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Published in:
Journal of Breath Research

DOI:
10.1088/1752-7155/6/3/036001

2012

Simultaneously recorded single-exhalation profiles of ethanol, water vapour, and CO_2 in humans: Impact of pharmacokinetic phases on ethanol airway exchange

Short title: Exhalation profiles of ethanol, H_2O and CO_2.

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Abstract:

The breath alcohol concentration (BrAC), standardized to the alveolar water vapour concentration has been shown to closely predict the arterial blood alcohol (ethanol) concentration (ABAC). However, a transient increase in the ABAC/BrAC ratio has been noticed, when alcohol is absorbed from the gastrointestinal tract (absorption phase) and the ABAC rapidly rise. We analysed the plot of simultaneously recorded alcohol, water vapour, and CO_2 against exhaled volume (volumetric expirogram) for respiratory deadspace volume (VD) , cumulative gas output and phase III slope within one breath to evaluate whether changes in the BrAC profile could explain this variability. Eight healthy subjects performed exhalations through pre-heated non-restrictive mouth pieces and the concentrations were measured by infrared absorption.

In the absorption phase the respiratory VD of alcohol was transiently increased and the exhaled alcohol was displaced to the latter part of the expirogram. In the post-absorption phase, the respiratory VD for alcohol and water vapour was stable and always less than the respiratory VD for CO_2, indicating that the first part of the exhaled alcohol and water originated from the conducting airway. The position of the BrAC profile between water vapour and CO_2 in the post-absorptive phase indicates an interaction within the conducting airway, probably including a deposition of alcohol onto the mucosa during exhalation. We conclude that the increase in the ABAC/BrAC ratio during the absorption phase of alcohol coincides with a transient increase in respiratory VD of alcohol and a delay in the appearance of alcohol in the exhaled air as the exhalation proceeds compared with the post absorption phase.

Keywords: expirogram, ethanol, water vapour, carbon dioxide, deadspace, phase III slope, breath alcohol.

1. INTRODUCTION

We have earlier shown that the breath alcohol concentration (BrAC) standardized to the alveolar water vapour concentration closely predicts ABAC in the post-absorptive phase and is as precise as blood alcohol analysis [1 2] . The ABAC is the best method to quantatively assess a person´s drunkenness from a scientifically point of view [3 4] . It governs the driving force for the distribution of alcohol in the body and particular into the brain where it produces its pharmacological and toxic effects [5]. The BrAC reflects ABAC, since it is in equilibrium with the alcohol concentration in the pulmonary capillary blood, which subsequently is ejected as arterial blood [6 7]. Unfortunately, arterial blood sampling is not feasible for routine use, due to the risk of bleeding and injuries to the artery [8]. Importantly, BrAC is not equilibrated with the peripheral venous blood alcohol concentration (VBAC), which is the current standard of sampled blood medium practiced in many jurisdictions. The VBAC has been compared with BrAC, in an attempt to find a stable blood to breath alcohol concentration ratio (BBR), which consequently is impossible [9-15]. In the alcohol absorption phase the VBAC only reflects the uptake of alcohol in the peripheral tissue. Since this uptake is rather substantial the VBAC is low and results in an irrelevant low VBAC/BrAC ratio. On the contrary, there is a transient increase in the ABAC/ BrAC ratio when alcohol begins to be absorbed from the gastrointestinal tract extending up to approximately 15-30 minutes [1 2]. Although, most drivers are tested in the post-absorptive phase, some may be tested in the absorptive phase, and it is, therefore, important to try to explain the reason for this increase in the ABAC/BrAC ratio [5].

In order to evaluate the reason for this variability, we have analysed volumetric expirograms (VE) [20 21]. The VE is essentially similar to a time-based expirogram, but adds some specific parameters, since it connects the expired gas concentration to a specific expired volume. The CO_2 VE has been extensively studied [16-19] and its division into three functional phases was used as the basis for the analysis (Fig 1). Phase I consists of ambient air coming from the analyzer (apparatus VD), followed by ambient air coming from the most proximal part of the airway of the subject, usually denoted respiratory VD. Phase I is the part of the exhaled air that do not take part in any gas exchange and consists thus of the total VD (respiratory and apparatus VD). Phase II represents the transition zone and contains the interface between the inhaled (ambient) air not taking part in any gas exchange and the air exchanged in the lung. For water vapour and alcohol phase II represents the transition from

VD to their exchange positions and for CO_2 from VD to exhalation of alveolar air. It is marked by a rapid S-shape upswing in measured gas concentration. The phase III for CO_2, a plateau of variable slope, has been shown to indicate that alveolar air is exhaled [20 21]. Several methods have been used for the analysis of the VE, but all are in variable degree susceptible to changes in both the slope of phases II and III [22]. Parameters such as dead space and the distribution of the gas in different parts of the exhaled volume can be evaluated.

The profile of the VE depends on where the gas is exchanged in the airways. This, in turn, depends on the blood:gas partition coefficient (λ), which is defined as the ratio of the concentrations in the blood and the gas phase at partial pressure equilibration. The equilibrium process is accordingly governed by the partial pressure the substance generates and not the concentrations. In the lung, it has been shown that the λ is related to the alveolar partial pressure of a gas that is generated by a particular mixed pulmonary venous partial pressure [23]. The temporal increase in the partial pressure of an alveolar gas with a particular λ depends on the pressure a solute generates in the pulmonary capillary blood, residence time, and ventilation/perfusion mismatch, as described by Opdam et al. [24]:

$$P_A = P_v + ((P_{A0} - P_v) * e^{-(\lambda * Q/V * t)} \quad \text{(Equation 1)}$$

where P_A is the partial pressure in alveoli, P_V is the pressure a solute generates in its particular solvent in pulmonary capillary blood, P_{A0} is the alveolar partial pressure at time zero, λ is the partition coefficient described as the concentration ratio between blood and air, Q is blood flow, V is effective alveolar ventilation and t is the residence time.

Equation 1 illustrates that when the term $(\lambda * Q/V * t)$ is >10 the term e^{-x} is less than 0.000045 and $P_A = P_v$. This means that CO_2, for which λ is 3, reaches near-full equilibration (95 %) in approximately 1 s at a normal ventilation-perfusion ratio of 1, but the process is strongly dependent on the ventilation-perfusion relationships. At 0.1 s the P_A has only reached approximately 25 % of the P_v. CO_2 was used as a reference gas as it has been studied extensively; it is transferred from blood to alveolus by diffusion and then exhaled with no or very little interaction within the conducting airways. Water vapour with λ of 15000-17000 achieves near instant equilibration between blood and air in the alveoli, since the equilibrium occurs in less than 0.0001 s according to the equation. However, with such a high λ, partial pressure equilibration occurs almost momentarily and already in the conducting airways to full saturation at body temperature of the inhaled air [25]. The position in the airway where this

partial pressure equilibrium occurs between mucosa and air depends, besides λ, on breathing pattern and ambient air conditions. This transition between inhaled ambient air and air in equilibrium with the partial pressure of alcohol in the mucosa of the airway is therefore, achieved at a dynamic position in the airways. It has been determined in pigs and found to be positioned around the bifurcation of the trachea [26] . This interface has been called the isothermic saturation boundary for water vapour, since water vapour saturation is highly temperature dependent. Alcohol with a λ of approximately 1800 would establish partial pressure equilibration across the capillary-alveolar membrane within hundredths or thousands of a second and equilibration would also be independent of ventilation-perfusion ratios, at least in the normal range of 0.1 – 10 [27]. Its λ also suggests that equilibration may occur within the conducting airways at a dynamic position in the airways depending on breathing pattern.

In addition, it has been shown that the bronchial circulation is important to provide water for hydration and for equilibration at the mucosal surface of the conducting airways. Since alcohol also has a high λ, it is likely that the bronchial circulation is important for the transfer of alcohol to the mucosa [28 29]. The concentration of alcohol in the blood of the bronchial arteries, which is identical to the ABAC, may therefore importantly impact the alcohol exchange, in addition to the instantaneous exchange taking place over the pulmonary capillary – alveolar membrane.

The aim of this study was, therefore, to evaluate whether the transient increase in the ABAC/ BrAC ratio after alcohol begins to be absorbed from the gastrointestinal tract is reflected in the VE and thereby, could be detected and explained. The VE of alcohol was compared with those of water vapour and carbon dioxide, which represent two reference gases with different partition coefficients and their VEs are expected to be stable. Mouth alcohol (MA) was used as a control to verify that changes in the BrAC origin were satisfactorily detected.

2. MATERIALS AND METHODS

2.1 Subjects and ethical consent.

Eight healthy volunteers, six men and two women, with ages ranging from 35 to 71 years participated in the study. All were moderate drinkers of alcoholic beverages and gave their

informed consent. The study was approved by the Ethics Committee of the University of Lund, Sweden (DNR558).

2.2 Experimental procedure

Subjects underwent a set of breath tests carried out after the administration of 0.4 g alcohol per kg body weight directly to the stomach by a thin gastric tube after fasting for two hours. The alcohol was prepared from gin (40% (v/v)), diluted to 20 % by the same volume of tap water. This maneuver allowed absorption of alcohol from the stomach without any contamination of the mouth and pharynx. Consecutive exhalations and readings were obtained for alcohol, water vapour, and CO_2 starting from the very beginning of the absorption phase of alcohol. Exhaled profiles were collected for analysis at 11 specific time points (3, 4, 5, 6, 7, 8, 9, 23, 40, 60 and 82 minutes) after gastric alcohol intake. Breath alcohol started to occur at measurable levels three minutes after intake in five of the subjects. Three breath samples had to be discarded at this point due to absent or very low concentrations of alcohol. When the elimination phase of alcohol was reached at 90 minutes after gastric intake, i.e. the alcohol level steadily decreased, subjects rinsed their mouths with 30 ml gin (40% (v/v)) for 30 seconds and then expectorated. Readings were thereafter taken for analysis at 8 specific time points (0.5, 1, 2, 5, 8, 10, 15, and 25 minutes).

2.3 Measurement of alcohol, water vapour and CO_2 concentrations in breath.

The subjects blew through a mouth-piece with no flow restriction (inner diameter of 13 mm) into the inlet of the cuvette of the analyser. The subjects were instructed to exhale fast and directly after a moderate-sized inhalation. The mouth piece was pre-heated between measurements in a warming-box at 60 °C to inhibit absorption of the gases. The analyzer itself is also heated and has no resistance to flow, making the exhalation unrestricted. This resulted in a totally unobstructive exhalation, which takes approximately 1-2 sec, during which all gases and airflow were measured.

Gas analysis was by a mainstream analyzer (Servotek AB, Sweden) utilizing absorption of infrared light. A combination of filters situated on a disc which rotates at 33 Hz, measure alcohol, water vapour, and CO_2 concentrations. Determination of alcohol concentration was made at 3.32, 3.40 and 3.48 μm, plus a reference wavelength to discriminate it from other infrared absorbing gases. For correct comparisons it is necessary to measure the concentrations of all three gases at a simultaneous time point. This is achieved by defining a

time point each time the disc rotates. Since the concentration of the gases is measured at different times depending on the location of the filter on the disc, the concentrations at the specified time point are calculated through linear interpolation between two successive measurements of each gas and the corresponding time to the time point. The determination of the concentrations, at the specified time point, is applied to all filters on the disc. The flow signal is sampled and synchronized to within ± 5 ms of this time point. Measurements take place in a cuvette, ventilated by a fan which allows a minimal inflow of ambient air. Ambient air humidity is continuously measured by the analyzer between measurements. When the subject blows into the inlet in the front of the analyzer, a low-resistance valve closes the ambient air flow, which thus is replaced by the exhaled air. The dead space of the analyzer is 18 ml. The analyzer was calibrated with carbon dioxide (4%) in air deoxygenated to 17% oxygen, alcohol at 0.05, 0.1, 0.2, 0.8 and 1.5 mg/L air containing 44 mg/L water vapour and water vapour at 10, 22, 30 and 44 mg/L air. Linearity was verified and no drift was detected in the infrared sensor.

Exhaled flow was measured with a pneumotachograph (Fleisch No 2, Gould Inc, Cleveland, OH, USA) and the pressure difference with a differential pressure transducer (Validyne model OP103-12, Northridge, CA, USA) externally fitted and connected to the gas outlet of the analyzer cuvette. The voltage output of ± 6 V at full scale and the linearity of the pneumotachograph was calibrated and controlled before use. Volume was obtained by integration of the flow signal. Airflow and all gas concentration signals were monitored on-line on a computer screen and sampled into a computer data acquisition system (MatLab) with a built in analogue-to-digital converter. Collected data were stored on the computer hard disc for subsequent analysis on a separate computer.

2.4 Data analysis

The following indices were obtained from the volumetric expirograms:

a) Total (respiratory (anatomical + airway) and apparatus) VD was determined with a modification of the method described by Koulouris [30]: The exhaled gas output was determined by integration of the gas concentration over exhaled volume ($\int F_E \, \text{gas} \, dV$) between 1 to 90 % of exhaled volume (Fig 1). A curvilinear line (Equation 2) was fitted to the exhaled cumulative gas amount vs exhaled volume by non-linear regression. The intersection of the

regression line and the x-axis (for water vapour ambient gas concentration) was an estimate of the total VD.

$y_2 = k_2x^b + c_2$ (Equation 2)

where y_2 is exhaled cumulative gas output, k_2 is the slope constant, x is the exhaled volume, and the coefficient *b* (deviation index) describes the deviation of the slope as the exhaled volume (x) change, c_2 is the intercept at an exhaled volume of zero.

The apparatus VD (18 ml) was subtracted from total VD to achieve respiratory VD.

Phase II and III was separately analyzed, since they represent two airway compartments with different kinetics profiles.

b) The distribution of the cumulative gas output in phase II was determined by using the coefficient b in Equation 1 as a deviation index aimed at the first part of the VE. This was done by fitting Equation 1 a second time to the exhaled cumulative gas amount vs exhaled volume by non-linear regression between 0 – 0.8 L of the exhaled volume (Fig 1).

Value of coefficient $b = 1$ indicates a linear relationship and a constant delivery of the gas to the exhaled air. Values of coefficient $b < 1$ indicate a decrease in the amount of gas delivered to the exhaled air with exhaled volume; more of the gas output is delivered early during the exhalation, whereas an increasing amount of gas is delivered to the exhaled air as the exhalation proceeds for values of coefficient $b > 1$ (Fig 2). Change in the deviation index (b) indicates a change in the gas distribution from the airway in the first part of the exhalation (phase II).

c) The slope of phase III was determined as the slope (k_1) of a straight line fitted to the phase III part of the exhaled CO_2 concentration in the interval between 40-80 % of exhaled volume [22] by linear regression (Equation 3):

$y_1 = k_1x + c_1$ (Equation 3)

Where y_1 is the normalized gas concentration in % of peak level, k_1 is the slope constant, x is exhaled volume and c1 is the intercept at an exhaled volume of zero (Fig 1).

All gas concentrations were normalized to peak gas concentrations (100%) for the comparison. The ‘relative slope’ was referred to as the changes of the normalized gas concentrations (%) per unit of exhaled volume and presented as %/L.

We illustrate how the three simultaneous recorded VEs occurred at 4 different occasions in one participant in order to give the reader an idea of how the gases are partitioned in the exhaled air at different pharmacokinetic phases, including mouth alcohol. Mean curves containing all three gases for all subjects with standard error of the mean merge with each

other, since the VEs occurs at different expired volumes in different subjects, although, their relative position were stable.

Data are presented as mean ± SEM. The parameters of the exponential functions were determined by nonlinear least-squares regression and the linear function with linear least-squares regression (Statistica, Statsoft, Tulsa, OK, USA). The software module provided preset values for the estimates. The paired *t*-test was used for statistical comparison of data determined for CO_2 and water vapour. One-way analysis of variance (ANOVA) with time as the independent factor was used for statistical comparison of the data determined for alcohol at different time points during the experiment and Fisher´s LSD (Least Significant Difference) test was used for post hoc test analysis when significant differences were detected. P-values < 0.05 were taken to indicate statistical significance.

3. RESULTS

In fig 3 a-d the VE of carbon dioxide showed a typical CO_2 profile; phase I was followed by a rapid rise (phase II) to a sloping alveolar plateau (phase III). Water vapour, which started at ambient water vapour concentration, had the shortest phase I, while phase II had a more rapid rise, but phase III slope was less compared with alcohol and CO_2. Both were stable throughout all parts of the experiment.

Figure 3a reveals that alcohol had a slightly shorter phase I; phase II occurred later and phase III was steeper compared with CO2 in the early absorption phase. Figure 3b shows that alcohol had a shorter phase I; phase II occurred earlier in relation to the exhaled volume and phase III had a flatter slope compared with CO_2 in the post-absorption phase. Figure 3c shows that in the presence of high levels of mouth alcohol the phase I for alcohol became shorter and was followed by a rapid rise in the concentration before the rise of exhaled water vapour. After an initial peak, the concentration decreased gradually, generating a transiently negative phase III slope. In Figure 3d it can be seen that this negative slope gradually disappeared as the mouth alcohol concentration decreased. The peak expired alcohol concentrations in the profiles of the subjects at 4, 40 minutes after GAI of 0.4 g alcohol per kg body weight, 2 and 8 minutes after MA rinsing were 0.058 ± 0.013, 0.313 ± 0.017, 1.918 ± 0.199, and 0.308 ± 0.042 mg/L respectively.

The total VD of CO_2 was greater than for water vapour and both were stable throughout the experiments (Fig 4). After subtracting apparatus VD, the respiratory VDs for CO_2 and water vapour were 158 ± 37 ml and 26 ± 10 ml respectively ($p<0.001$).

Exhaled alcohol had a respiratory VD of 62 ± 3 ml (n = 32) 23 – 82 minutes after gastric alcohol intake (GAI). It was larger in the early absorption phase, 3 and 4 minutes after GAI started (95 ± 19 and 90 ± 10 respectively), compared with the level achieved between 23 to 82 minutes ($p < 0.05$). Mouth alcohol decreased VD immediately to a level similar to or slightly lower than that of water vapour. Five minutes after alcohol mouth rinse, VD gradually increased and regained similar values as before the mouth rinse.

The cumulative gas output in phase II presented as the deviation index (Fig 5) was stable for CO_2 at 1.72 ± 0.02 and water vapour at 1.07 ± 0.003 throughout all parts of the experiment ($p < 0.001$). An increasing amount of CO_2 was consequently delivered to the exhaled air as the exhalation proceeded. The deviation index for alcohol was 1.76±0.24 during early absorption and decreased to 1.22± 0.02 23-82 minutes after GAI ($p < 0.001$)

indicating a change from where the alcohol was delivered to the exhaled air as the experiment proceeded from the absorption to the post absorption phase. When mouth alcohol was introduced to the subjects 90 minutes after GAI the deviation index decreased to 0.89 ± 0.02 and increased gradually as mouth alcohol disappeared ($p < 0.001$).

The phase III slopes (Fig 6) of CO_2 and water vapour were stable and significantly different at 8.96 ± 0.70 %/L and 0.58 ± 0.12 %/L respectively ($p<0.001$). The phase III slope tended to decrease as alcohol was absorbed from 13.45 ± 5.78 to 5.76 ± 1.43 %/L, at 82 minutes after GAI. The introduction of mouth alcohol caused a negative slope -5.49 ± 1.90 ($p<0.001$), which reverted gradually to positive levels as mouth alcohol disappeared.

4. DISCUSSION

In the early absorption phase, when alcohol starts to be absorbed from the gastrointestinal tract and the arterial blood alcohol concentration (ABAC) rapidly increases, the breath alcohol concentration (BrAC) is lower than expected and the ABAC/BrAC ratio is consequently higher than in the post-absorptive phase [1 2]. This coincides with a significant increase in the airway dead space (VD) for alcohol compared with the VD in the post-absorptive phase. It indicates that alcohol in the absorption phase is exhaled in air from deeper parts of the airways than in the post absorption phase. Secondly, the analysis of the deviation index for alcohol also shows that the alcohol output in the most early absorption phase was shifted towards deeper airway compartments. Thirdly, the slope of phase III for alcohol seemed to decrease gradually from the absorption phase until it stabilized in the post-absorptive phase.

Our interpretation of these findings is that the diffusion time of the alcohol molecules from the bronchial arteries to the mucosa in the conducting airways is longer than across the capillary-alveolar membrane, which occurs instantaneously. Consequently, in the early absorption phase the alcohol in the mucosa generates a lower pressure compared with the partial pressure it generates in the alveoli. During the following exhalation the alcohol excreted in the alveoli is presumed to be reabsorbed by the airway mucosa, since the pressure gradient is directed to the mucosa. This displaces exhalation of alcohol to a later part of the exhaled breath volume.

At 23 minutes after gastric alcohol intake (GAI) both the VD and the deviation index for alcohol decreased and returned to stable levels. At these levels both water vapour and alcohol have a smaller VD than CO_2 and reach their phase III slopes before alveolar air was exhaled, according to the phase III slope of CO_2 [16]. This shows that the exchange of water vapour and alcohol starts proximal to the alveoli, where the CO_2 is known to be released.

Alcohol has a VD exceeding that of water vapour by approximately 40 ml, indicating that the transition zone of alcohol occurs deeper down in the airways compared with water vapour. Different diffusion characteristics for alcohol may be the reason for this [29]

It can be argued that the changes in the phase II and III slopes contributed to the overestimation in the dead space volume of alcohol in the absorption phase. This has been systematically studied by Tusman et al [22] and they find that the VD increased up to 4 ml when the slope of phase III increased and up to 6 ml when the phase II decreased, i.e. much less than our findings. In addition, our technique to use a curvilinear line to fit to the exhaled cumulative gas amount vs exhaled volume for the determination of VD may show a slightly

lower VD than fitting it to a linear regression line. A linear regression line crosses the x-axis at a slightly higher value. The curvilinear line fit takes all available data points into account and avoids the overestimation of the total VD calculation known to occur with the use of a linear regression fit [22]. We, therefore believe that our technique is reliable, which also was evident by stable values of VD for water vapour and CO_2, and that the respiratory VD for CO_2 was comparable with earlier publication [31]. Our study was intended to detect intra-subject differences in the position of the VE for alcohol compared with water vapour and CO_2 at different phases of the pharmacokinetic of alcohol and not end-tidal values. Differences in vital capacity between the subjects did therefore not influence our findings.

Our finding of an exchange of alcohol in the conducting airway supports an explanation for the difference in partition ratio between the alcohol concentration in blood and air in vitro (λ) and between blood and expired breath in vivo (BBR). The inspired ambient air, which lack alcohol, resorbs alcohol from the mucosa of the conducting airway during inhalation, thereby decreasing the alcohol concentration in the mucosa. The inspired air equilibrates with ABAC at least at the level of the alveoli, where the partial pressure of alcohol in air would instantaneously increase. During the following exhalation the alcohol is reabsorbed back by the mucosa, thereby decreasing the BrAC. The redistribution of alcohol in inspired/expired air delays the airway losses of alcohol, like an exchanger, and makes the ABAC/BrAC ratio higher than the ABAC/alveolar alcohol concentration. The partition of alcohol between blood and the head-space air in vitro is 1783:1 to 1830: 1 in men respectively women (hematocrit dependent) [33]. This partition follows thermodynamic and kinetic principles, depends on the solubility of alcohol in blood and air and should accordingly agree with the partition of alcohol in the alveoli. In expired breath air, however, the ABAC/BrAC ratio is approximately 2251:1. This means that the BrAC is approximately 20-25 % less than the alcohol concentration in the alveoli, at similar ABAC [1 32 33]. Interestingly, the fraction of reabsorption may be altered by manipulation in the breathing pattern, but seems otherwise to be relatively constant, thereby, explaining the finding of close agreement between BrAC and ABAC in the post-absorption phase [1 32 34 35].

However, in the absorption phase, when alcohol starts to be absorbed from the gastrointestinal tract the partial pressure in the mucosa has still not reach a level that corresponds to its blood concentration and this results in a more marked decrease in BrAC, explaining thus the increase in the ABAC/BrAC ratio in this particular phase. However, since this process occurs within 15-20 minutes after alcohol intake, it is normally hidden behind the presence of mouth alcohol and subsequently of minor importance for measurements of BrAC

for legal purpose. However, it is of physiological interest to understand how alcohol is exchange in the lung. In addition, under real-world drinking conditions when the alcohol is consumed in smaller portions over a longer time period the ABAC/BrAC ratio may be much more stable and the BrAC standardized to alveolar water vapour concentration much more reliable.

Mouth alcohol was used as a control to show that changes in the position of alcohol output were detected. As soon as mouth alcohol was introduced, the dead space volume for alcohol decreased to the level of water vapour, the deviation index became < 1 showing that alcohol mainly was exhaled early and the phase III became transient negative, indicating that the bulk of alcohol originates from the mouth. The deviation index and the determination of the dead space volume detected the contamination easily.

Conclusions

The transient increase in VD and deviation of alcohol gas output to a more distal part of the airway according to the comparative analysis of volumetric expirograms, indicates that alcohol comes from a more distal part of the airway in the absorption compared with the post-absorption phase. This may provide a foundation for increase deposition of alcohol onto the mucosa during exhalation. Although the number of subject is small, these findings may provide an explanation for the increase in the ABAC/BrAC ratio in the absorption phase. The position of the cumulative alcohol output in phase II and the phase III slope of alcohol between water vapour and CO_2 in the post-absorptive phase, indicate that alcohol interacts within the conducting airway. This forms a basis to understand that reabsorption of alcohol does occur in the conducting airways during exhalation and explains the finding in earlier studies of a difference of 20-25 % in the partitioning between blood and air in vitro compared with in vivo calculations in the post-absorption phase.

Legends:

Figure 1:
Illustrates how the volumetric expirogram is analysed. The measured fraction (concentration) of an exhaled gas (F(gas) on the left y-axis and the cumulative exhaled gas amount on the right y-axis, determined by integration of the gas concentration over exhaled volume ($\int$ F (gas) dV) are plotted against exhaled volume (x-axis). For this illustration we have chosen an expiration of alcohol. I-III denotes phases of the volumetric expirogram. For further information see text (Data analysis).

Figure 2:
Illustration of the concept of using the exponent b in the equation $y_2 = k_2x^b + c_2$ to describe the deviation of an exhaled cumulative gas amount. For further information see text (Data analysis).

Figure 3 a-d:
Illustrates typical simultaneous volumetric expirograms obtained from alcohol, water vapour, and CO_2 at 4 and 40 minutes after gastric alcohol intake (GAI) and 2 and 8 minutes after mouth alcohol rinsing had been introduced 90 minutes after GAI.

Figure 4:
Total deadspaces (VD) for alcohol, water vapour and CO_2 at different time points during the experiments after gastric alcohol intake (GAI) and after introduction of mouth alcohol (MA) 90 minutes after GAI. * $p < 0.05$, *** $p < 0.001$ statistically compared with total VD determined at 82 minutes after GAI. (Respiratory VD is achieved by subtracting apparatus VD (18 ml) from total VD)

Figure 5:

The deviation indexes for alcohol, water vapour and CO_2 in phase II (expired volume of 0-0.8 L) at different time points during the experiments after gastric alcohol intake (GAI) and after introduction of mouth alcohol (MA) 90 minutes after GAI. * $p < 0.05$, ** $p< 0.01$, *** $p < 0.001$ statistically compared with deviation index determined at 82 minutes after GAI.

Figure 6:

The phase III slopes for alcohol, water vapour and CO_2 at different time points during the experiments after gastric alcohol intake (GAI) and after mouth alcohol rinsing (MA) 90 minutes after GAI. ** $p < 0.01$, *** $p < 0.001$ statistically compared with phase III slope determined at 82 minutes after GAI.

Acknowledgements

The authors would like to thank SG Olsson (inventor of the analyzer), Mikael Finnhult and Daniel Dencker at Servotek AB, Arlöv, Sweden, for technical assistance. This work was supported by grants from “Anna och Edwin Bergers Stiftelse”

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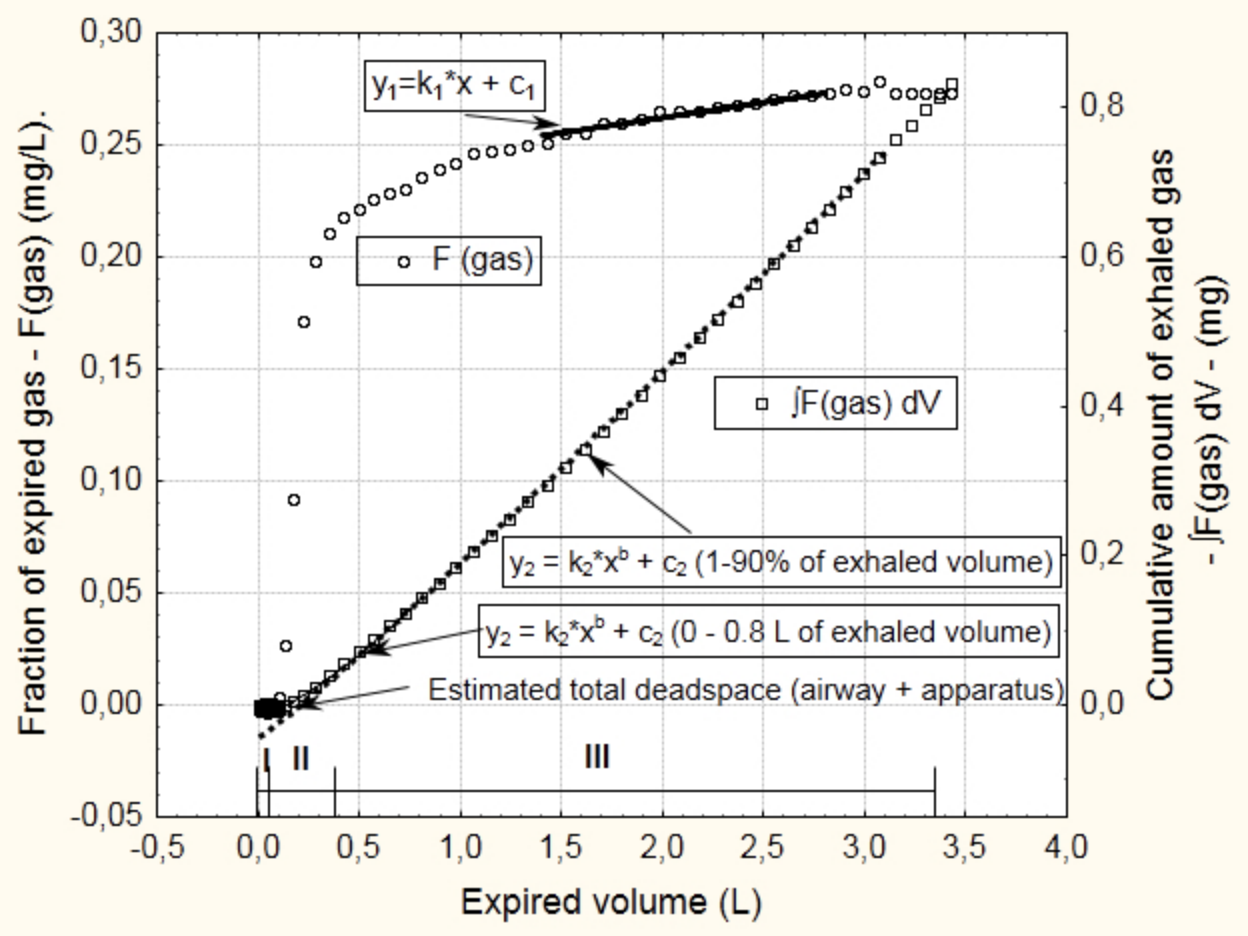
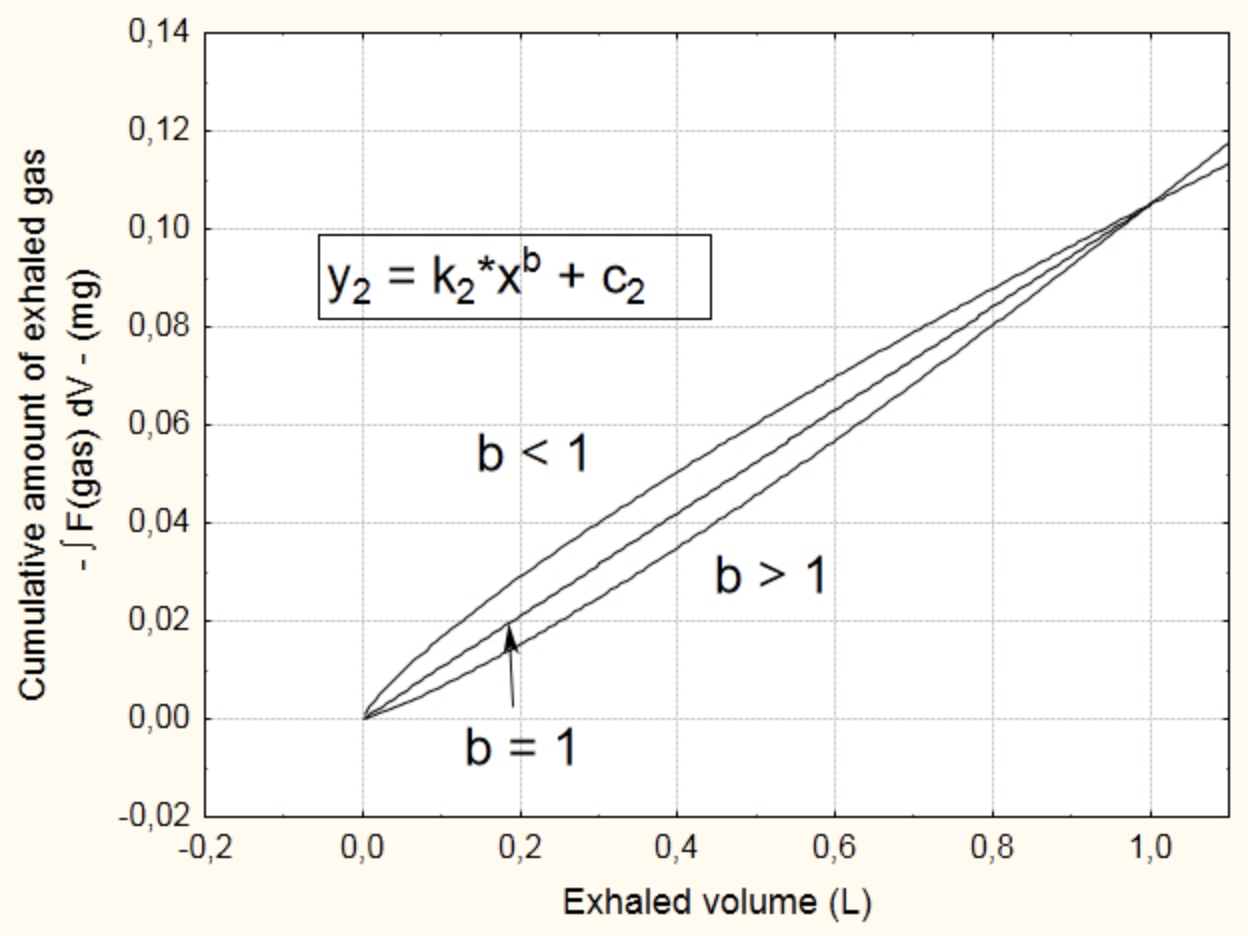
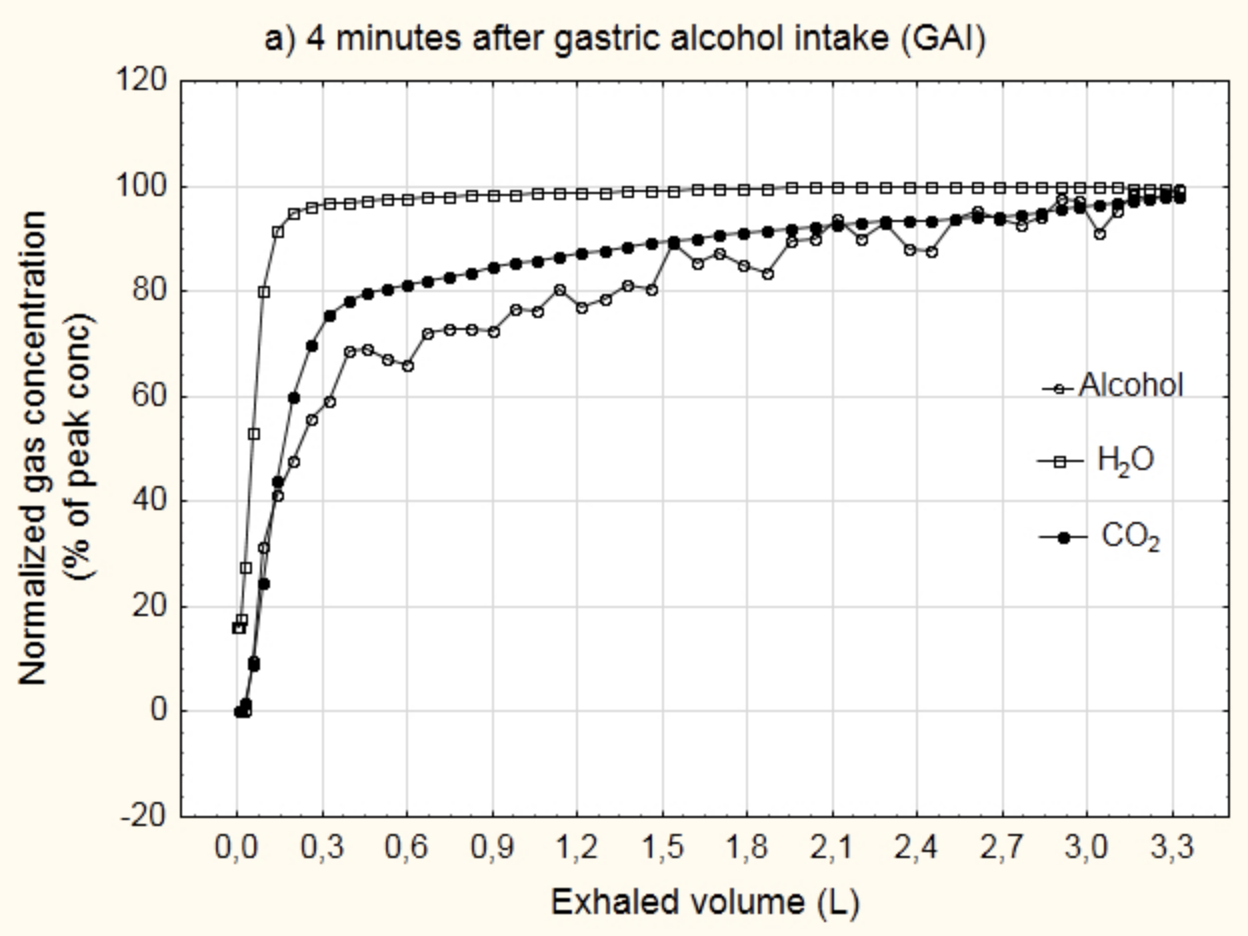
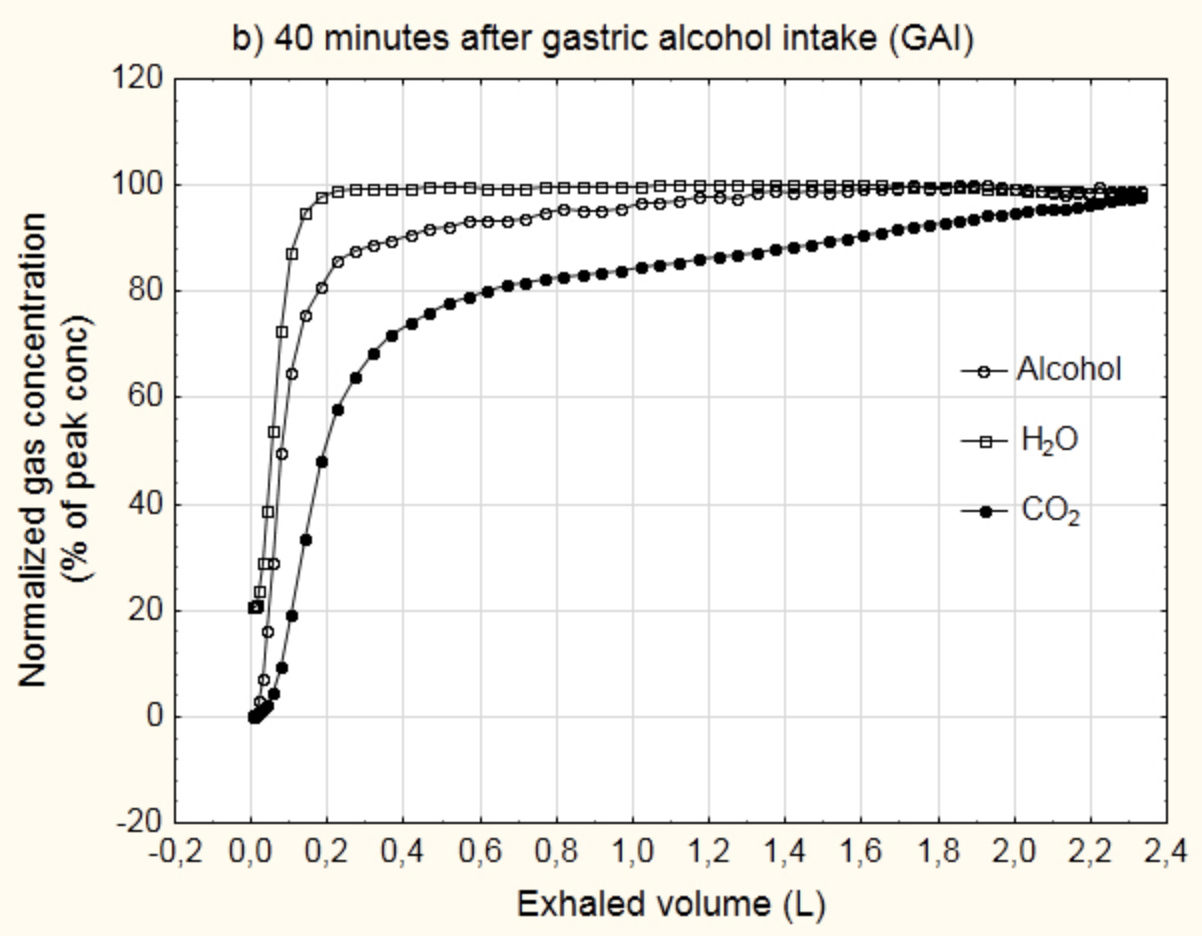
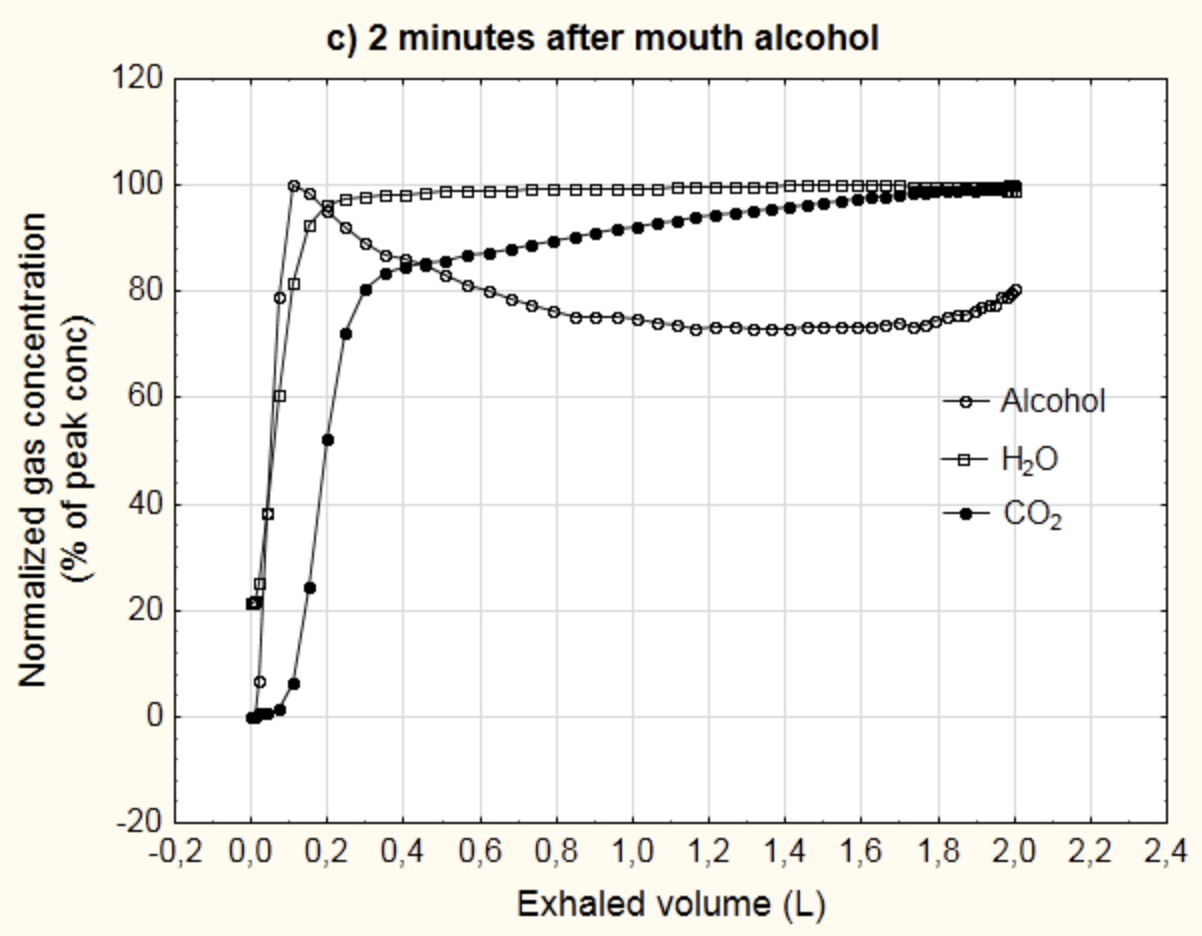
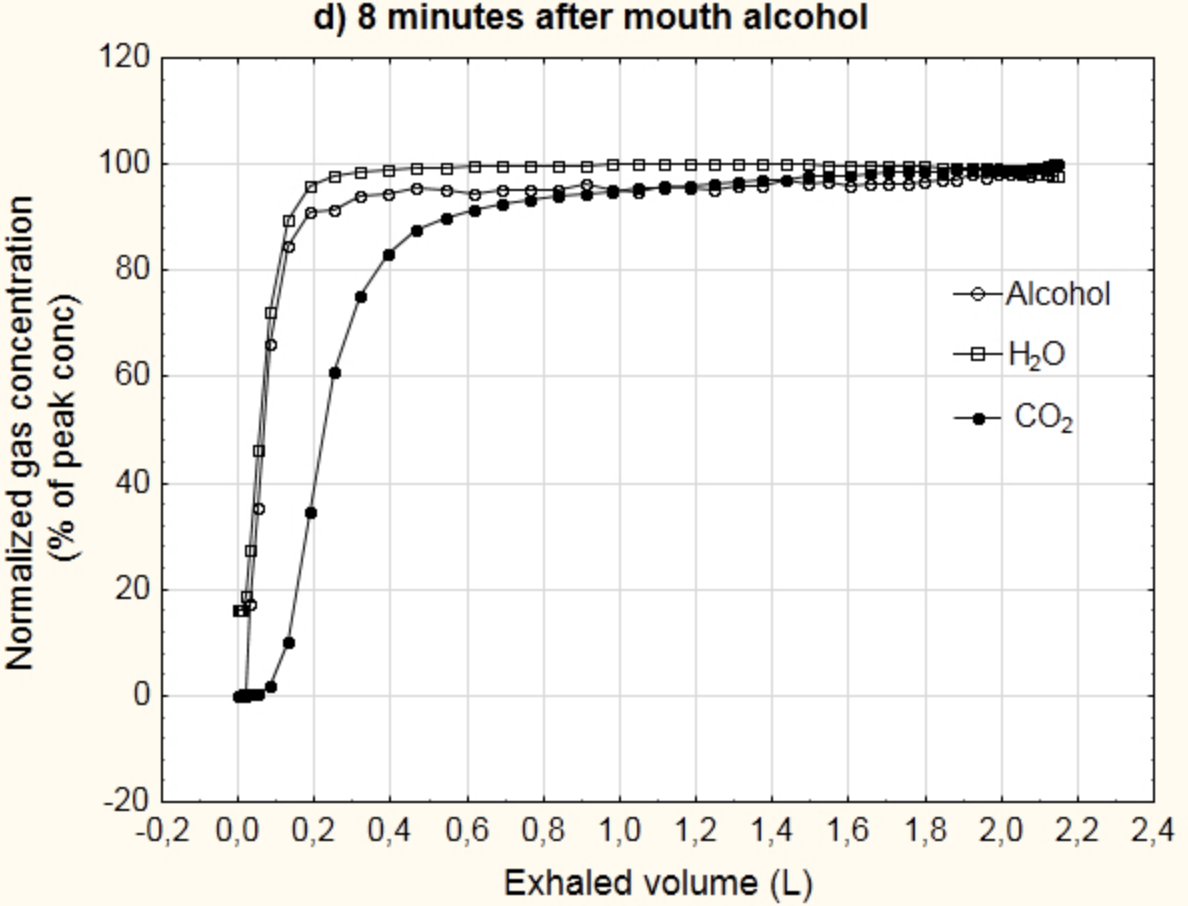

0,30
0,25
0,20
0,15
0,10
0,05
0,00
-0,05
0,8
0,6
0,4
0,2
0,0
-0,5
0,0
0,5
1,0
1,5
2,0
2,5
3,0
3,5
4,0
Fraction of expired gas - F(gas) (mg/L).
Cumulative amount of exhaled gas
- ∫F(gas) dV - (mg)
Expired volume (L)
y1=k1*x + c1
F (gas)
∫F(gas) dV
y2 = k2*x^b + c2 (1-90% of exhaled volume)
y2 = k2*x^b + c2 (0 - 0.8 L of exhaled volume)
Estimated total deadspace (airway + apparatus)
I
II
III

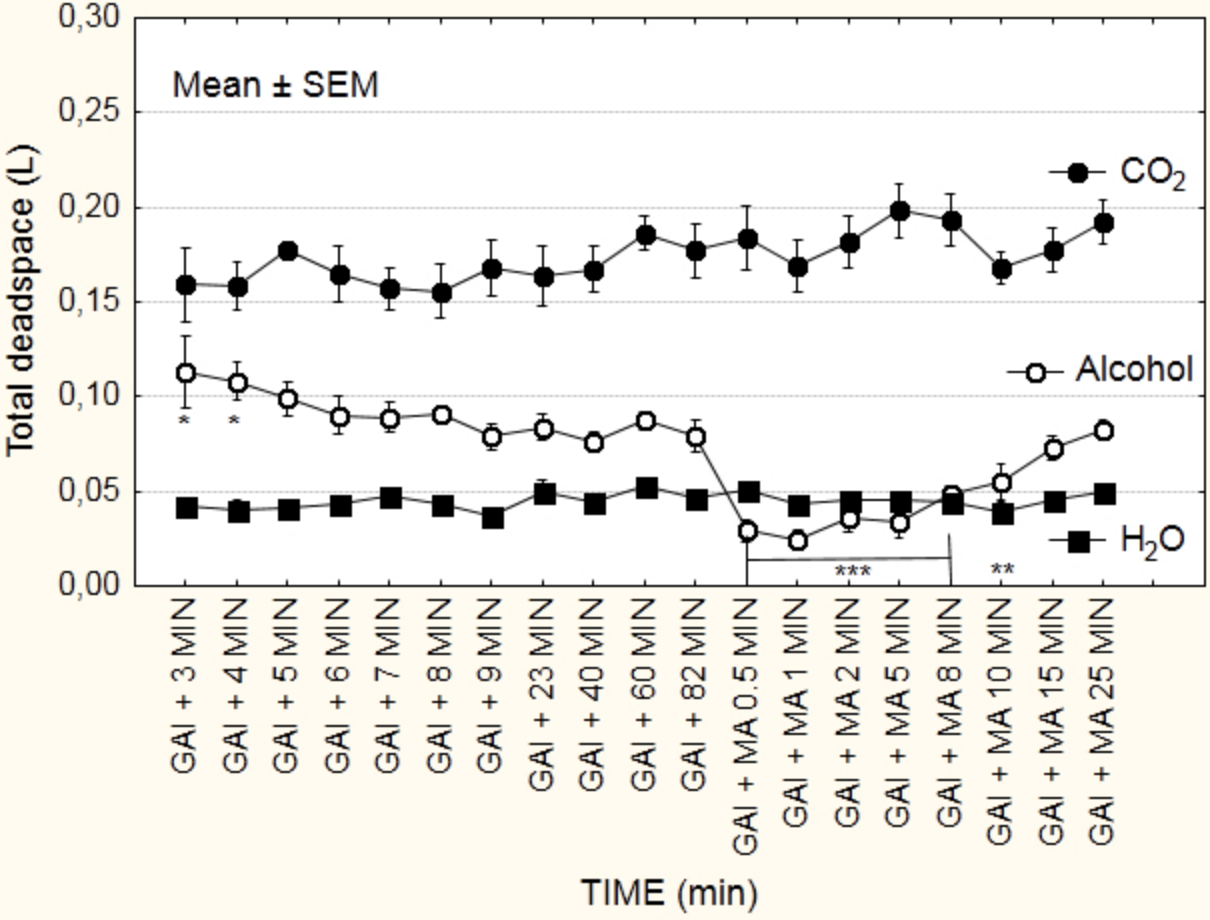

0,14
0,12
0,10
0,08
0,06
0,04
0,02
0,00
-0,02
-0,2
0,0
0,2
0,4
0,6
0,8
1,0
Cumulative amount of exhaled gas
- ∫F(gas) dV - (mg)
Exhaled volume (L)
y2 = k2*x^b + c2
b < 1
b > 1
b = 1

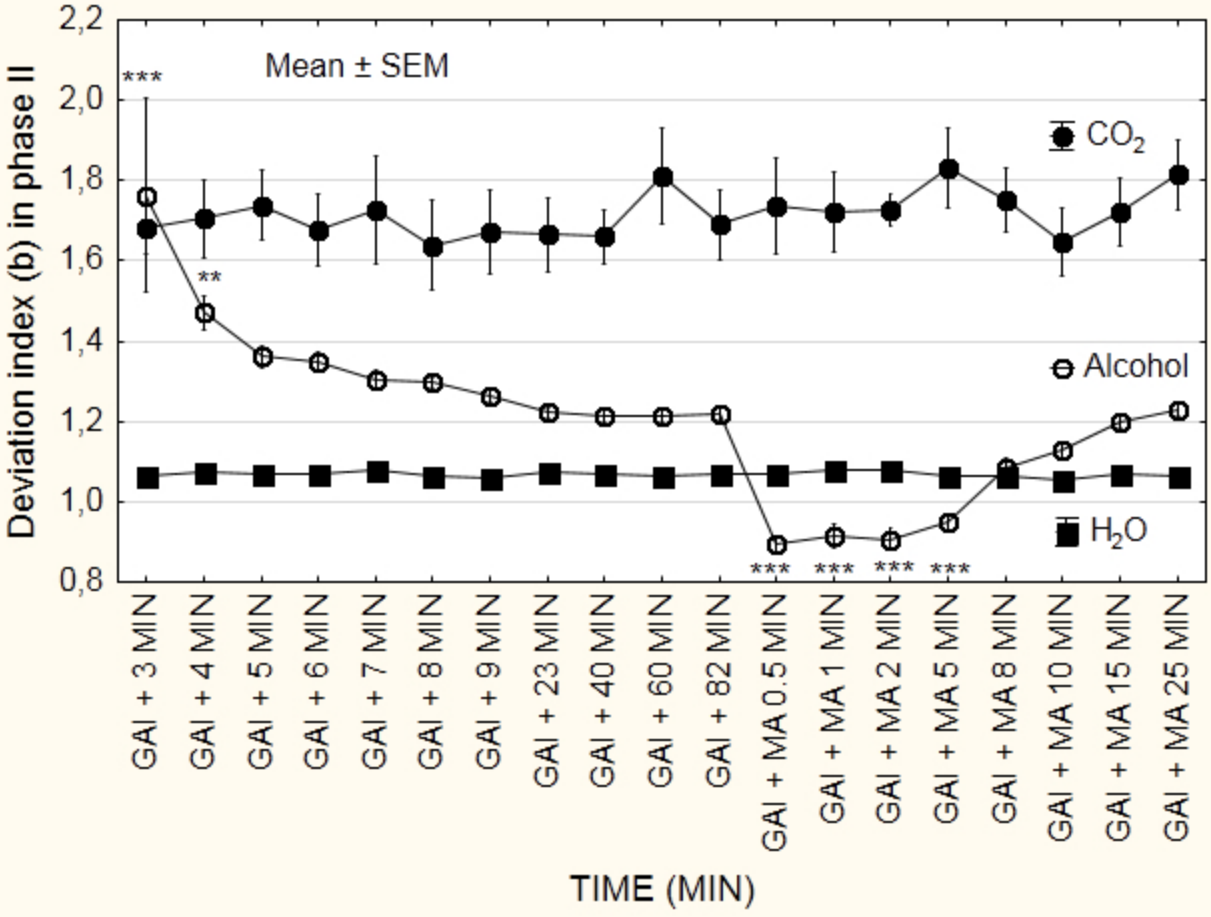

a) 4 minutes after gastric alcohol intake (GAI)
Normalized gas concentration (% of peak conc)
120
100
80
60
40
20
0
-20
0,0
0,3
0,6
0,9
1,2
1,5
1,8
2,1
2,4
2,7
3,0
3,3
Exhaled volume (L)
Alcohol
H2O
CO2

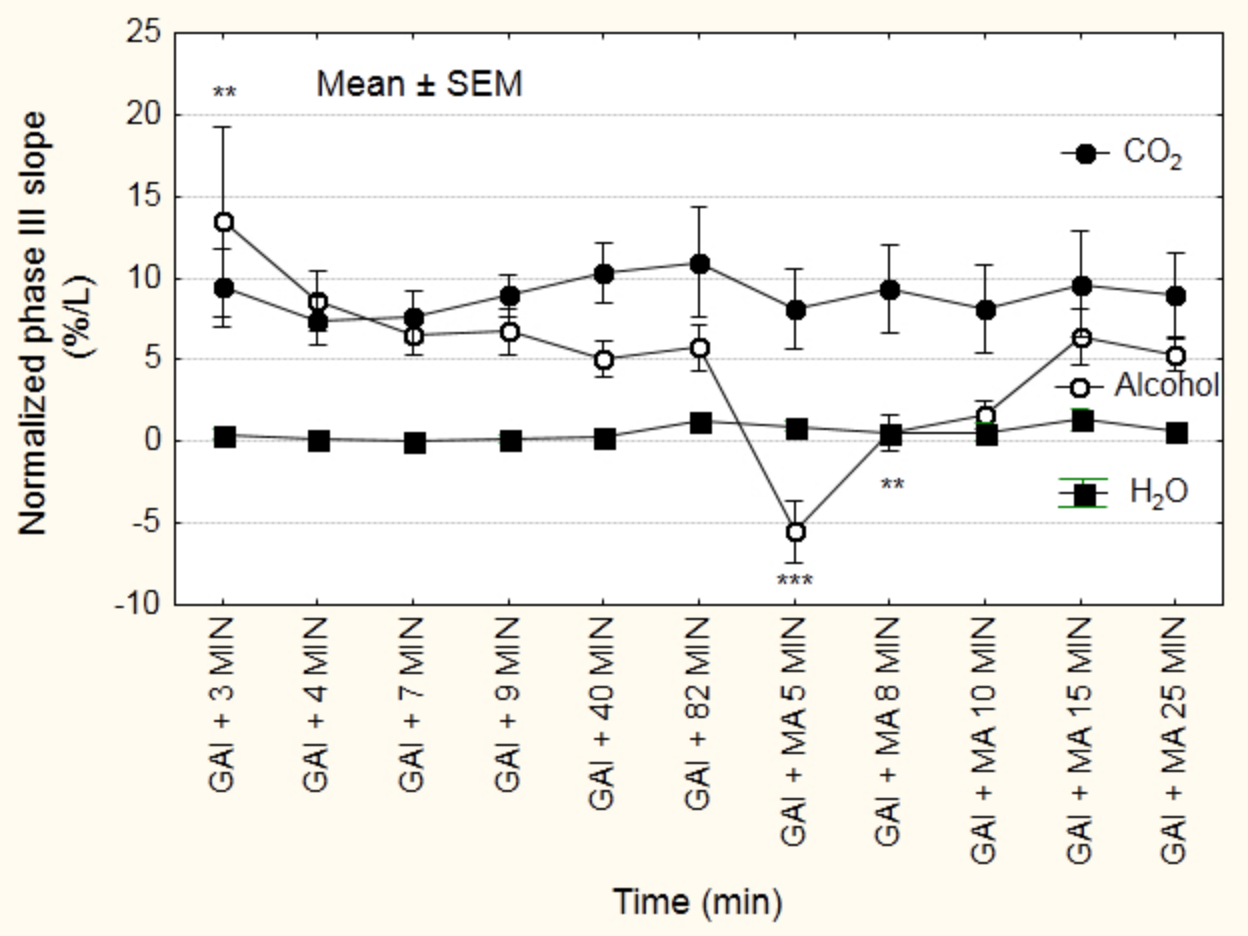

b) 40 minutes after gastric alcohol intake (GAI)
Normalized gas concentration (% of peak conc)
120
100
80
60
40
20
0
-20
-0,2
0,0
0,2
0,4
0,6
0,8
1,0
1,2
1,4
1,6
1,8
2,0
2,2
2,4
Exhaled volume (L)
Alcohol
H2O
CO2


c) 2 minutes after mouth alcohol
Normalized gas concentration (% of peak conc)
120
100
80
60
40
20
0
-20
-0,2
0,0
0,2
0,4
0,6
0,8
1,0
1,2
1,4
1,6
1,8
2,0
2,2
2,4
Exhaled volume (L)
Alcohol
H2O
CO2


d) 8 minutes after mouth alcohol
Normalized gas concentration (% of peak conc)
120
100
80
60
40
20
0
-20
-0,2
0,0
0,2
0,4
0,6
0,8
1,0
1,2
1,4
1,6
1,8
2,0
2,2
2,4
Exhaled volume (L)
Alcohol
H2O
CO2


Mean ± SEM
Total deadspace (L)
0,30
0,25
0,20
0,15
0,10
0,05
0,00
CO2
Alcohol
H2O
*
*
***
**
GAI + 3 MIN
GAI + 4 MIN
GAI + 5 MIN
GAI + 6 MIN
GAI + 7 MIN
GAI + 8 MIN
GAI + 9 MIN
GAI + 23 MIN
GAI + 40 MIN
GAI + 60 MIN
GAI + 82 MIN
GAI + MA 0.5 MIN
GAI + MA 1 MIN
GAI + MA 2 MIN
GAI + MA 5 MIN
GAI + MA 8 MIN
GAI + MA 10 MIN
GAI + MA 15 MIN
GAI + MA 25 MIN
TIME (min)


Mean ± SEM
Deviation index (b) in phase II
2,2
2,0
1,8
1,6
1,4
1,2
1,0
0,8
CO_2
Alcohol
H_2O
***
**
***
***
***
***
GAI + 3 MIN
GAI + 4 MIN
GAI + 5 MIN
GAI + 6 MIN
GAI + 7 MIN
GAI + 8 MIN
GAI + 9 MIN
GAI + 23 MIN
GAI + 40 MIN
GAI + 60 MIN
GAI + 82 MIN
GAI + MA 0.5 MIN
GAI + MA 1 MIN
GAI + MA 2 MIN
GAI + MA 5 MIN
GAI + MA 8 MIN
GAI + MA 10 MIN
GAI + MA 15 MIN
GAI + MA 25 MIN
TIME (MIN)


Mean ± SEM
Normalized phase III slope (%/L)
Time (min)
CO2
Alcohol
H2O
**
**
***
25
20
15
10
5
0
-5
-10
GAI + 3 MIN
GAI + 4 MIN
GAI + 7 MIN
GAI + 9 MIN
GAI + 40 MIN
GAI + 82 MIN
GAI + MA 5 MIN
GAI + MA 8 MIN
GAI + MA 10 MIN
GAI + MA 15 MIN
GAI + MA 25 MIN