

Research Article

Comparison of Breath and Blood Alcohol Concentrations in a Controlled Drinking Study

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Abstract

In the research of influence of alcohol on the mental and physical state of the body alcohol concentration in the blood plays an important role which must be properly defined. Therefore, the aim of our study was to examine breath and blood alcohol concentration in ten young, healthy, non-smoker male volunteers after consuming a red wine, containing 13 vol % of alcohol. Red wine (3 ml/kg) was consumed in the morning, after an overnight fast. Breath alcohol concentration (BrAC) and blood alcohol concentration (BAC) were determined before (baseline) and 15, 30, 45, 60, 90, 120, 150 and 240 min after beverage consumption. BAC was determined by headspace gas chromatography and BrAC was determined with Alcotest Dräger 6810 instrument. The concentration time profiles of alcohol in blood and breath following wine intake, poorly matched. Results show that the averages BrAC are significantly higher than average BAC in 15 min, 30 min and 45 min after beverage consumption. One hour after beverage consumption there were not statistically significant difference between BrAC and BAC measures. These results indicate that factual blood alcohol concentration and related expert toxicological decision based solely on the results obtained from breath analyzer might not be correct.

INTRODUCTION

Breath alcohol (ethanol) measurement is widely used in law enforcement as an evidence for prosecution of drunk drivers. It represents an indirect and non-invasive way of estimating an individual's blood alcohol concentration (BAC) [1-4]. Such breath-testing eliminates the need for taking blood samples, it can be used at the roadside and the result is immediately available for police officers to charge the suspect on the scene without delay [5,6]. Legal systems that use breath alcohol concentration for detecting drinking drivers do not attempt to relate the breath alcohol level to the blood alcohol level, but declare the offence solely on the basis of exceeding certain level in the breath [7].

Many studies have been performed on the variables, which affect absorption, distribution and elimination of alcohol. Some of these studies involved measurement of breath alcohol concentration (BrAC) since this is the primary evidential specimen, its measurement is less invasive than blood sampling and it is thought to correlate well with blood alcohol concentration (BAC) [8-12].

Several studies have shown high correlations ($r = 0.95 - 0.98$) between the concentrations of alcohol determined in blood and breath when specimens are taken nearly simultaneously [13,14]. Consequence, forensic toxicologists may retrogradely calculate BAC corresponding to the exact time of an accident using only the BrAC values determined with Dräger's analyzer as the input data.

Breath-analysis with alcometer gives an estimate of BAC by the use of a breath-to-blood conversion factor known as the blood/breath ratio. This blood/breath factor was used to calibrate the breath-analyzers and a value of 2100:1 became generally accepted for legal purposes to produce readings directly in BAC units [6,14].

However, due to significant inter- and intra-individual variations in the rate of alcohol absorption and elimination the reliability of this conversion has been challenged [15]. Several studies showed that the BAC/BrAC ratios might vary between absorptive, distributive and eliminative stages of alcohol metabolism. [15-17] shortly after drinking, during the absorption phase, BAC/BrAC ratios tended to be less than 2100:1. Numerous

investigations have been carried out with regard to the BAC/BrAC ratios [16]. Furthermore, there are many conditions that may influence BrAC to BAC conversion. For example, lower hematocrite level may influence BrAC resulting in the error range from -2 to +5%. An elevation in body temperature of 1°C results in 7% higher BrAC level. Physical activity and hyperventilation decrease BrAC levels up to 25%. Lower atmospheric temperature changes the levels of water that condenses in expired air and can also give falsely lower BrAC [1,2,18,19].

Kovacic *et al.* shown that BrAC and BAC calculated to the time of breath alcotest, were not within 95% predicament range in 30 out of 570 individuals (5.3%) [19]. Also, limited information exists on the alcohol kinetics when analyzed from series of blood and breath samples [13].

Therefore, in the present study we determined and compared breath and blood alcohol concentrations in the series of blood and breath samples, after consumption of red wine, following an overnight fast.

MATERIALS AND METHODS

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University of Split, School of Medicine. All subjects signed a written consent prior to their participation in the study. Ten healthy male volunteers, aged 20 to 35 years (mean body mass index of 24.2 ± 1.8 kg/m²) were normolipidemic, nonsmokers, taking no medications and had no history of alcohol abuse. Subjects were asked to abstain from exercise, fruits, vegetables, dietary supplements, tea, alcoholic beverages and caffeine- or theobromine-containing foods for 24 h before experiment. After an overnight fast, the subjects consumed 3 mL/kg body wt red wine.

Breath alcohol concentration was measured before and 15, 30, 45, 60, 90, 120, 150 and 240 min after beverage consumption. For each time point two independent breath samples were taken within 1 min and blood-samples were collected. A venous cannula (22G) was inserted into large subcutaneous vein in the left antecubital fossa to allow blood sampling.

Measures

Wine: Red wine (Vinagra, vintage 2005, 13% v/v alcohol) was provided by the Bric winery, Slovenia.

Blood alcohol concentration measurements: Blood alcohol (ethanol) concentration was measured by Shimadzu 2010 gas chromatography with headspace and flame ionization detector (FID). Headspace conditions: thermostating 12 min at 75°C with rotation speed 500 rpm, needle transfer temperature 95°C. Ultra-pure grade helium was used as the carrier gas at a flow rate of 11.70 ml/min. The chromatographic column was RTX-BAC2 (Fused Silica, 30m, and 0.53mm i.d, with a film thick 0.20µm). Injection temperature was 200°C, column conditions 3 min at 60°C, FID 200°C.

Breathe alcohol concentration measurements: A measure of breath alcohol concentration was carried out with a calibrated electrochemical system, Dräger Alcotest 6810 (Draeger Safety Pacific Pty. Australia).

Preparation of blood samples: Blood samples were collected into blood collection system with citrate buffer 9NC (PFA), (Monovette, Sarstedt, Germany). Sample preparation involved a mixture of 300µL sample and 1000µL of t-butanol as internal standard into headspace vial. All solvents and chemicals were analytical-grade-purity products of Merck Darmstadt, Germany).

Statistical analysis

Statistica 10™ software package (StatSoft, Inc., Tulsa, USA) was used to perform statistical analysis of the data. Repeated measures analysis of variance (rmANOVA) was used to determine if there were any differences between the means of post-drink time conditions. Bland-Altman plot was used to calculate bias and imprecision between the measurements of alcohol in blood and breathe [20-22].

We used Bonferroni test for post hoc analysis. Statistical values were considered significant at 99% ($p < 0.01$).

RESULTS AND DISCUSSION

For each volunteer BrAC and BAC measurements were obtained and average values were plotted on the figure (Figure 1). Average maximum values of BAC after consuming red wine were 0.28 g/kg respectively while BrAC values were 0.41 g/kg.

The repeated measures ANOVA shows that difference in BrAC between 15, 30, 45, 60, 90, 120, 150 and 240 min after beverage consumption condition are statistically significant, $F(8,10) = 88.33$, $p < .001$, $\eta^2 = 0.91$. Post hoc analyses using the Bonferroni Post Hoc test criterion for significance shows that differences between the averages of BrAC measurements in 15, 30, 45 and 60 min after beverage consumption did not significantly significant, $p > 0.05$.

The repeated measures ANOVA shows that difference in BAC between 15, 30, 45, 60, 90, 120, 150 and 240 min after beverage consumption condition are statistically significant, $F(8, 10) = 117.62$, $p < .001$, $\eta^2 = 0.93$.

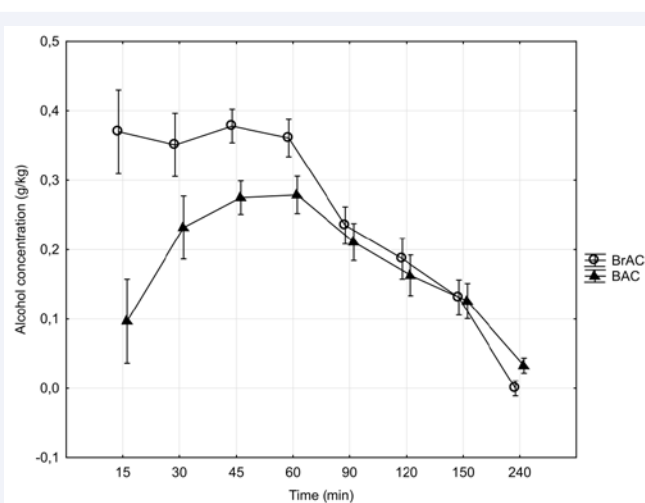


Figure 1 Comparative means plot (vertical bars denote 0.95 confidence intervals) of ethanol in blood (BAC) and end-expired breath (BrAC) for ten volunteer subjects who drank a red wine with 0.31 g of alcohol per kg body weight.

ANOVA shows that difference between BrAC and BAC measurements are statistically significant, $F(1,18) = 33.95$, $p < .001$, $\eta^2 = 0.65$. Post hoc analyses using the Bonferroni Post Hoc test criterion for significance shows that the average BrAC is significantly higher, $p < 0.01$, than average BAC in 15 min, 30 min and 45 min after beverage consumption. One hour after beverage consumption there were not statistically significant difference between BrAC and BAC measures.

In (Figure 1) the regression relationship was $\text{BrAC} = 0.505 \times \text{BAC} + 0.040$. The slope (0.505 ± 0.136) differed significantly from unity (95% CI were 0.184 - 0.826) and the intercept (0.04 ± 0.04) did not differ significantly from zero (95% CI were -0.049 - 0.129), indicating the presence of a proportional bias.

In the absorption phase the mean bias (BrAC-BAC) was 0.141 ± 0.105 g/kg and the 95% limits of agreement from -0.065 to 0.346 g/kg. However, in the elimination phase the mean bias (BrAC-BAC) was 0.005 ± 0.030 g/kg and the 95% limits of agreement from -0.054 to 0.065 g/kg by Bland - Altman plot (Figure 2).

In (Figure 2) elimination rate β_{60} , was calculated according to the Widmark's equation [23]: $C_{\text{BAC } t_1} = C_{\text{BAC } t_2} + \beta \Delta t$, where β represents an alcohol elimination factor (g/kg/h), C_{BAC} represents blood alcohol concentration at different times (g/kg) and Δt equals the time, in hours, elapsed between two blood samplings. The elimination rates β_{60} , which can be used for minimum and maximum back calculations for the BAC.

DISCUSSION

Using the Dräger Alcotest 6810 breath analyzer in volunteers ingesting a moderate dose of alcoholic drinks (0.31 g/kg of ethanol was ingested in the morning after an overnight fast), we tried to define correlation between their BrAC and BAC. The main finding from this study was a significantly higher BrAC than BAC after drinking red wine. In the absorption phase, BrACs were always higher than BACs when determined with the Alcotest. Our results are in accordance with previous reports [24,25], where it was suggested that BrAC could be higher than alcohol concentration in peripheral venous blood (BAC), due to two facts: 1) during the absorption phase the alcohol concentration in arterial blood is higher than in venous blood, and 2) end-expiratory air (substrate for BrAC) equilibrates with the pulmonary arterial blood. [26]

In many countries before BrAC measurements can be legally performed, a waiting time of 15 minutes must be observed whilst our results show that 15 minutes between consumption of alcoholic beverage and BrAC measurement is not enough time to make the BrAC results reliable, if the alcoholic beverage was consumed on an empty stomach. [27]

Figures 1 and 2 shows that BrAC and BAC values for all subjects have reached matching values only 90 minutes after alcohol intake (elimination phase). There are a number of possible explanations for this phenomenon. If alcohol is consumed on an empty stomach it can cause spasm of the pyloric sphincter, thus keeping the alcohol in the stomach for longer and retrogradely elevating breath alcohol levels. This phenomenon is largely related to gastric emptying. [28] On the other side, drinking ethanol with or after a meal not only decreases peak in BAC, but it also decreases alcohol's total bioavailability. Also, the amount of sugar in an ethanol-containing beverage can slow ethanol absorption by slowing gastric emptying. [29] Several other variables further complicate an individual's ethanol absorption rate. The slight deviation in the relationship between BrAC and BAC in the absorption phase, lasting for approximately 90 min, may also be caused by a fast redistribution of alcohol between different body compartments. This redistribution may be influenced by fraction of water in the blood and/or the measured BrAC [30,31]. This kinetic energy produces the distribution force, which governs the diffusion equilibration of alcohol with other biological fluids including the air spaces of the alveoli. [32] At equilibrium the average kinetic energy of alcohol is the same in all compartments, but the concentration of alcohol differs, since it depends on the solubility of alcohol in a particular compartment.

Matching airflow with blood flow is critical for normal gas exchange and requires a delicate balance between the blood and air distribution systems. [33] The physiological basis of the breath alcohol test depends on the continuous equilibration of alcohol between the pulmonary capillary blood and the alveolar air. [34] Breath alcohol testing relies on the fact that alveolar alcohol concentration can be measured in the expired air. It is believed that the partial pressure of alcohol in the alveoli corresponds to alcohol concentration in arterial blood, which in turn reflects the brain alcohol concentration at equilibrium. This equilibration of alcohol between arterial blood and the brain tissue is likely to occur rapidly, since the brain receives a large cardiac output. [35-38] Average velocities of alcohol molecules and alcohol

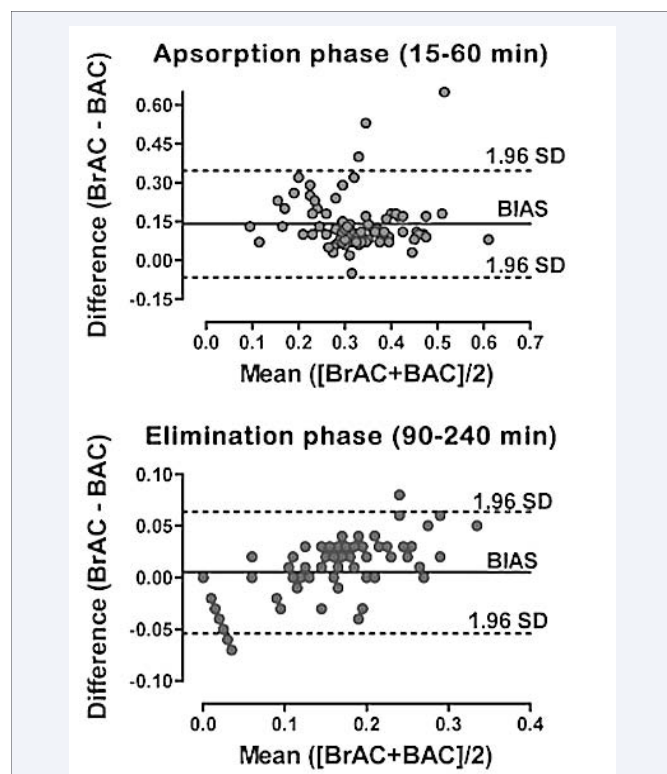


Figure 2 Bland-Altman plots of individual differences (BrAC-BAC) against mean $(\text{BrAC} + \text{BAC})/2$, in the absorption and elimination phase.

concentrations vary between compartments and depend on liposolubility of alcohol. Volatile substances, such as alcohol, are highly soluble in water, and do not bind with plasma proteins, all of which makes it suitable for analysis in bodily fluids and exhaled air. Distribution of alcohol in the body is proportional to water content in the tissue. [39]

Many studies showed high correlations between the concentrations of ethanol determined in blood and breathe when specimens are taken nearly simultaneously. Much less information exists about the pharmacokinetics of ethanol derived from analyzing a series of breath samples. [13,14] In forensic casework, it is sometimes necessary to make forward or retrograde extrapolations of a suspect's blood- or breath- alcohol concentration, e.g. from the time of sampling back to the time of driving. [40,41]

CONCLUSION

The use of results obtained with an alcometer without confirmation of alcohol levels through blood testing, is not acceptable in court processes, even if the breath was tested shortly after the accident or any other legal offence. Unique and reliable correlation factor for comparing the BrAC and BAC which would possibly determine BAC using solely BrAC, has been not found. Legal classification of being under the influence of alcohol refers to blood alcohol concentration, not breathe alcohol concentration.

The results indicate that conclusions on the factual blood alcohol concentration and related expert toxicological decision based solely on the results obtained from Dräger Alcotest breath analyser might not be correct.

As the number of subjects participating in this comparative study was rather small, there is a need for future studies, with larger sample size and other co-factors tested (e.g. influence of food) in order to fully elucidate the comparability between blood alcohol concentration and breath alcohol concentration (utilizing this particulate measuring device).

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