nose-exhaled airway tissue CO plotted against both the corresponding ambient air CO concentration (red round markers) and nose-exhaled ETCO at 60 ml s\(^{-1}\) (black square markers), together with linear fits (Spearman’s correlation coefficients indicated).

As for the alveolar eCO parameters, the airway CO concentration for mouth- and nose-exhalations are in a similar range and show a comparable spread. The median airway CO concentration is consistently higher than ambient air CO and the two parameters correlate strongly (\(p < 0.001, r = 0.92\)). However, airway CO also correlates weakly, but significantly (\(p = 0.02, r = 0.42\)), with ETCO.

### 3.4. Result summary and correlations across EFRs

In figure 6(a) the individual mouth-exhaled (red square markers) and nose-exhaled (black round markers) ETCO values, alveolar diffusing capacities and ambient CO concentrations at an EFR of 120 ml s\(^{-1}\) are shown for all 32 subjects. In the lowest panel, the corresponding ambient air concentrations measured just before the mouth-exhalations (open triangular markers) are plotted for comparison. Figure 6(b) displays mouth-exhaled ETCO plotted against the mouth-exhaled ETCO (markers) for each breathing maneuver. The solid line shows a linear fit to the BH data (Spearman’s correlation coefficient indicated). It is evident from the data presented in figure 6 that for all breathing maneuvers the absolute mouth- and nose exhaled ETCO concentrations are in very good agreement and correlate strongly for each study participant individually. This is true also for airway CO, but less so for the alveolar diffusing capacity, probably due to a moderate negative correlation with exhalation volume (data not shown).

In order to further investigate ETCO and strengthen the confidence in the integrity of the extended eCO analysis approach, figure 7(a) presents mouth-exhaled ETCO at 120 ml s\(^{-1}\) plotted against mouth-exhaled ETCO determined with the other breathing maneuvers (250 ml s\(^{-1}\), 60 ml s\(^{-1}\) and BH 10). The figure also shows linear fits to the data with Spearman’s correlation coefficients indicated. Figure 7(b) displays the mouth-exhaled alveolar CO diffusing capacity at 120 ml s\(^{-1}\) versus the mouth-exhaled alveolar diffusing capacity measured at 250 ml s\(^{-1}\), 60 ml s\(^{-1}\) and BH 10 together with linear fits (Spearman’s correlation coefficients indicated).
Table 3. Breath CO and respiratory parameters for an exhalation flow rate of 120 ml s$^{-1}$. ETCO—end-tidal CO, $C_{ACO}$—equilibrium alveolar CO, $J_{ACO}$—maximum alveolar flux, $D_{ACO}$—alveolar diffusing capacity, $J_{awCO}$—maximum airway flux, $D_{awCO}$—airway diffusing capacity, $C_{tiss}$—equilibrium airway tissue CO, $C_{amb}$—ambient air CO, IFR—inhalation flow rate, EFR—exhalation flow rate, $V$—inhaled/exhaled volume, ETCO$_2$—end-tidal CO$_2$. The parameters are defined in table 1.

| Parameter | Unit | Mean | SD | Median | Range          | Mean | SD | Median | Range          |
|-----------|------|------|----|--------|----------------|------|----|--------|----------------|
| ETCO      | ppb  | 1771 | 370| 1882   | 1483 (874–2357)| 1724 | 367| 1776   | 1514 (851–2365)|
| $C_{ACO}$ | ppb  | 1864 | 386| 1964   | 1550 (935–2485)| 1817 | 389| 1874   | 1639 (870–2509)|
| $J_{ACO}$ | pl s$^{-1}$ | $1.3 \times 10^7$ | 3.4 $\times 10^6$ | $1.3 \times 10^7$ | $1.4 \times 10^7$ (4.5 $\times 10^6$–1.8 $\times 10^7$) | $1.2 \times 10^7$ | 3.3 $\times 10^6$ | $1.2 \times 10^7$ | $1.4 \times 10^7$ (6.5 $\times 10^6$–2.1 $\times 10^7$) |
| $D_{ACO}$ | pl s$^{-1}$ ppb$^{-1}$ | 6903 | 1055 | 6769 | 5162 (4824–9986) | 6652 | 920| 6535 | 4014 (5380–9394) |
| $J_{awCO}$ | pl s$^{-1}$ | 344 | 87 | 350 | 386 (200–586) | 343 | 104| 311 | 453 (193–646) |
| $D_{awCO}$ | pl s$^{-1}$ ppb$^{-1}$ | 1.6 | - | 1.6 | - | 1.6 | - | 1.6 | - |
| $C_{tiss}$ | ppb  | 215 | 56 | 219 | 241 (125–366) | 218 | 66 | 202 | 283 (121–404) |
| $C_{amb}$ | ppb  | 190 | 67 | 174 | 292 (96–388) | 192 | 74 | 181 | 338 (71–409) |
| IFR       | ml s$^{-1}$ | 124 | 12 | 121 | 53 (111–164) | 123 | 7 | 123 | 26 (111–137) |
| EFR       | ml s$^{-1}$ | 119 | 16 | 117 | 95 (93–117) | 120 | 6 | 120 | 26 (111–137) |
| $V$       | ml   | 1137| 161| 1118| 1000 (860–1860) | 1167| 75 | 1165| 377 (975–1166) |
| ETCO$_2$  | %    | 5.9 | 0.4| 5.9 | 1.5 (5.2–6.7) | 5.8 | 0.3| 5.7 | 2.4 (4.4–6.6) |
Table 4. Selected mouth-exhaled parameters at EFR 250, EFR 120, EFR 60 and BH 10 illustrating the dependence on exhalation flow-rate. EFR 120 shows the lowest spread. ETCO—end-tidal CO, $J_{ACO}$—maximum alveolar flux, $D_{ACO}$—alveolar diffusing capacity, EFR—exhalation flow rate, ETCO$_2$—end-tidal CO$_2$. The different parameters are in detail defined in table 1.

| EFR        | Parameter | Unit | Mean | SD  | Median | Range          |
|------------|-----------|------|------|-----|--------|----------------|
| 238 ± 11 ml s$^{-1}$ | ETCO | ppb | 1686 | 387 | 1743   | 1598 (801–2399) |
|            | $J_{ACO}$ | pl s$^{-1}$ | $1.8 \times 10^7$ | $4.5 \times 10^6$ | $1.9 \times 10^7$ | $1.7 \times 10^7$ (8.4 x 10$^6$–2.6 x 10$^7$) |
|            | ETCO$_2$ | %   | 5.4  | 0.4 | 5.5    | 1.6 (4.6–6.2)   |
|            | ETCO | ppb | 1771 | 370 | 1882   | 1483 (874–2357) |
| 119 ± 16 ml s$^{-1}$ | $J_{ACO}$ | pl s$^{-1}$ | $1.3 \times 10^7$ | $3.4 \times 10^6$ | $1.3 \times 10^7$ | $1.4 \times 10^7$ (4.5 x 10$^6$–1.8 x 10$^7$) |
|            | ETCO$_2$ | %   | 5.9  | 0.4 | 5.9    | 1.5 (5.2–6.7)   |
|            | ETCO | ppb | 1855 | 373 | 1926   | 1478 (1001–2479) |
| 68 ± 14 ml s$^{-1}$ | $J_{ACO}$ | pl s$^{-1}$ | $1.0 \times 10^7$ | $3.7 \times 10^6$ | $9.8 \times 10^6$ | $1.6 \times 10^7$ (3.7 x 10$^6$–2.0 x 10$^7$) |
|            | ETCO$_2$ | %   | 6.2  | 0.5 | 6.2    | 2.1 (5.2–7.2)   |
|            | ETCO | ppb | 1889 | 411 | 1941   | 1750 (860–2610) |
| 10 s BH 112 ± 9 ml s$^{-1}$ | $J_{ACO}$ | pl s$^{-1}$ | $1.3 \times 10^7$ | $4.0 \times 10^6$ | $1.2 \times 10^7$ | $1.5 \times 10^7$ (4.6 x 10$^6$–2.0 x 10$^7$) |
|            | ETCO$_2$ | %   | 6.0  | 0.4 | 6.0    | 1.7 (5.3–6.9)   |

Figure 5. (a) TMAD-derived mouth- and nose-exhaled airway tissue concentrations determined from the BH 10 maneuver for all 32 subjects together with the inhaled ambient air CO levels. (b) TMAD-derived airway tissue concentration versus nose-exhaled ETCO at 60 ml s$^{-1}$ and corresponding ambient air CO levels together with linear fits (Spearman’s correlation coefficients indicated).

Figure 6. (a) Mouth- and nose-exhaled (EFR 120) end-tidal CO, alveolar diffusing capacity and airway tissue CO concentration for all 32 subjects. The measured ambient air concentrations are shown for comparison in the lower panel. (b) Mouth- versus nose-exhaled end-tidal CO for all four breathing maneuvers. Solid line shows linear fit to BH 10 data (Spearman’s correlation coefficient indicated).
The data in figure 7 shows that the measured ETCO values are consistent and strongly correlate throughout the four breathing for every study participant, while there is only a weak correlation for the diffusing capacity, again most likely related to the observed correlation with exhalation volume, which was not directly controlled in the breath sampling procedure (standard deviation of 120 ml around a mean 1054 ml over all breathing maneuvers). In general, for each exhalation flow rate, ETCO, alveolar CO and maximum alveolar flux correlate strongly with each other, but not with ambient air CO and alveolar diffusing capacity. A special case is the EFR 60 maneuver, for which weak, but significant, correlations of ETCO were also observed with ETCO2, ambient air CO and exhalation volume (negative). As expected, ambient CO correlates across all EFRs, because for each subject all breath samples were collected within one hour in the same laboratory.

4. Discussion

The range of end-tidal CO concentrations measured in the present study (0.8–3 ppm) is well in line with the expected healthy non-smoker baseline due to endogenous CO production at normal HbCO levels [5, 9], and considering the low indoor (and outdoor) air CO levels (table 3) at the measurement location. The obtained TMAD parameter ranges agree with previous results obtained in the author’s laboratory [23, 24]. The observed spread in the data mainly originates from inter-individual differences in physiological parameters (e.g. HbCO level, lung diffusion properties, body height) and/or breath sampling conditions (e.g. exhalation flow rate and volume, inhaled CO). Since the eCO parameters did not correlate with ETCO2 (except at EFR 60), hypo- and hyperventilation presumably played a minor role in this work.

As evident from table 3 and figures 3, 4 and 6, for all breathing maneuvers, the individual values for mouth- and nose exhaled ETCO agree very well, and the mean values of all eCO parameters are virtually indistinguishable within the intra-individual uncertainty. Contrary to the results of a study by Andersson et al [27], who analyzed air sampled directly from the nostrils and paranasal sinuses, this suggests that there is no nasal CO production in healthy non-smokers. Thus, if equivalent sampling procedures are employed, nose- and mouth exhalations can be used interchangeably, and deviations between mouth and nose eCO could point to abnormalities in the nasal tract.

The observed dependence of the expirogram shape (figure 2), and, subsequently, the eCO parameters, on the exhalation flow rate (table 4), confirms previous findings [16, 28] and is in agreement with recent studies performed in our group [21, 23]. Median ETCO was consistently lower than the model-predicted median alveolar CO and both parameters increased slightly with decreasing EFR, with the highest concentrations obtained for BH, which is probably due to the longer gas residence time in the alveoli. In general, the shorter alveolar residence time at higher IFRs and EFRs is compensated by a higher alveolar CO diffusing capacity and maximum CO flux (figures 3 and 4). Taking into account that CO gas exchange is limited by the diffusion process across the capillary membrane and equilibrium between alveolar and blood CO is not established instantaneously, this could imply that equilibrium was never reached during the breathing maneuvers investigated in this work.

The fact that the alveolar parameters ETCO, C_ACO and J_ACO strongly correlated at each EFR and across flow rates indicates that they are related to a common factor, most probably HbCO. End-tidal CO was also found to correlate with body height, body weight
and gender, but not with age, which is in accordance with [29] and could be connected to differences in endogenous CO production. The observed decrease in ETCO towards the end of the expirogram, i.e. a bending of exhalation phase III, for EFR 60 (figure 2(a)) has also been measured by Fritsch et al [28]. One reason for this behavior could be a momentary depletion of blood oxygen at the end of the long exhalation, which would allow more CO to stay bound to hemoglobin and lead to a decrease in CO elimination [30].

The alveolar CO diffusing capacity, represented by the slope of the expirogram plateau (i.e. exhalation phase III) in figure 2, does not correlate with ETCO for any of the breathing maneuvers, which renders the parameter largely independent of endogenous CO, as was also concluded for the standard D_{LCO} test [31]. Since D_{LCO} depends on the exhalation flow rate rather than the gas residence time in the lung and exhibits a negative correlation with exhaled volume, the diffusing capacity seems considerably influenced by the breath sampling conditions. However, despite these confounding factors, the individual diffusing capacity values obtained for the 32 subjects still exhibit a weak correlation across the four breathing maneuvers (figure 7(b)), which gives confidence in the accuracy and repeatability of the analytical method. For EFR 120, the alveolar diffusing capacity has a relative, intra-individual uncertainty of ±15% (twice the standard deviation). Hence, a deviation from the healthy non-smoker median D_{LCO} by more than 15% could imply abnormal lung diffusing capacity. This is comparable to the deviation necessary to identify clinically relevant changes in the standard D_{LCO} value, which is generally assumed to be around 10% [26, 32], but could be as high as 20%–25% [33].

The TMAD-derived equilibrium airway (tissue) CO concentrations are only slightly higher than, and correlate strongly with, ambient air, which points to negligible endogenous airway CO production in healthy non-smokers. However, the moderate correlation with ETCO (figure 5), indicates that, while airway CO is mainly determined by the inhaled CO concentration, it may also include a contribution from blood CO diffused through airway tissue, or be influenced by the perpetual exposure to alveolar CO during exhalations. Of note, the fact that the small influence of blood or alveolar CO could be resolved implies that the method can be used to extract potentially elevated airway CO levels in diseased cohorts.

It is important to emphasize that the quantitative relationship between momentarily inhaled CO and exhaled CO is complex and not necessarily additive. Uptake and elimination of CO follow a non-linear behavior with a half-life of around 5 h [34]. Moreover, despite the low water-solubility of CO, inhaled CO may participate in airway gas exchange before reaching the alveolar sacs. For inhaled CO much lower than alveolar CO, the latter will usually converge to the prevailing (endogenous) blood CO level independently of the inhaled CO concentration. In accordance with this, it was found here, that ambient air CO does not affect end-tidal CO concentrations (except for a weak correlation at low flow rate EFR 60). Therefore, in general, it cannot be recommended to directly subtract the ambient CO concentration from ETCO or alveolar CO in order to retrieve endogenous CO [8].

Some of the limitations of the present study are the relatively small cohort size and the lack of spirometry (lung function, D_{LCO}) and blood (HbCO, CO) data. In addition, since the airflow parameters for each subject are solely derived from the BH 10 maneuver, the information on their variation with EFR is limited. Nevertheless, a good indication of the healthy population baseline and natural variation of the extended eCO parameters is provided. Overall, the parameters were found to vary in a sufficiently narrow range to be able to define confidence intervals and cut-off levels. Given the superior confidence intervals in the respiratory and eCO parameters and the high convenience during continuous online breathing, an exhalation flow rate of around 120 ml s⁻¹ can be recommended for CO (and potentially other) breath tests.

The added value of extended breath CO analysis clearly is that more parameters than the end-tidal CO concentration are obtained from a single exhalation. The novel approach can contribute to the standardization of breath sampling and eCO assessment, and thereby facilitate eCO interpretation and comparison between results of clinical studies. For example, the method could help to identify, whether elevated eCO levels are caused by increased blood CO levels or by abnormal lung diffusion properties. The former is a sign of high HbCO due to e.g. systemic oxidative stress or environmental exposure, while the latter could be associated with chronic obstructive pulmonary disease (COPD). Moreover, a method to measure airway CO could aid in evaluating eCO as biomarker of inflammation in the respiratory tract, which is of interest for patients with COPD or asthma. It remains to be seen, whether the technique can also help to discriminate between systemic CO and inflammatory conditions deep in the lungs, or to measure endogenous CO in the presence of external CO exposure.

5. Conclusions

The impact of breath sampling conditions, including exhalation flow rate, ambient air CO and exhalation route (mouth/nose), on eCO parameters, such as end-tidal CO, airway CO and alveolar CO diffusing capacity, was investigated in a cohort of 32 healthy non-smokers. Precise, real-time eCO detection was performed with laser absorption spectroscopy by acquisition of single-exhalation profiles from mouth and nose at three exhalation flow rates and after BH. A morphological model was employed to simulate pulmonary CO gas exchange and fit the real-time breath
data. End-tidal CO concentrations were in the expected healthy population range, and individual values for end-tidal CO and alveolar diffusing capacity were consistent across all breathing maneuvers, giving confidence in the method. No significant difference was observed between mouth- and nose exhalations and no evidence for endogenous airway CO production in healthy subjects was found, but the eCO parameters clearly showed a dependence on exhalation flow rate. End-tidal CO is largely independent of ambient air and alveolar diffusing capacity, which could serve as an indicator for abnormal lung diffusion. An exhalation flow rate around 120 ml s\(^{-1}\) can be recommended for eCO measurements. The extended eCO analysis approach provides means to measure variations in endogenous CO, which can improve the interpretation of exhaled CO concentrations and the diagnostic value of eCO tests in clinical studies.

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Author contributions statement

All authors reviewed the manuscript.

Competing Interests

The authors declare no competing interests.

ORCID iDs

Ramin Ghorbani @ https://orcid.org/0000-0002-7272-533X
Anders Blomberg @ https://orcid.org/0000-0002-2452-7347
Florian M Schmidt @ https://orcid.org/0000-0002-5065-7786

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