CHAPTER 3

A comparative study of two methods for attaining constant alcohol levels

Br J Clin Pharmacol 2008, 66 (5), 674-681

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ABSTRACT

BACKGROUND Alcohol effects or drug-alcohol interactions are preferably studied at constant blood levels. To achieve pseudo-steady state levels, various methods are used, which usually produce adequate averages but variable individual concentration profiles. In this study two modes of alcohol administration were compared: a 'two-step prekinetic procedure' and a 'clamping method'.

METHODS The two-step prekinetic procedure started with determination of individual pharmacokinetic parameters, during a prestudy occasion. Individual infusion regimens were calculated afterwards, based on a pseudo-steady state breath alcohol concentration (BrAC) of 0.65 $g{\cdot}L^{-1}$ and applied during a separate occasion. For the clamping procedure, a spreadsheet-based paradigm was developed using BrAC guided adjustments of infusion rates, to maintain stable BrAC levels of 0.6 $g{\cdot}L^{-1}$.

RESULTS The mean BrAC during clamping (0.61 $g{\cdot}L^{-1}$ (95% CI: 0.58 - 0.63 $g{\cdot}L^{-1}$)) did not differ from its intended level of 0.6 $g{\cdot}L^{-1}$ (1.0% on average). In contrast, the mean BrAC during the prekinetic procedure was significantly lower than the 0.65 $g{\cdot}L^{-1}$ set-point (0.59 $g{\cdot}L^{-1}$ (95% CI: 0.54 - 0.63 $g{\cdot}L^{-1}$)), and deviated from this target by 9.7% on average. The clamping method also showed less variation between subjects (CV: 6.2%), compared to the prekinetic procedure (CV: 14.6%).

CONCLUSIONS Although the two methods differ considerably in their approach, clamping of BrAC resulted in more accurate alcohol levels than infusion based on PK-modelling and does not require an extra prestudy occasion. The novel alcohol clamping paradigm can be of value in future studies of alcohol interactions or the pharmacodynamics of acute alcohol administration.

INTRODUCTION

Alcohol, or ethanol is widely used in social settings and causes dose-dependent central nervous system (CNS) depression (Little, 1991). Its metabolising systems show a large ethnic (Eng *et al.*, 2007; Reed *et al.*, 1976) and interindividual variability, and there are many potential drug interactions. Alcohol intake can inhibit drug oxidation by competing with metabolising enzyme systems. On the other hand, chronic use of alcohol induces liver microsomal enzyme activity, which can lead to lower plasma levels of susceptible drugs when given concomitantly. Besides this pharmacokinetic interplay, alcohol consumption can also interact with drugs or substances on a pharmacodynamic level (Sellers and Holloway, 1978). Hence, research for possible interactions between alcohol and other pharmacological compounds is often necessary when developing a safe drug therapy. Interactions with alcohol are particularly important for psychoactive drugs, since such compounds are often metabolised by the liver and they may affect the CNS-effects of alcohol or vice versa. At the same time, alcohol abuse occurs with many psychiatric disorders (Kushner *et al.*, 2000; Soyka *et al.*, 1993) and alcohol consumption is an accepted and frequent habit in Western society. Therefore, alcohol interaction studies are performed in many early drug development programs of psychoactive agents.

In determining the effects of alcohol or assessing alcohol-drug interactions, it is helpful if alcohol plasma concentration can be maintained at pseudo-steady state levels. Most of the effects of alcohol are concentration- and time-dependent (Hiltunen *et al.*, 2000; Vogel-Sprott, 1979), and interpretation of the results can be complex if plasma levels change over time. Nonetheless, many studies of alcohol effects or drug interactions are not performed at pseudo-steady state levels, or under other conditions in which these complexities are regulated. In many cases, alcohol exposure is merely controlled by adaptation of the dose to weight, and sometimes to gender or other demographic variables (Wilkinson, 1980). Measurements of the alcohol effects are usually performed at fixed time intervals after

intake. Often, breath alcohol levels are measured, but efforts to maintain constant levels are rare (Wilkinson, 1980). A major reason for this lack of methodological stringency is the complexity and variability of alcohol's pharmacokinetic characteristics.

To achieve pseudo-steady state alcohol plasma concentrations, various methods have been reported in the literature. One method described by Hartmann *et al.* (Hartmann *et al.*, 1988), used a two-step infusion rate protocol with a prestudy day. The pharmacokinetic profiles for alcohol were established after intravenous administration of a fixed alcohol dose on a prestudy day, and the individual parameters were used to calculate the infusion regimens needed to achieve a certain plasma concentration profile. On the actual study day, plasma samples were taken for determination of alcohol concentration, during the first hour of infusion. The results were available within the next hour and were used to adapt the infusion rate. In practice however, Hartmann *et al.* showed that these adaptations decreased the stability of the plasma alcohol concentrations (Hartmann *et al.*, 1988). Therefore, the method was performed without these adjustments during several studies in our centre (van Steveninck *et al.*, 1993; van Steveninck *et al.*, 1996). This technique produced a reliable average concentration profile with occasional over- or undershoots.

To avoid major fluctuations altogether and produce a more stable steady state serum level of alcohol with lower variability, it would be necessary to adapt the infusion rate to the plasma alcohol concentration instantaneously. O'Connor *et al.* (O'Connor *et al.*, 1998) developed an algorithm that uses breath alcohol concentration (BrAC) to guide infusion rate adjustments for a steady alcohol plasma concentration. Their initial method started with an oral loading dose, adapted to body weight. After one hour, an alcohol infusion was initiated and BrAC samples were taken with ten minutes intervals. An experienced technician adapted the infusion rate to the BrAC-changes. The authors were able to keep alcohol plasma concentrations within 10% of the desired steady state, thus reducing variance introduced by changing alcohol plasma levels. Ramchandani *et al.* (Ramchandani *et al.*, 1999), replaced the oral loading dose with a preprogrammed intravenous infusion rate profile. Using a physiology-based pharmacokinetic model, a steady BrAC within 5% window of the target concentration could be achieved.

Despite its apparent advantages, the alcohol BrAC clamping method has not gained wide application. This might be related to the perceived complexity of the procedure, which in addition to an intravenous route of administration seems to require specific expertise and frequent BrAC-measurements for adaptations of the infusion rate. In addition, although it seems clear that the alcohol clamp will improve the stability of alcohol levels, it has not yet been established how this compares to other procedures, such as two-step prekinetic methods. We describe a new method, based on O'Connor's principles in comparison to the adapted two-step infusion rate method, which had been used previously (van Steveninck *et al.*, 1993; van Steveninck *et al.*, 1996).

METHODS

All of the studies described below were approved by the Medical Ethics Review Board of Leiden University Medical Centre. Both oral and written informed consent were obtained from all participating subjects after protocol approval. All studies were performed in compliance with the law on clinical trials of the Netherlands (WMO).

'Two-step prekinetic procedure'

The alcohol data obtained from eight healthy male and eight healthy female volunteers participating in a drug-alcohol interaction study (CHDR0111 – data on file) were used to evaluate the feasibility of the adapted two-step prekinetic procedure as described by Hartmann (Hartmann *et al.*, 1988). Here, we only report alcohol data from the treatment arm that consisted of alcohol administration alone. On the prestudy day, an infusion of 50 grams of alcohol was administered for one hour (500 mL of 100 $g \cdot L^{-1}$ alcohol solution

in 5% glucose). Breath alcohol concentrations were obtained at t = 0, 30, 55, 75, 90, 120, 180, 240, 300 and 360 minutes. A hand-held Alco-Sensor IV meter was used for BrAC assessments during the study. To avoid an 'overshoot' in the alcohol level, the infusion of alcohol was terminated whenever the level of 1.00 g·L^{-1} was reached.

INDIVIDUAL INFUSION REGIMENS

For each subject, individual pharmacokinetic parameters were determined as follows. Previously, a nonlinear mixed effects population pharmacokinetic model had been built, based on the data from an earlier CHDR study with a similar design (van Steveninck *et al.*, 1996), using nonlinear mixed effect modelling with NONMEM version V software (Beal S.L. *et al.*, 2006). A two-compartment model was used with Michaelis-Menten elimination from the central compartment. V1, V2, V_{max}, K_m and Q (inter-compartmental clearance) were estimated. Exponential models for inter-individial variabilty were used but inter-individual variability for K_m was fixed to zero to avoid numerical problems. An additive residual error model was used to avoid overestimating the importance of low alcohol measurements. Parameters were calculated using first-order conditional estimation (FOCE). These population parameters were used as priors in a Bayesian non-linear regression analysis of the BrAC-levels of the prestudy day, to generate pharmacokinetic parameters. Next, individual infusion regimens were simulated using the Bayes estimates. The regimen that approached and stayed at 0.65 g·L^{-1} was applied to the subsequent alcohol infusion occasions.

This infusion regimen consisted of a constant intravenous administration over one hour (loading phase), followed by a lower constant rate infusion over three hours (maintenance phase). Breath alcohol levels were obtained at t = 0, 60, 100, 150, 180, 210 and 300 minutes after the start of the loading phase. Contrary to Hartmann's original procedure, no further adjustments in infusion rate were made during the infusion period.

'Alcohol clamping procedure'

The O'Connor method (O'Connor *et al.*, 1998) is based upon the theory that for substances with marked saturable elimination in the relevant concentration range (like alcohol), an approximately linear relationship exists between the applied infusion rate and the resulting change in alcohol concentration. When alcohol elimination is fully saturated, it is excreted at a constant (g/min) rate, independent of concentration. Therefore, when the input (g/min) is changed, this will result in a proportional change in alcohol concentration. The change in alcohol level required to achieve the target concentration can then be used to back-extrapolate the infusion rate that corresponds with, and should hence lead to this necessary change. The original BrAC clamping algorithm (O'Connor *et al.*, 1998) consisted of an oral loading dose and BrAC samples, with possible infusion rate adaptations every ten minutes. It was anticipated that an intravenous loading infusion would reduce variability and shorten the time required to reach pseudo-steady state. Additionally, an increase in sampling interval to 30 minutes (after steady state was reached) would improve the feasibility of other pharmacodynamic or functional assessments, as a ten minute interval may interfere with the required measurements. Simulations were performed for a large range of scenarios using alcohol population parameters determined from previous CHDR studies (88128, CHDR0111, CHDR0210 – data on file). These parameters are shown in table 1. The same population model was used as for the two-step prekinetic procedure, but the increase in information from using data from two extra studies, resulted in the possibility to estimate correlations between population parameters and inter-individual variability for K_m as well.

These simulations indicated that the original O'Connor (O'Connor *et al.*, 1998) procedure would result in infusion rate suggestions that would be too large, and that the increase to a 30 minute measurement interval would result in alcohol increases that would be difficult to recover from (even by shutting down the alcohol infusion for the next 30 minutes). As a result, an adapted

regime was empirically determined consisting of a time dependent reduction of the suggested O'Connor infusion rate. This regime is fully described in the Appendix and was implemented in a simple user-friendly Microsoft Excel® spreadsheet (available from the authors on request). During the procedure, online adjustments in infusion rates are made using the BrAC samples as a guideline to achieve a pseudo-steady state alcohol serum level of 0.6 $g \cdot L^{-1}$. This target level was expected to result in saturated alcohol elimination, since K_m was estimated at 0.0452 $g \cdot L^{-1}$. At more than 10 times this concentration (during clamping at 0.6 $g \cdot L^{-1}$), elimination will be saturated, hence complying with the prerequisite for the clamping technique. This new infusion paradigm was tested for its feasibility in a subsequent study.

Clamping study

Six healthy male and six healthy female volunteers were recruited for this alcohol clamping study, where alcohol was infused for five hours. BrAC samples were taken at baseline and every five minutes for the first 30 minutes after the start of the infusion, every ten minutes for the next 30 minutes and then every half hour until the end of the study. Again, the hand-held Alco-Sensor IV meter was used for BrAC sampling. Since product specifications for this apparatus state that sampling intervals shorter than five minutes cause fatigue of the BrAC sensor, two different measurement devices were alternated during the clamping study.

The clamping study was performed in a randomised, double-blind and placebo-controlled fashion. The intravenous alcohol infusion (alcohol 10% w/v solution in glucose 5% or placebo (glucose 5%)) took place for five hours in total. An infusion assistant, who was not a member of the study team, was made responsible for the BrAC measurements and the execution of the clamp, to maintain blinding during the study. During the whole study day, volunteers performed a battery of both cognitive and psychomotor CNS tests between sampling time points. The results of these pharmacodynamic assessments will be described elsewhere.

SPREADSHEET-BASED PROCEDURE

In advance of a study occasion, the study statistician prepared individual computer spreadsheets, according to a randomisation schedule. For alcohol occasions, this spreadsheet contained a blank 'measurement column', in which the measured BrAC-values were entered by the infusion assistant. Based on these results, the spreadsheet calculated the new infusion rate. For placebo occasions, the spreadsheet contained a separate column with 'sham' BrAC-values, determined from kinetic simulations. At each protocol time, the infusion assistant entered the corresponding 'sham' value into the measurement column. This value was used by the spreadsheet to calculate a new 'sham' infusion rate, which was subsequently applied. In this way, operations during the alcohol clamping and sham procedures were exactly similar for the subject.

Alco-Sensor IV Intoximeter

Two Alco-Sensor IV Intoximeters (Honac, Apeldoorn, the Netherlands) were used for BrAC measurements during the studies. The Alco-Sensor utilizes an electrochemical sensor (fuel cell) to quantify alcohol in the provided sample. Prior to the study, the reliability of the BrAC devices was confirmed in a validation study. Twelve subsequent samples of a 1.08 $g \cdot L^{-1}$ calibration gas (taken at t = 0, 10, 20, 35, 55, 115, 175, 235, 295, 355, 415 and 475 min.) resulted in a mean value of 1.07 $g \cdot L^{-1}$ (95% CI: 1.06, 1.08 $g \cdot L^{-1}$) for the first apparatus and a mean value of 1.08 $g \cdot L^{-1}$ (95% CI: 1.07, 1.09 $g \cdot L^{-1}$) for the second apparatus. An important error in the measurement of alcohol levels during the clamping study could be exhaustion of the BrAC sensor. Since, exhaustion of the sensor, which was most likely during the ascending limb of the BrAC curve (where frequent BrAC measurements are required) could result in incorrect alcohol values, two BrAC devices were alternated. The instruments were calibrated prior to the start of the studies.

Subject characteristics

Subjects participating in the two-step prekinetic study were on average 27 years of age (range 18 - 53 years old), had an average weight of 77 kg (range 53 - 95 kg) and an average height of 175 cm (range 155 - 187 cm). Subjects participating in the alcohol clamping study were on average 22 years old (range 18 - 39 years old), had an average weight of 74 kg (range 56 - 89 kg) and an average height of 173 cm (range 161 - 195 cm). All subjects participating in the previous alcohol studies (88128, CHDR0111, CHDR0210 – data on file) used to construct the pharmacokinetic model were on average 24 years of age (range 18 – 53 years old), had an average weight of 77 kg (range 53 - 97 kg) and an average height of 180 cm (range 155 – 194 cm). All subjects were occasional users of alcohol.

Safety

To prevent local pain in the beginning of the alcohol infusion, an additional diluting glucose 5% infusion (100 $mL \cdot hr^{-1}$) was given to all participants, during the first ten minutes after the start of the alcohol infusion over the same infusion line. No further specific safety measurements were planned for both the alcohol clamping procedure and the two-step prekinetic procedure. During both studies, alcohol was infused to a level of 0.6 $g \cdot L^{-1}$, just above the legal limit of 0.5 $g \cdot L^{-1}$ for adult motor vehicle drivers in the Netherlands.

RESULTS

Subjects

During the prestudy day of the two-step prekinetic procedure, the infusion of one of the subjects was stopped 38 minutes after the start of the alcohol infusion, because BrAC exceeded 1.0 $g \cdot L^{-1}$ and concomitant nausea and inebriation occurred. The available data from this subject were used for the analyses, and for the determination of well-tolerated infusion regimens for the 'pseudo-steady state' part of the two-step procedure.

In the clamping study, one subject dropped out after the first occasion for personal reasons. This subject was replaced by another volunteer receiving the same order of alcohol/placebo administration. Another subject repeated his first occasion because he had hardly slept the night before the study occasion, despite instructions to maintain a regular diurnal rhythm during the study period. Both repeated occasions were used for the pharmacokinetic analysis. All other subjects completed the studies according to protocol.

Two-step prekinetic procedure

The average BrAC-profiles over time for both steps of the two-step prekinetic procedure are presented in figure 1. A large variability in BrAC values was observed after the fixed intravenous alcohol dose in the prekinetic occasion. Individual peak BrAC levels ranged from 0.71 $g \cdot L^{-1}$ to 1.71 $g \cdot L^{-1}$. During the loading phase of the pseudo-steady state occasions, the maximum initial overshoot reached up to 0.93 $g \cdot L^{-1}$, which is an excursion of almost 50% above the target value. A slight, but clear declining trend in average BrAC level could be observed during the maintenance phase. During this period BrAC values ranged from 0.56 to 0.63 $g \cdot L^{-1}$. A 'plateau phase' could be observed between 100 and 210 minutes post-dose. During this part, BrAC values were 9.7% below the desired concentration on average.

Alcohol clamping procedure

The average BrAC profiles of subjects participating in the alcohol clamping study are presented in figure 2. The 0.6 $g \cdot L^{-1}$ pseudo-steady state level was reached within 25 minutes after the start of the alcohol infusion. The intravenous loading regimen did not produce an overshoot as could be observed from the figure. A pseudo-steady state phase can be observed between 25 and 300 minutes. During this period, BrAC values ranged from

0.55 to 0.65 g·L^{-1}. On average, the clamping procedure slightly exceeded its target level by 1.0% during the plateau phase. The graph also shows that the stability of the clamp was not affected by the simultaneous performance of the pharmacodynamic test battery.

The applied infusion rates during clamping may serve as an indirect parameter for alcohol clearance. This parameter can be used to compare alcohol metabolism between certain subgroups. Differences in alcohol metabolism between sex and race or changes in alcohol pharmacokinetics due to drug-alcohol interactions can be assessed and quantified, using infusion rates as a marker. In this study the mean individual infusion rates during the plateau phase were somewhat higher for males than for females (143.5 mL·hr^{-1} (SD: 36.1) and 108.3 mL·hr^{-1} (SD: 23.7) respectively), but the difference did not reach significance in this small group (p = 0.08).

Comparison of the alcohol clamping procedure and the two-step prekinetic procedure

To compare the two different infusion regimens the mean BrAC level during the relevant steady state interval and the coefficient of variation were calculated. During the two-step prekinetic procedure (target level 0.65 g·L^{-1}) a mean BrAC of 0.59 g·L^{-1} (95% CI: 0.54 - 0.63 g·L^{-1}) was found. The mean BrAC of the novel alcohol clamping procedure (target level 0.60 g·L^{-1}) was 0.61 g·L^{-1} (95% CI: 0.58 - 0.63 g·L^{-1}). The calculated coefficient of variation of the clamping method (6.2%) was more than twice as low as the two-step prekinetic procedure (14.6%). Thus, the novel clamping paradigm resulted in more accurate pseudo-steady state levels of alcohol compared to the mean BrAC during the two-step prekinetic procedure, with two-fold lower variability.

Adverse events

No serious adverse reactions occurred during either study. The most frequently reported adverse events included inebriation, a painful arm at the start of the infusion, sleepiness and headache (see table 2). These symptoms were all transient and mild in severity.

DISCUSSION

The two-step prekinetic procedure resulted in a substantial overshoot prior to the maintenance phase, and a declining plateau phase entirely located below the desired target level. Some fluctuations were expected since online corrections were not implemented in this method. Despite these inaccuracies, the adapted paradigm was able to maintain serum levels of alcohol that were on average relatively stable. The clamping method did not require prior determination of individual disposition parameters and was able to keep constant alcohol levels over an extended period of time. Online adjusted BrAC levels could easily be targeted to a preset target level and showed very little interindividual variability. The higher variability obtained with the two-step prekinetic procedure might in part be attributable to the interoccasion variability related with the study design of the two-step procedure. In this respect, the design of the alcohol clamp study appeared to be superior to the two-step design of the prekinetic procedure.

A less obvious, but potentially important advantage of the clamping method is that if a drug modifies alcohol kinetics, the BrAC guided method will instantly adapt. This will improve the safety and tolerability of drug-alcohol interaction studies. Using applied infusion rates and BrAC assessments, alcohol kinetics can still be determined; but a much simpler comparison of alcohol metabolism can be obtained from the total amounts of infused alcohol that were needed to maintain pseudo-steady state BrAC-levels.

Contrary to O'Connor's originally described procedure, the clamping study presented here used an intravenously administered loading dose. Although it was not investigated in this study, it is likely that intravenous loading will cause less variability than oral loading because between subject variability in absorption is removed. Two other practical advantages of the

new alcohol clamping method compared to the one originally proposed by O'Connor are its user-friendliness and the incorporation of wider inter-sampling periods. By converting the paradigm (see Appendix) electronically into an easy-to-handle spreadsheet, the procedure can be executed by anyone able to modify an infusion pump and use a breath alcohol meter, after a short introduction. Furthermore, reducing the number of BrAC samples increased the time between two sequential BrAC sampling moments. This allows for the performance of other measurements, which makes the clamp more suitable for pharmacodynamic alcohol effect or interaction studies. This study showed that a battery of pharmacodynamic CNS measurements could be integrated in the course of the clamp without affecting the stability of the target level.

Since the clamping method is based on empirical principles it is not necessarily state of the art. Systematic procedures like target-controlled infusions or physiology-based pharmacokinetic methods may result in more accurate alcohol concentrations over time. The procedure described by Ramchandani, based on precalculation of infusion rates, probably approaches this more closely (Ramchandani *et al.*, 1999). However, these state of the art approaches are rarely implemented in practice, because of the perceived complexity during execution. The clamping procedure presented here, is a simple to implement procedure that allows everyone to use it with good results.

Future studies should focus on further optimisation of the alcohol clamp. Both over- and undershoots that appear particularly during the transition from the ascending (loading) limb to the plateau (maintenance) phase of the BrAC curve can be minimised by individualisation of the initial infusion rates during the first ten minutes. The initial fixed infusion rates for the first ten minutes, could for example be replaced by infusion rates that are based on individual demographic characteristics (e.g. gender, age, height and weight). More research is needed to find out if the clamping method is also able to produce stable BrAC levels for longer periods than the five hours used in this study, or at different target BrAC levels.

In conclusion, the novel alcohol clamping paradigm was an accurate, user-friendly method, with low variability, able to maintain constant alcohol levels for hours. The current paradigm shows an opportunity to perform intensive pharmacodynamic or functional assessments during the execution of the clamp, which could be of great value for future studies of alcohol.

FIGURE 1 RESULTS OF THE 'TWO-STEP PROCEDURE'

Left: Average BrAC-profile of the participating subjects during the prekinetic occasion and their SD's. Minimum and maximum values are also presented. The straight vertical line marks the end of the one-hour infusion period. Right: Average BrAC-profile of the participating subjects during the pseudo-steady state experiment and their SD's. Minimum and maximum values are also presented. The area between the two straight vertical lines at t=0 and t=60 minutes marks the 'loading phase'. The area between the two straight vertical lines at t=60 and t=240 minutes marks the 'maintenance phase'.

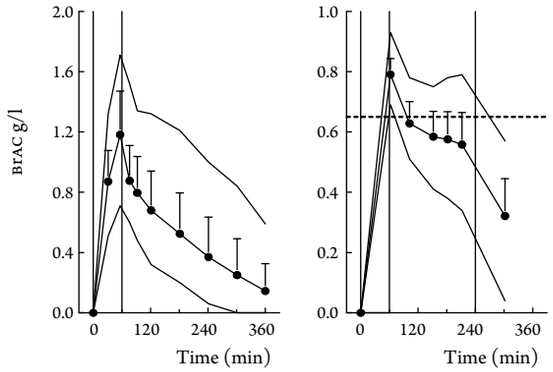

FIGURE 2 AVERAGE BrAC-PROFILE OF THE PARTICIPATING VOLUNTEERS DURING ALCOHOL CLAMPING PROCEDURE WITH THEIR SD'S

Minimum and maximum values are also presented. The vertical lines mark the start and the stop of the infusion.

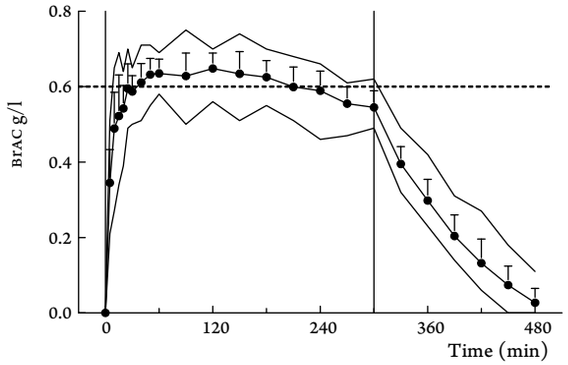

TABLE 1 NONMEM ALCOHOL POPULATION PARAMETERS

Mean: population average, IICV: inter-individual coeffcient of variation. These population parameters are based on 68 subjects and 6052 BrAC samples.

	mean	**IICV**	**between subject correlations**			
			V_c	V_p	K_m	V_{max}
V_c (L)	13.6	51%				
V_p (L)	36.0	36%	-0.13			
K_m (g·L^{-1})	0.0452	46%	-0.56	-0.49		
V_{max} (g·L^{-1}·min^{-1})	0.0117	49%	-0.90	0.34	0.51	
Q (L·min^{-1})	1.22	25%	-0.35	0.81	-0.50	0.50
SD residual error (g·L^{-1})	0.035					

TABLE 2 DISTRIBUTION OF ADVERSE EVENTS DURING BOTH EVALUATED METHODS

The numbers represent the occurrence of the symptoms as a percentage of the total number of adverse events per study.

	'Clamping method'		**'Two-step prekinetic procedure'**	
	alcohol	placebo	alcohol	placebo
"inebriation"	22%	3%	26%	3%
"painful infusion"	22%	3%	8%	0%
"sleepiness"	16%	3%	20%	16%
"headache"	8%	3%	8%	9%

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ALCOHOL CLAMPING PARADIGM

Alcohol was infused for a total of five hours with a set point of 0.6 $g \cdot L^{-1}$.

BRAC measurement were obtained at 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 390, 420 and 480 minutes postdose.

Infusion rate between 0 and 5 minutes was fixed at 72 $g \cdot hr^{-1}$.

Infusion rate between 5 and 10 minutes was fixed at 60 $g \cdot hr^{-1}$.

Infusion rates were modified at 10, 15, 20, 25, 30, 40, 50, 60, 90, 120, 150, 180, 210, 240 and 270 minutes.

Regression equations of applied infusion rate vs. resulting change in alcohol concentration were calculated for all available pairs of data (i.e. starting with the measurement at 5 minutes) and updated with each new data point, up to 270 minutes.

If a negative infusion rate is suggested by the regression equation (e.g. to correct for an overshoot), then the infusion rate was set at 1.0 $g \cdot hr^{-1}$ (the lowest possible rate on the infusion apparatus).

At times (especially at early sampling points) it is possible for the regression equation to arrive at a negative slope. This means that the equation suggests that in order to increase the concentration, less alcohol needs to be infused. This anomaly was corrected by replacing the regression equation in this case by a population average equation, determined using a large number of simulated regression equations. The population average equation was: *new_rate = (required_rate_of_change + 0.013) / 0.06*.

In order to correct for the suggested infusion rates, they were reduced in a time dependent manner:

- at 10, 15, 20, 25 and 30 minutes the correction percentage was 75%,
- at 40 minutes the correction percentage was 70%,
- at 50 minutes the correction percentage was 60%,
- at 60 minutes the correction percentage was 50%,
- from 90 minutes up to 210 minutes the correction percentage was 45%,
- (a correction of 75% implies that the suggested rate was reduced by 25%).

If, after all these adjustments, rates were still too high, the following maximum time-dependent rates were applied:

- from 10 to 30 minutes (not incl.) the maximum allowed rate was 144 $g \cdot hr^{-1}$,
- from 30 to 60 minutes (not incl.) the maximum allowed rate was 72 $g \cdot hr^{-1}$,
- from 60 minutes onwards the maximum allowed rate was 36 $g \cdot hr^{-1}$.

The average total amount of infused alcohol is approximately 75 grams when a pseudo-steady state of 0.6 $g \cdot l^{-1}$ is achieved for five hours. This corresponds to the consumption of less than one bottle of wine, over that same time period.