CHAPTER 7

Comparison of the pharmacokinetics and effects of alcohol on objective and subjective biomarkers between healthy adolescents and adults

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ABSTRACT

Although the acute effects of alcohol consumption on the central nervous system (CNS) have been studied extensively in adults, these effects have not been studied in adolescents. It is likely that the effects of alcohol reported in adults cannot simply be extrapolated to adolescents, as animal studies have shown that adolescent and adult animals have different sensitivities to alcohol. Here, we used a pharmacokinetics-pharmacodynamics (PK/PD) modeling approach to compare the objective and subjective responses to alcohol between adolescent and adult subjects. The acute effect of consuming a socially accepted dose of alcohol (two standard units) was determined in 16-18-year-old adolescents. Blood alcohol concentration was measured non-invasively using end-expired breath samples. A PK/PD model was then developed by combining the data obtained from this study in adolescents with data obtained from previous alcohol studies performed in adults. This model was used to characterize alcohol's pharmacokinetics and effects on an objective biomarker and a subjective biomarker and to explore potential sources of variability, including age. A two-compartment structural model with first-order absorption and Michaelis-Menten elimination provided the best description of estimated plasma alcohol PK.

Inter-individual variability was identified for several kinetics parameters, with lean body weight-dependent variability in peripheral compartment volume and maximum elimination, weight-dependent variability in central compartment volume, and height-dependent and age-dependent variability in intercompartment clearance. The relationship between alcohol concentration and the effect on baseline smooth pursuit performance and the Visual Analogue Scale (VAS) Alertness score was described best as a dose-dependent effect without indications of delay or tolerance. Higher baseline performance in smooth pursuit was correlated with a larger absolute decrease in performance. No covariates were identified for the relationship between alcohol concentration and effect with respect to baseline smooth pursuit performance or VAS Alertness score. Whether sensitivity to other alcohol-related pharmacodynamics effects changes with age remains to be determined.

Introduction

Ethyl alcohol (ethanol; referred to hereafter as simply 'alcohol') is the most commonly used recreational compound among adolescents[1-3]. In most Western countries, adolescents experiment with alcohol, and alcohol consumption usually becomes 'normal' during adolescence. In 2009, approximately 85% of 15-16-year-old Dutch adolescents reported having consumed alcohol, and more than 60% were current users[2,4]. Concerns regarding the deleterious effects of early or excessive alcohol consumption on brain development and the increased risk of alcohol abuse in adulthood has led to a plethora of experimental animal studies and observational human studies of adolescents with alcohol abuse/dependency or binge drinking (for reviews, see[5-7]). However, although a small subpopulation of adolescents use alcohol with high frequency and are exposed to the risks of heavy drinking, most adolescents ultimately establish a drinking pattern that is considered socially acceptable[2,8-10]. Although adolescents cannot legally purchase alcohol in the Netherlands, alcohol consumption by adolescents is – in itself – not prohibited by Dutch law. Nevertheless, the social and legal acceptability of moderate underage alcohol consumption has no scientific basis, as the functional effects of alcohol in adolescents have not been investigated in a placebo-controlled study.

The effects of acute alcohol consumption on the central nervous system (CNS) have been quantified extensively in adults, and consistent effects on tests that evaluate divided attention and visuomotor control have been reported after a relatively low dose (e.g., BAC <0.5 g/L)[11]. Adolescence is a period of intense development, and animal studies have revealed differences in sensitivity to the acute effects of alcohol between adolescents and adults[12-26]. Therefore, it is conceivable that the effects of alcohol that have been reported in adults cannot simply be extrapolated to adolescents. If human adolescents and adults also have differential sensitivities to the acute effects of alcohol, adolescents may experience functional cognitive impairments at lower alcohol doses than adults, which is a serious issue given the substantial cognitive demands that adolescents face in school and early in their developing careers.

In addition, because motor impairment and sedation are direct effects of moderate alcohol intake[14], lower sensitivity to these effects in adolescence may contribute to increased alcohol use among this age group[27]. Conversely, if adolescents develop severe ataxia or sedation after only a relatively low dose of alcohol, this could impair their ability to ride a bike or drive a car safely. Thus, it is important to determine whether ingesting a 'socially acceptable' quantity of alcohol affects the nervous system with a different time course in adolescents than in adults.

Here, we studied the effect profile of consuming two standard alcohol units by 16-18-year-old adolescent subjects. The acute effects of alcohol were determined using a limited number of well-characterized, sensitive biomarkers[11], and the effects of alcohol on the autonomic nervous system were assessed by measuring systemic blood pressure and heart rate. To determine the correlation between the measured effects and alcohol concentration, blood alcohol concentration was measured non-invasively using end-expired breath samples. A pharmacokinetics-pharmacodynamics (PK/PD) model was developed by combining the data obtained from this study in adolescents with data obtained from alcohol studies in adults performed previously by our research group[28-35]. The purpose of this model was to characterize alcohol pharmacokinetics and alcohol's effects on one objective biomarker and one subjective biomarker and to explore potential sources of variability, including age. Objective and subjective biomarkers for PK/PD modeling were selected based on an exploratory meta-analysis of all relevant alcohol data.

Methods

Clinical trial in healthy adolescents

SUBJECTS

Healthy male and female subjects aged 16-18 years were included. The subjects had to be non smokers. They had to be current users of alcohol (i.e., use of at

least 4 units during the month preceding study participation), but were not allowed to use on average more than 7 units alcohol per week. After signing informed consent (in case age < 18 years, also by parents or legal guardian), subjects were medically screened within three weeks prior to study participation and excluded in case relevant clinical abnormalities were found. Use of medications and compounds known to affect CNS performance (including nicotine, xanthines, drugs or alcohol) were not allowed and were screened during screening and prior to each study day. Ethical approval of the study protocol was obtained from the Central Committee on Research involving Human Subjects, the Netherlands.

STUDY DESIGN

This was a randomized, double-blind, placebo-controlled, two-way crossover study of 16 healthy, adolescent subjects with a wash-out period of at least 3 days. Prior to the study days, subjects were instructed to remain fasted from midnight. Smoking and the use of alcohol and xanthine-containing foods or beverages were not allowed during the study days. A standardised light breakfast and lunch were offered at approximately 1 hr prior and 2.5 hr after alcohol intake respectively. Water was allowed ad libitum. Subjects remained in house until 5 hr after alcohol intake.

The sample size of 16 was calculated (two-sided test, alpha = 0.05) for randomization using Williams Squares. This sample size was estimated to have 80% power of detecting a difference in means of -4.6 points for smooth pursuit eye movement assuming a standard deviation of differences of 6.1 (as found in a previous ethanol study by our group in which an effect of -3.9 points was found for an ethanol level of 0.3 g/L).

INTERVENTIONS

All subjects received 2 beverages (200 ml each) containing an oral dose of approximately 10 gram ethanol each (e.g., appropriate amounts of Malibu Coconut Rum, a rum with natural coconut extract with an alcohol percentage by volume of 21.0%, mixed with coconut milk and orange juice) or placebo (e.g., coconut milk mixed with orange juice and vanilla aroma) on different study days. The total alcohol dose of 20 gram was anticipated to lead to a peak blood concentration of 0.3 g/L. Intervention arms were made as comparable as possible regarding expectancy, sensory effects and the presence of biologically active substances other than ethanol. To avoid confounds from circadian variability, alcohol was administered at the same time of day in all subjects. Subjects were instructed to drink one beverage at a time during a 5 minute interval.

PHARMACOKINETICS

Breath alcohol concentrations (BrAC) were determined through a breath test, using a hand-held Alco-Sensor IV meter (Honac, Apeldoorn, The Netherlands). Subjects were instructed how to properly use the device during a training session and 2 breath tests were taken prior to alcohol or placebo intake. To eliminate residual alcohol in the mouth, subjects were instructed to rinse their mouths thoroughly with water directly after alcohol intake. The first breath test after intake was taken at 17 minutes after alcohol intake to avoid residual alcohol contamination of the mouth despite mouth washing. Breath tests were taken every 20 minutes (until 1 hour after intake), every 30 minutes (until 2 hours after intake) and then at hourly intervals (until 5 hours after intake).

PHARMACODYNAMICS

The NeuroCart is a test battery of sensitive tests for a wide range of CNS domains that has been developed at the Centre for Human Drug Research (CHDR, Leiden, The Netherlands) to examine different types of CNS-active drugs. This test battery was incorporated to provide background information on general CNS performance and functional CNS domains, which could be affected by alcohol based on previous findings in adults[11].
All pharmacodynamic measurements (PD) measurements, with exception of the visual verbal learning task (VVLT), were performed at t= 30, 60, 90, 120, 180, and 300 minutes after alcohol intake. The VVLT was administered at approximately 70 and 140 minutes after alcohol intake to assess immediate and

delayed recall. The PD measurements were performed in a quiet room with ambient illumination with only one subject in the room per session. Prior to the first study day, subjects were familiarized with the experimental procedure and given a practice session on the tasks to minimize learning effects during study days. The tests were performed as described below. In addition, a short questionnaire was taken at screening to evaluate the subjects' current perceptions on alcohol use.

ADAPTIVE TRACKING The adaptive tracking test as developed by Hobbs & Strutt, according to specifications of Borland and Nicholson[36] was used. The adaptive tracking test is a pursuit-tracking task in which a circle of known dimensions moves randomly about a screen. The study subject was instructed to try to keep a dot inside the moving circle by operating a joystick. If this effort was successful, the speed of the moving circle was increased. Conversely, the velocity was reduced if the subject could not maintain the dot inside the circle. Performance was scored after a fixed period and the average performance and the standard deviation of scores over a 3.5-minute period was used for analysis.

SACCADIC EYE MOVEMENTS Saccadic peak velocity is one of the most sensitive parameters for sedation[37–39]. The use of a computer for measurement of saccadic eye movements was originally described by Baloh and colleagues[40], and has been validated at CHDR by Van Steveninck and colleagues[39,41].
Recording and analysis of saccadic eye movements was conducted with a microcomputer-based system for sampling and analysis of eye movements. The nystagmo stimulator used for stimulus display is from Nihon Kohden (Nihon Kohden Corporation, Tokyo, Japan), the program for signal collection and the AD-converter from Cambridge Electronic Design (CED Ltd., Cambridge, UK), the amplification by Grass (Grass-Telefactor, An Astro-Med, Inc. Product Group, Braintree, USA) and the sampling and analysis scripts are developed at the CHDR. Disposable electrodes were applied on the forehead and beside the lateral canthi of both eyes of the subject for registration of the electro-oculographic signals. Head movements were minimised with the aid of a head support placed opposite the target. The target consisted of an array of light emitting diodes on a bar, fixed at 50 cm in front of the head support. Saccadic eye movements were recorded for stimulus amplitudes of approximately 15 degrees to either side. Fifteen saccades were recorded with interstimulus intervals varying randomly between 3 and 6 seconds. Average values of latency (reaction time), saccadic peak velocity of all correct saccades and inaccuracy of all saccades were used as parameters. Saccadic inaccuracy was calculated as the absolute value of the difference between the stimulus angle and the corresponding saccade, expressed as a percentage of the stimulus angle.

SMOOTH EYE PURSUIT The same system as used for saccadic eye movements was also used for measurement of smooth pursuit. For smooth pursuit eye movements, the target moves sinusoidally at frequencies ranging from 0.3 to 1.1 Hz, by steps of 0.1 Hz. The amplitude of target displacement corresponds to 22.5 degrees eyeball rotation to both sides. Four cycles are recorded for each stimulus frequency. The method has been validated at the CHDR by Van Steveninck and colleagues[39,41] based on the work of Bittencourt and colleagues[42] and the original description of Baloh and colleagues[40]. The time in which the eyes were in smooth pursuit of the target was calculated for each frequency and expressed as a percentage of stimulus duration. The average percentage of smooth pursuit for all stimulus frequencies was used as parameter.

DUAL TASK TEST The Dual Task (DT) can be used to measure mental workload, as multiple tasks should produce interference when they compete for the limited capacity resources. During this task, subjects were required to perform two separate tasks, each involving an unrelated mapping of a set of possible stimuli to a set of possible responses[43] in an adapted version of the Pashler's DT[44–46]. This adapted DT consists of 54 trials, with three blocks of 18 trials each. The subject was required to give a response as quickly and as accurately as possible indicating whether the tone is either low or high pitched and which letter is presented with a line below or above it. The subjects were instructed not to postpone their reaction for Stage 1 (S1) up until they knew the response

for Stage 2 (S2). At the end of each block, the subject was provided with feedback of percentage correct responses and mean correct RT for both S1 and S2.

BODY SWAY The body sway meter allows measurement of body movements in a single plane, providing a measure of postural stability and was measured with an apparatus similar to the Wright ataxiameter[37,47]. With a string attached to the waist of the subject, all body movements over a period of time were integrated and expressed as mm sway on a digital display. Before starting a measurement, the subjects were asked to stand still and comfortable, with their feet approximately 10 cm apart and their hands in a relaxed position alongside the body. The subjects were instructed to keep the eyes closed.

VISUAL VERBAL LEARNING TASK The visual verbal learning test (VVLT) is a comprehensive memory task for immediate and delayed recall and contains three different subtests that cover merely the whole scope of learning behaviour. Subjects were asked to complete a training version of the VVLT within three weeks before study start. The subjects were presented 30 words in three consecutive word trials at approximately 75 minutes after alcohol/placebo administration, i.e. word learning test (VVLT30). Each trial ended with a free recall of the presented words, i.e., immediate recall, to determine acquisition and consolidation of information. Approximately two hours after start of the first trial, the subjects were asked to recall as many words as possible, i.e. delayed recall, to measure active retrieval from long term memory. Immediately thereafter, the subjects underwent a memory recognition test, which consisted of 15 presented words and 15 'distractors', i.e. delayed recognition, to test memory storage. The subjects were not allowed to write words down at any time during the whole test procedure.

VISUAL ANALOGUE SCORES At various times, the subject indicated (with a mouse click on the computer screen) on sixteen horizontal Visual Analogue Scales how he/she felt. From these measurements, three main factors were the calculated as described by Bond and Lader[48]: alertness (from nine scores), contentedness (often called mood; from five scores), and calmness (from two scores). To make the VAS more age-appropriate, the words 'incompetent' and 'recalcitrant' in the frequently used Dutch translation of this VAS were replaced by 'onbekwaam' and 'tegendraads' respectively. In addition to this, a separate 100 mm-line was added, asking the subject to indicate 'how large is the effect of alcohol that you feel' (alcohol effect)?

BLOOD PRESSURE AND HEART RATE Automated oscillometric blood pressures were measured using a Nihon-Kohden BSM-1101K monitor, a Colin Pressmate BP 8800 or a Dash 4000 monitor. Pulse rates were determined by oscillometry.

Alcohol studies in adults

Pharmacokinetic and selected pharmacodynamic data from several alcohol studies in adults previously performed by our research group were included in the PK/PD model (see Table 1 for an overview). In these studies, the alcohol clamping method (according to the methods of Zoethout and colleagues[49]) was used in which the infusion rate is adjusted according to the estimated blood alcohol concentration. All studies were performed in healthy adults, with exception of one study in adult patients with essential tremor[30]. In all studies, subjects with a history of ethanol abuse were excluded from the study. In most studies subjects who drank regularly (up to 3-4 alcoholic beverages a day) were allowed to participate. In three studies[28,29,31] familiarity with the use and effects of ethanol was required for inclusion of a subject. All subjects had a normal body mass index (BMI).

Statistical analysis and pharmacometrics

PHARMACODYNAMICS

The pharmacodynamic end-points of the adolescent study were analysed by mixed-model analyses of variance (using SAS PROC MIXED) with treatment,

study day, time and treatment by time as fixed effects, and subject, subject by treatment and subject by time as random effects and the average baseline value as covariate. Contrasts were estimated within the overall treatment effect and contrasts between treatments over 120 min and 300 min were calculated within the statistical model. Body Sway and VAS alcohol scores variables were analysed after log-transformation and back-transformed after analysis (results may be interpreted as percentage change). VAS alcohol scores were log transformed (10log) after 2 was added to each score to avoid log transformation from zero. VVLT parameters were analyzed by mixed model analysis of variance with treatment and study days as fixed factors and subject as random factor. The statistical hypothesis was 'there is no difference between alcohol and placebo'.

PHARMACOMETRICS

DATA PK data from study days in which only alcohol was administered of the adolescent study and several adult studies (see Table 1) were used. Exploratory individual and summary concentration-time profiles were generated to identify potential outliers, understand the influence of censoring concentrations below the limit of detection (BLOD) and give indications regarding the base structural model. As the device reports a value of zero when alcohol breath concentrations are BLOD, all PK observations with a value of zero after plasma peak concentrations were removed from the PK dataset. Blood alcohol concentrations were calculated according to the specifications of the manufacturer (using ratio (2300:1) between blood- and breath ethanol concentrations) and used to develop the PK model.

MODELING STRATEGY Pharmacometric analyses were performed using nonlinear mixed effect modeling (NONMEM version 7.2.0 (Beal). First order conditional estimation with interaction (FOCEI) was used for estimation with a convergence criterion of 5 significant digits in the parameter estimations. NONMEM reports an objective function value (OFV) which is the -2 times log likelihood (-2LL). Model comparison testing was done using the likelihood ratio test under the assumption that the difference in -2LL is Chi-square distributed with degrees of freedom determined by the number of additional parameters in the more complex model. Hence, with a decrease in OFV of at least 10.8 points the model with one additional parameter is considered superior over its parent model ($p<0.001$). Different models with increasing complexity were compared to find the simplest model that described the data adequately. Graphical analysis was used to assess model performance during model development. The goodness of fit plots used included: observed concentration (dependent variable, DV) versus population predicted concentration (PRED) and versus individual predicted concentration (IPRED); weighted residuals (CWRESI) versus IPRED and versus time; combined PRED, IPRED and DV versus time, per individual, and distribution of interindividual variability (ETA). Covariate analysis was performed using a stepwise approach. Selection of the best PK- and best PK/PD models was based on the likelihood ratio test, diagnostic plots, visual predictive check (VPC) and precision in parameter estimates. Calculation of the relative standard error (RSE) was used to derive the uncertainty in the parameter estimates and was considered acceptable when less than 10%. NONMEM input file preparation and processing (tables and graphs) of the model results was performed using R version 2.12.0 (V2.12.0, R Foundation for Statistical Computing, Vienna, Austria, 2010).

POPULATION PK MODEL DEVELOPMENT First, a population PK model for oral and intravenous alcohol was developed. One and two compartment structural models, and different compartmental and elimination submodels were tested. All models used a first order process to describe the oral absorption of alcohol in the adolescent data set. Inter-individual variability (IIV) was assessed separately on each of the PK parameters using a stepwise bottom-up approach. Correlations between the IIV of the various parameters were graphically explored. When correlations were significant, either by shape or Piersons correlation coefficient, covariance between the terms was assessed by application of an omega block on selected parameters and accepted based on the likelihood ratio test. For the parameter estimation, shrinkage was considered

acceptable when below 30%[50]. Proportional, additive, and combined error structures were evaluated to best describe the residual error. After graphical identification, the most promising covariates were tested in the model and included based on decrease in OFV in a stepwise manner (forward inclusion of covariates, followed by a backward elimination step). All covariates were implemented in normalized power function (Eq. 1), where the normalization values for LBW, WGT, AGE and HGT were 60, 70, 30 and 1.75 respectively.

$$\theta i = \exp(\theta TV + \varepsilon) \times ([COV]i / [COV]n) \wedge (\theta cov) \quad \text{Eq. 1}$$

where θi; individual parameter estimate, θTV; typical (population) value, ε; interindividual variability, *COVi*; individual covariate value, *COVn*; normalization value for covariate, θCOV; parameter estimate for the exponent.

PK/PD MODEL DEVELOPMENT After development of the PK model, exploratory PD and PK/PD profiles were generated to identify the most suitable objective- and subjective PD parameter for development of a PK/PD model. Only PD parameters that were measured in at least two different studies and more than once on a single occasion were included. Selection of PD parameters for PK/PD model development was based on strong response to alcohol and indications of presence of a direct relationship between alcohol concentration and effect. For the development of the PK/PD model, all PK parameters were fixed to the individual parameter estimates of the best PK model. The simulated individual plasma alcohol concentration time profiles were used in the exploration of linear- and exponential concentration-effect relationships, to best describe the observed PD response. Model development was performed as described for the best PK model (e.g. incorporation of IIV, residual error and covariates). Model description of the data was considered acceptable if time-, concentration- or performance-dependent bias seemed absent.

Results

Clinical trial in healthy adolescents

SUBJECTS

In the adolescent study, a total of 17 subjects (8 males and 9 females) 16-18 years of age were enrolled in 2010 and 2011. All of the subjects attended pre-university secondary education (vwo, Voortgezet Wetenschappelijk Onderwijs). One of the female subjects discontinued the study because of a migraine experienced following the first study day (an alcohol occasion); this subject was replaced by another female subject. Three of the female subjects used an oral contraceptive; other medications used during the study included levocetirizine 5 mg p.o. as needed (by one subject; stopped two days before the first study occasion), paracetamol 500 mg p.o. 2-6 times daily (by 1 subject during the first study occasion), terbinafine cream 10 mg/g and terbinafine 250 mg p.o. once daily (by one subject during both study occasions). The mean alcohol consumption by the subjects in the months preceding the study was 3 standard units per week (range: 1-6 units per week). The reasons cited by the subjects for drinking alcohol included: they liked it (n=13); 'it is socially enjoyable' (n=14); and it reduces inhibition (n=2). None of the subjects reported that they drank alcohol because their friends also drank alcohol or to become drunk. The subjects either had never been drunk (n=6) or were drunk only occasionally (n=11); nine subjects reported an occasional black-out episode after consuming alcohol. Most of the subjects considered the short-term effects of alcohol (n=7) or the dangers of alcohol consumption (n=11). The majority of subjects (n=11) reported that they considered the long-term effects of alcohol only when pointed out by others.

The mean alcohol dose in the study was 0.29 g/kg (range: 0.24-0.34 g/kg) for males and 0.31 g/kg (range: 0.27-0.38 g/kg) for females. After ingesting the alcohol or placebo on the study day, the subjects were asked which of the two they thought they had received. After ingesting the placebo, 13/16 subjects believed they had taken the placebo, and the other three did not know whether they

had taken placebo or alcohol. After alcohol intake, 14/17 subjects believed they had taken alcohol, two believed they had taken the placebo, and one did not know.

PHARMACODYNAMICS RESULTS

The pharmacodynamics results are summarized in Table 2. Least Square Means graphs of the parameters that differed significantly between alcohol and placebo are presented in the Supplemental Data.

PHARMACOMETRICS

An overview of the demographics for the studies from which data were used to develop the model is presented in Table 3.

PK MODEL DEVELOPMENT A total of 3,112 PK observations were obtained from 27 adolescents (16-18 years of age) and 122 adult subjects (19 years of age and older). In general, the percentage of PK data that was below the level of detection was <15%, except for the adult study in healthy Caucasian and Japanese volunteers[29] (34%, excluding pre-dose observations) and the adult study with the compound GSK598809[32] (29%, excluding pre-dose observations). In these two studies, PK sampling continued relatively long after ethanol infusion had stopped, and during the last few PK measurements, most of the subjects' alcohol concentrations were below detectable levels. None of the data points between the start of dosing and reaching peak plasma alcohol concentration were below detectable levels. The entire data set from one occasion from one adult subject was excluded because the subject was not 'well-rested', and the occasion was repeated on a later day. Finally, data from four adult subjects were excluded due to the suspected presence of erroneous dosing information.

A two-compartment structural model with first-order absorption and Michaelis-Menten elimination provided the best description of estimated alcohol plasma PK. A schematic representation of the alcohol PK model structure is presented in Figure 3. Parameter estimates, relative standard error (RSE), and IIV are presented in Table 4. A combined residual error structure was identified for the model. IIV could be identified with respect to VM (the maximum elimination rate parameter), V2 (the central compartment), V3 (the volume of the peripheral compartment), and Q (intercompartment clearance). The subsequent addition of an ETA to the Michaelis constant (KM) caused a significant decrease in OFV; however, this decrease (38%) was above the acceptance criterion and this covariate was not incorporated in the model. The investigated covariates included age, gender, height, weight, BMI, and lean body weight (LBW[51]). The following five covariate relationships were implemented in the model: LBW on V3 and VM, weight on V2, and height on Q, and age on Q. For the covariate weight on V2, the exponent was fixed at 1, as the confidence interval of the estimated exponent overlapped 1.0. Backwards elimination was than performed; however, this approach resulted in a significantly poorer model in all cases. Covariance was identified between VM and Q, VM and V2, and V2 and V3.

In the best PK model, the predicted concentrations were accurate and no time-dependent bias was observed (Figure 4 and 5). The Loess regression curve of the conditional weighted residuals (CWRESI) versus individual predicted concentration (Figure 5) suggested a slight bias at higher concentrations (>1.0 g/L); this bias is likely due to the low number of observations at high concentrations (there were only two observations between 1.5 and 2.0 g/L) reducing the reliability of the Loess curve, rather than to model misspecification. NONMEM assumes that ETAs are normally distributed around zero. The estimated ETAs were distributed normally (Figure 6).

Because alcohol was administered orally in only one study (adolescent study), and because this study did not include crossover design with an intravenous group, oral bioavailability (F) could not be estimated reliably by the PK model. The individual parameter estimates of V2, V3, and VM of the subjects in the adolescent study actually represent V2/F, V3/F, and VM /F, which can affect parameter estimation and/or the best model structure. A sensitivity analysis was performed using the data set (excluding the oral data). Removal of the oral data from the analysis did not change any decisions made at key steps in the model development; the resulting best model structure was the same, and

none of the parameter estimates changed significantly. Therefore, we conclude that including oral data – without estimating oral bioavailability – did not have a negative impact on the model development.

POPULATION PK/PD MODEL DEVELOPMENT Based on the exploratory plots of PD and PK/PD, smooth pursuit performance was chosen as the most suitable objective PD measure (3643 data points collected from 24 adolescent subjects and 64 adult subjects), and VAS Alertness (2671 data points collected from 19 adolescent subjects and 68 adult subjects) was chosen as the most suitable subjective PD measure. For these parameters, the exploratory plots suggested a strong, dose-dependent alcohol effect with no placebo effect (Figures 1 and 2). The exploratory plots also suggested relatively large inter-individual differences in subject susceptibility to alcohol's effects on smooth pursuit performance and VAS Alertness.

Smooth pursuit was included as a PD measure in the adolescent study and in four of the adult studies[28,29,32,35], and VAS Alertness was included as a PD measure in the adolescent study and in four of the adult studies[29,31,32,35]. One of the adolescent pre-dose smooth pursuit measurements reflected extremely poor performance (5.2%), which was likely related to a technical or subject attention-related problem; this measurement was therefore excluded from the analysis.

SMOOTH PURSUIT PK/PD MODEL The relationship between alcohol concentration and the effect on baseline smooth pursuit performance was described best as a direct, linear concentration-effect relationship. A concentration-effect relationship proportional to the baseline characterized the effects of ethanol on smooth pursuit performance better than an absolute effect independent of baseline (lower OFV and comparable parameter estimates). Because our exploratory plots revealed no apparent placebo effect on smooth pursuit performance, we used a fixed baseline. We also attempted to estimate a population value for the baseline performance; however, the best results were obtained using the mean performance of the pre-dose smooth pursuit tests as the baseline. Implementing an additional population value did not significantly improve the model ($p>0.05$).
The data were described using the following equation:

$$SMP = BL \times (1-(KE \times C)) \qquad \text{Eq. 2}$$

where SMP is smooth pursuit performance (%), BL is baseline performance (%), KE is effect constant (L/g), and C (g/L) is the alcohol concentration simulated by the plasma PK model.

An additive error structure performed better than a proportional error structure in all models (including the best PK/PD model). A combined error structure did not improve the model and was abandoned. IIV could be identified for the baseline smooth pursuit performance (BL) and the effect constant (KE), with acceptable shrinkage in the ETA on BL and KE (<20%). A covariate analysis of age and gender was performed. Including age as a continuous covariate did not improve model performance. Age was also implemented as a categorical covariate, parsing the subjects into the following three groups: adolescents (16-18 years of age), young adults (19-29 years of age), and older adults (>29 years of age). This approach did not reduce OFV, and the estimated age effect did not differ significantly from 'no effect'. As a result, no covariate was included on the model parameters BL and KE. Implementation of covariance between ETA1 and ETA2 did not improve the model and was therefore not added to the model.

The parameter estimates of the best model are summarized in Table 5. The uncertainty of the parameter estimates was considered to be acceptable. As expected based on the exploratory plots, the IIV of KE was relatively high (60.7%). Both the shrinkage on the ETAs and the additive error were below 20% and were therefore considered to be acceptable.

The ability of the PK/PD model to describe the effect of alcohol on smooth pursuit performance was acceptable (Figures 7 and 8). NONMEM assumes a normal distribution of the ETAs around zero. In the best model, the ETAs follow this assumption, although the distribution of the ETA1 suggests a slight skew (Figure 9).

Because the PK/PD model was developed by sequentially modeling PK and PD, mis-fitting of the PK can cause a bias in the predicted PD (for example, if the ethanol concentrations are overestimated, the predicted effect might be overestimated as well). Therefore, the conditional weighted residuals of the best PK model were plotted against the residuals of the best PK/PD model. We found no indication that the residual error in the best PK/PD model was caused by the residual error in the best PK model (Figure 10).

VAS Alertness PK/PD model

The relationship between alcohol concentration and VAS Alertness score was described best as a direct, linear effect on baseline. An additional concentration-effect relationship characterized the effects of ethanol on VAS Alertness best when compared to a proportional relationship. Because there were no indications of a placebo effect, a fixed baseline was chosen. We tested the following four baseline sub-models: (1) the estimated population value; (2) the occasion-specific mean of the pre-dose measurements; (3) the fraction of the occasion-specific mean of the pre-dose measurements, and (4) the occasion-specific mean of the pre-dose measurements as a normalized, linear covariate on the estimated population value. Of these four sub-models, the fourth performed the best ($p<0.001$) and was therefore implemented in the structural model. A combined error structure provided the best characterization of the residual error in the best model.
The data were described using to the following equation:

$$\text{VAS Alert} = BL - (KE \times C) \qquad \text{Eq. 3}$$

where VAS Alert is the VAS Alertness score (mm), BL is the baseline score (mm), KE is the effect constant (mm*g/L), and C (g/L) is the predicted alcohol concentration.

Removing either the additive or proportional error component resulted in a significantly poorer model ($p<0.001$). Implementing IIV on KE significantly improved the model ($p<0.001$), but because of high ETA-shrinkage (42.7%), the model without IIV on BL was deemed superior. The occasion-specific mean of the pre-dose measurements was used as a covariate on BL during the structural model development. Because none of the covariates showed a particularly strong correlation with the individual parameter estimates (based on Pearson's correlation coefficient and a visual check of the scatterplot), no additional covariate relationships were included in the model.

The parameter estimates are presented in Table 6. The uncertainty of the residual error was rather high, and the other parameters had a reasonable degree of uncertainty. As expected from the exploratory plots, the IIV of KE was high compared to the population estimate of KE. Both the shrinkage of ETA and the error were <20%.

The PK/PD model described the effect of ethanol on VAS Alertness score relatively well, with an absence of both time-dependent and concentration-dependent bias (Figures 11 and 12). The Loess curve in the CWRESI vs IPRED plot suggests a bias towards overestimating the VAS Alertness score at very low scores (<30 mm). However, given the relatively small contribution of these few values in the total number of observations and given the fact that the low CWRESI values seemed to lie within acceptance limits (-2 to 2), this apparent bias was not considered a factor in the model's performance. NONMEM assumes a normal distribution of the ETAs around zero. The IIV of KE followed a normal distribution, although the mean of ETA was not significantly different from zero (Figure 13). Thus, it might not be appropriate to assume a normal distribution of ETA when performing a simulation using this PK/PD model.

Discussion

This is the first study to use a PK/PD modeling approach to compare the objective and subjective responses to alcohol between adolescents and adults. Most previous observational studies of alcohol use in adolescent subjects focused on potential negative effects on the brain and the associated long-term

risks of heavy drinking. Only a few clinical studies investigated the effects of acute alcohol in children and adolescents; however, these studies were not placebo-controlled and included relatively alcohol-naïve subjects[52] or they evaluated responses in adolescents who had (or were at high risk for developing) an alcohol abuse disorder[52,53]. Data from these studies may not have direct implications for how alcohol interferes with psychomotor and cognitive abilities in more common situations, for example when relatively low, socially accepted doses are consumed by healthy adolescents with a limited history of drinking alcohol. In our study, a PK/PD model was developed by combining the adolescent data with adult data obtained from alcohol studies previously performed by our research group in order to investigate whether PK and/or PD has any age-related differences. The clamping method, which was used in previous adult studies, is best suited to evaluating potential sources of variance in PK, including age-related effects. Due to ethics considerations, ethanol clamping could not be performed in adolescents. We anticipated that the accuracy of the clamping method in adults would provide the basis for an accurate PK/PD model, which would be robust enough to accommodate the more variable adolescent data collected after oral ingestion.

The PK model was built using a combination of estimated blood alcohol concentration in adolescents (following oral administration) and adults (using an infusion-based clamping method). A two-compartment structural model with first-order absorption and Michaelis-Menten elimination provided the best description of estimated alcohol plasma PK. The estimated combined volume of the central and peripheral compartments (46 liters) and estimates of elimination (maximum elimination rate and Michaelis constant) were consistent with published values[54–57]. The population parameter estimates of absorption and distribution had low uncertainties. Combining oral (low concentration range) and intravenous (higher concentration range) PK data provided an accurate estimate of the absorption rate, distribution parameters, and Michaelis constant, thereby yielding accurate descriptions of the ascending and descending limbs of the alcohol concentration-time curve (the PK phases in which most of alcohol's effects occur). In contrast, the relative standard error of the population Km value in our PK model was rather high. Because the majority of data was collected during clamping experiments, a relatively low percentage of the data was in the low concentration range, thereby reducing the informative value of the data with respect to estimating Km. In the adolescent study, alcohol was given as a fixed dose of 20 mg rather than being adjusted for weight (or total body water, which might have been preferred[58]). While this approach might reflect common drinking practices, it likely added variability. Interindividual variability was identified for various kinetic parameters, with LBW-dependent variability on peripheral compartment volume and maximum elimination, weight-dependent variability on central compartment volume, and height- and age-dependent variability on intercompartment clearance. Body composition is important for the equilibrium distribution of alcohol between the blood and various body compartments[58]. Because alcohol is distributed into the total body water, differences in age, gender, and body weight can affect alcohol's concentration-time profile[59–61]. In our evaluation, gender per se was not identified as a potential covariate, but gender was factored indirectly into in the calculation of LBW. The covariates that were identified in our PK model may be related – either directly or indirectly – to differences between adolescents and adults, as considerable age-related and maturity-related changes in body composition occur during adolescence, including changes in weight, height, and fat-free mass[62]. Because all of our subjects had normal BMI, differences in the prevalence of obesity between the adolescents and adults could not have accounted for the weight-related variability. The rate of alcohol distribution is dependent on factors that govern peripheral distribution[58]. In our PK model, age-dependent variability was found with respect to intercompartment clearance; specifically, clearance increases with age. Changes in intercompartment distribution can be caused by changes in peripheral circulation (e.g., due to stress), muscle contraction, hormonal changes, vasoconstriction, changes in body and environmental temperature, and circulatory impairments in the cardiovascular system[58]. Moreover, it is conceivable that adolescent subjects have different stress reactions to the testing environment than adults.

However, age-dependent factors unrelated to the testing environment may play a role as well, as equilibration can also reflect the development of other age-dependent processes, including alcohol- or metabolite-related changes in local muscle and/or CNS blood flow.

Although many studies have examined the acute effects of alcohol in adults[11], to date no alcohol PK/PD model has been presented. This might be due to the complex PD of alcohol, as both acute tolerance and a lag in the recovery from alcohol-induced impairment have been observed for several biomarkers[65-68], thus complicating the development of a PK/PD model. In our study, two biomarkers were selected based on an exploratory meta-analysis of several alcohol studies that were performed by our research group. Smooth pursuit performance and VAS Alertness showed a clear response to alcohol with no indications of an indirect effect or acute tolerance. Thus, a relatively simple model would likely describe the data well, and the presence or absence of an age-related effect could be investigated. As expected, the relationship between alcohol concentration and the effects of alcohol on baseline smooth pursuit performance and VAS Alertness score was described best as being dose-dependent, with no indications of delay or tolerance. Higher baseline performance for smooth pursuit was correlated with a larger absolute decrease in performance. The best model accounted for 61% and 13% of the inter-individual variability in smooth pursuit performance and VAS Alertness score, respectively, and the best model predicted the observed performance accurately. No significant covariates were identified, including no clear effect of age. However, VAS alertness and smooth pursuit eye movements may not necessarily represent all of the alcohol-related effects on the CNS, and we cannot exclude the possibility that sensitivity to other alcohol-related pharmacodynamics effects change with age. Age-related differences between adolescents and adults with respect to acute sensitivity to alcohol are believed to reflect PD –rather than PK –factors[72], and these differences may be related to the faster onset of acute tolerance and developmental changes that occur in the neural substrates underlying alcohol's effects. For example, age-related differences in sensitivity and tolerance have been related to age- and brain region-related differences in the expression NMDA and $GABA_A$ receptor isoforms[14]. In our study, smooth pursuit performance and VAS Alertness score were analyzed, as the data were likely to be described best using a simple PK/PD model. Other sensitive functional biomarkers[11] that were included in the adolescent study (for example, adaptive tracking, saccadic peak velocity, and VAS alcohol effect) were less suitable for developing a PK/PD model, as the small effect in adolescents and the high number of non-responders precluded our ability to quantify the adolescent data and evaluate an age-dependent effect. Although a higher dose of alcohol would likely have yielded a quantifiable effect, using a higher dose in adolescents would have been prohibitive from an ethics perspective. Other age-dependent effects may have be seen at higher doses as well, including effects on memory, given that a placebo-controlled study[73] found that 0.6 g/kg alcohol caused significantly more memory impairment in 21-24-year-old subjects than in 25-29-year-old subjects. In addition, adolescents and adults may also differ with respect to the development of acute tolerance to alcohol effects, as has been reported for animals[14]. Tests that evaluate postural stability (for example, body sway) may also reveal an age-dependent effect and may reflect a difference in the threshold to the aldehyde metabolite rather than the alcohol itself. Age-related differences in the PK and/or PD of acetaldehyde, which can mediate physiological responses such as facial redness, pulse rate, and blood pressure[63], may account for some of the differences observed between adolescents and adults[64], although this topic needs further study.

Research into age-dependent differences in the effects of alcohol is potentially complicated by differences in cumulative baseline drinking (affecting PK and/or PD) and age-related differences in motivation and expectations (affecting PD). In all of the adult studies, alcohol was administrated intravenously using the clamping method, which provides precise control over blood alcohol content (and therefore the brain's exposure to alcohol), thus minimizing variation between subjects. Due to ethics considerations, ethanol clamping was not performed in adolescents. The PK model adequately described both the oral and intravenous PK data. Adult subjects were exposed to alcohol longer than

the adolescents were exposed, although the clamp duration had no impact on VAS Alertness or smooth pursuit eye movement. However, it is formally possible that the route of administration can affect the brain's response to alcohol[87]. For example, the brain's functional response to alcohol can depend on both the alcohol concentration and the rate of change of alcohol concentration[88], which can differ depending on the route of administration.

The clear effects revealed by our study (including effects on motor coordination, subjective alertness and intoxication, postural stability, and vital signs) are an insightful illustration of the acute functional impact that healthy adolescents experience following a low dose of alcohol that is generally considered to be socially acceptable. Based on the clear effects measured in our study, the Dutch upper limit for blood alcohol concentration for drivers up to 23 years of age (0.2 g/L) seems justified and should perhaps be lowered to 0.0 g/L, at least for 18-year-old drivers. Importantly, the risk of auto accidents among adolescents is higher than in adults, particularly at low and moderate blood alcohol concentrations[86]. In our PK model an age-dependent variability was found for intercompartmental clearance of alcohol, which may be related to age-dependent factors related to the testing environment or reflect development of post-synaptic age-dependent processes. No covariates could be identified for the relationship between alcohol concentration and effect on baseline smooth pursuit performance or VAS Alertness score. It remains to be investigated if the sensitivity to other pharmacodynamic effects of alcohol does change with age.

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TABLE 1 Overview of adult studies of which data were incorporated in the PK/PD model development.

Ref	Study title	Ethanol treatment(s)	Placebo control for ethanol effect?	PD parameters in study which were also included in adolescent study
33	A Double-Blind, Randomized, Placebo- and Active-Comparator, Controlled, Triple-Dummy, 2-Period Crossover Study to Investigate the Psychomotor and Cognitive Effects of MK-0869 and Ethanol in Healthy Subjects	Constant ethanol infusion of 50 g/hr. Duration of infusion up to 1 hr (infusion terminated when blood alcohol concentrations exceeds 1.2 g/L)	No	N/A*
28	A placebo-controlled study on the effects of a novel method for alcohol infusion by clamping of breath alcohol concentration	Ethanol clamping; 5 hours at blood alcohol concentrations of 0.6 g/L	Yes	Adaptive tracking, Saccadic eye movement, Smooth pursuit
30	A double-blind, double-dummy, randomized, placebo-controlled, 3 period crossover study to investigate the effects of ethanol and L-000830982 on essential tremor	Ethanol clamping; 4 hours at blood alcohol concentrations of 0.6 g/L	Yes	--
29	Ethanol clamping at two levels of breath alcohol concentrations in Caucasian and Japanese subjects	Ethanol clamping; 5 hours at blood alcohol concentrations of 0.3 g/L and 0.6 g/L	Yes	Adaptive tracking, Saccadic eye movement, Smooth pursuit, VAS Bond and Lader, VAS alcohol effect, Body Sway
31	A randomized, double blind, placebo-controlled, double dummy, three-way crossover study to investigate the effects of both an intravenous ethanol clamp and a target controlled morphine infusion on resting state functional magnetic resonance imaging in healthy male volunteers	Ethanol clamping; 2.5 hours at blood alcohol concentrations of 0.6 g/L	Yes	VAS Bond and Lader, VAS alcohol effect
32	GSK598809 and Ethanol-Interaction study	Ethanol clamping; 5 hours at blood alcohol concentrations of 0.6 g/L	Yes	Adaptive tracking, Saccadic eye movement, Smooth Pursuit, VAS Bond and Lader, VAS alcohol effect, Body sway
34	ACT078573 and ethanol interaction	Ethanol clamping; 5 hours at blood alcohol concentrations of 0.6 g/L	Yes	Adaptive tracking, Saccadic eye movement, Smooth Pursuit, VAS Bond and Lader, VAS alcohol effect, Body sway
35	A double-blind, randomized, placebo-controlled, three-way crossover study to investigate the drug-drug interactions of brivaracetam and ethanol in healthy subjects	Ethanol clamping; 5 hours at blood alcohol concentrations of 0.6 g/L	No	Adaptive tracking, Saccadic eye movement, Smooth pursuit, VAS Bond and Lader, VAS alcohol effect, Body Sway, VVLT

Only tasks performed multiple times during a single occasion were included in the exploratory meta-analysis of PD parameters (i.e., VVLT data were not included). *Not applicable, as pre-dose alcohol concentrations (used to determine target level) were used for development of the PK model and therefore no pd was included in the PK/PD model.

TABLE 2 Summary of pharmacodynamic effects of alcohol compared to placebo (adolescent study)

	LS Means		Contrast
Parameter	Placebo	Alcohol	Alcohol vs Placebo Estimates of difference, 95% CI and *p*-value
Body sway (mm)	236.1	276.1	16.9% (5.2%, 30.0%) p=0.0069
Saccadic Inaccuracy (%)	6.22	6.27	0.05 (-0.65, 0.75) p=0.8728
Saccadic Peak Velocity (deg/sec)	454.1	452.5	-1.5 (-9.3, 6.2) p=0.6766
Saccadic Reaction Time (sec)	0.207	0.207	-.001 (-.006, 0.005) p=0.8502
Smooth Pursuit (%)	43.1	42.4	-0.7 (-2.3, 0.9) p=0.3744
Adaptive tracking (%)	26.36	25.48	-0.88 (-2.14, 0.39) p=0.1575
VAS Alertness (mm)	53.4	52.3	-1.1 (-2.4, 0.2) p=0.0879
VAS Calmness (mm)	55.5	55.4	-0.1 (-2.0, 1.8) p=0.9150
VAS Mood (mm)	56.6	57.1	0.5 (-0.5, 1.4) p=0.2946
VAS Alcohol effect (log(mm))	0.329	0.464	0.135 (0.033, 0.237) p=0.0134
Heart rate (beats per minute)	68.5	72.7	4.3 (2.6, 5.9) p<.0001
Diastolic blood pressure (mmHg)	61	61	-0(-3, 2) p=0.6988
Systolic blood pressure (mmHg)	116	113	-3 (-6, -0) p=0.0377
Correct acoustic stimuli at 650 msec	17.7	17.7	0.0 (-0.2, 0.2) p=0.8473
Average RT of visual stimuli (msec)	785.2	802.7	2.2 (-1.1%, 5.7%) p=0.1795
Correct visual stimuli at 650 msec	16.7	16.8	0.1 (-0.2, 0.4) p=0.6275
Correct visual stimuli at 150 msec	17.1	17.1	0.1 (-0.2, 0.3) p=0.5380
Correct visual stimuli at 50 msec	17.2	17.1	-0.1 (-0.4, 0.2) p=0.4435
Average RT of acoustic stimuli at 650 ms (msec)	638.64	644.21	0.9% (-7.3%, 9.8%) p=0.8296
Delayed word recall correct	15.9	15.1	-0.8 (-2.9, 1.2) p=0.3871
Word recognition correct	26.6	26.0	-0.6 (-2.1, 0.9) p=0.3917
Word recognition RT correct (msec)	857.1	837.3	-2.3% (-10.5%, 6.7%) p=0.5798
Word recall correct trial 1	11.0	9.4	-1.5 (-3.6, 0.6) p=0.1453
Word recall correct trial 2	15.9	14.3	-1.6 (-3.8, 0.6) p=0.1460
Word recall correct trial 3	18.8	18.0	-0.8 (-2.3, 0.6) p=0.2382

TABLE 3 Overview of study demographics among studies from which data were used of development of a PK/PD model

Ref	Number of subjects	Gender (male/ female)	Age (years)	Weight (kg)	Height	Study population
33	20	9/11	26.4 ± 9.2	75.1 ± 12.8	1.75 ± 0.08	Healthy adults
28	12	6/6	21.8 ± 6.1	75.5 ± 11.1	1.74 ± 0.10	Healthy adults
30	9	7/2	47 ± 21.3	81.0 ± 10.8	1.75 ± 0.11	Essential tremor patients
29	24	24/0	26.9 ± 5.6	76.1 ± 14.3	1.80 ± 0.09	12 Caucasian and 12 expatriate Japanese healthy adults
31	11	11/0	22.2 ± 2.2	80.8 ± 12.6	1.85 ± 0.08	Healthy adults
32	18	10/8	34.2 ± 14.4	72.4 ± 12.3	1.76 ± 0.09	Healthy adults
34	21	10/11	33.9 ± 15.6	73.9 ± 11.0	1.75 ± 0.08	Healthy adults
35	17	17/0	30.5 ± 8.3	81.7 ± 12.3	1.81 ± 0.07	Healthy adults
Adolescent study	17	8/9	17.1 ± 0.6	66.4 ± 8.6	1.76 ± 0.11	Healthy adolescents
Total	149	102/47	28.4 ± 12.6	75.4 ± 12.5	1.78 ± 0.09	

TABLE 4 Population parameter estimates of final PK model

Population parameters	Estimate [%RSE]	IIV (CV%)
vm (θ_1) (g/hr)	9.21 [3.6]	27.5
Km (θ_2) (g/L)	0.051 [26.2]	NE
v2 (θ_3) (L)	12.1 [3.0]	18.0
v3 (θ_4) (L)	33.8 [2.0]	29.3
Q (θ_5) (hr^{-1})	56.8 [3.0]	14.5
K_A(θ_6) (hr^{-1})	1.59 [7.6]	NE
Power of LBW on vm (θ_7)	0.692 [8.3]	
Power of LBW on v3 (θ_8)	0.407 [12.9]	
Power of age on Q (θ_9)	-0.283 [25.7]	
Power of height on Q (θ_{10})	2.29 [20.5]	
Residual error, proportional (%CV)	2.87 [38.9]	
Residual error, additive (g/L)	0.031 [11.6]	

Parameter uncertainties and interindividual variability (IIV) are provided when applicable. NE=not estimated.

TABLE 5 Parameter estimates of best PK/PD model for Smooth Pursuit Performance

Population parameters	Estimate [%RSE]	IIV (CV%)
BL (MPRD)	NE	10.0
KE (θ_1)	0.304 [7.9]	60.7
Residual error, additive (smooth pursuit performance [%])	5.40 [8.9]	

Parameter uncertainties and interindividual variability (IIV) are provided when applicable. NE=not estimated.

TABLE 6 Parameter estimates of VAS Alertness PK/PD model

Population parameters	Estimate [%RSE]	IIV (SD)
BL (mm) (θ_1)	53 [0.68]	
MPRD on BL (θ_2)	0.708 [10.0]	
KE (mm / (g/L)) (θ_3)	9.4 [18.0]	13.23
Residual error, additive (mm)	4.29 [42.1]	
Residual error, proportional (%CV)	6.67 [67.9]	

RSE, relative standard error; SD, standard error.

FIGURE 1 Individual change from baseline smooth pursuit performance over time of study CHDR0502[29]. Treatments: placebo (Placebo); ethanol clamping at 0.3 g/L (Clamp0.3); ethanol clamping at 0.6 g/L (Clamp0.6). The clamping was continued for 300 minutes. Similar effects of ethanol were seen in other studies. Open circles represent data; solid black lines represent a Loess curve through these points.

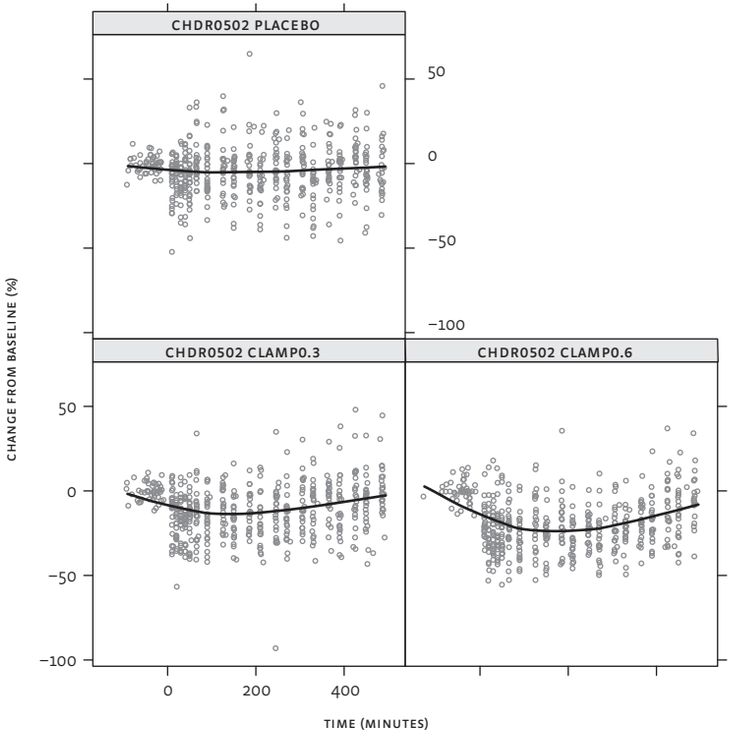


FIGURE 2 Ethanol concentration versus change from baseline smooth pursuit performance of CHDR0714[32], CHDR1011 (adolescent study), CHDR1214[35], CHDR0313[28], and CHDR0502[29]. 0.3 = target concentration of 0.3 g/L; 0.6 = target concentration of 0.6 g/L.

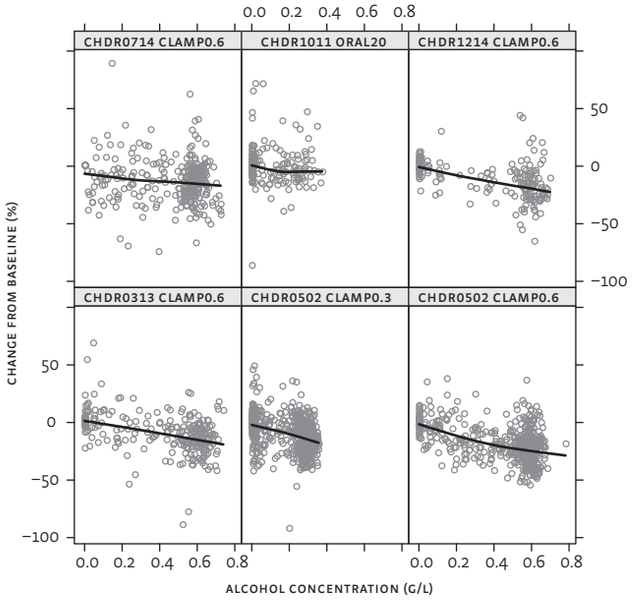


FIGURE 3 Schematic representation of the final PK model structure of ethanol

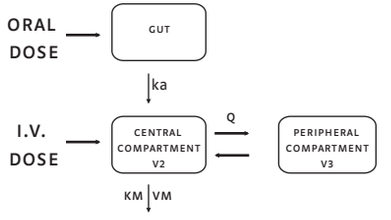


FIGURE 4 Individual predicted alcohol concentrations (IPRED) versus observations (DV). A linear regression of IPRED vs. DV is plotted as a continuous line.

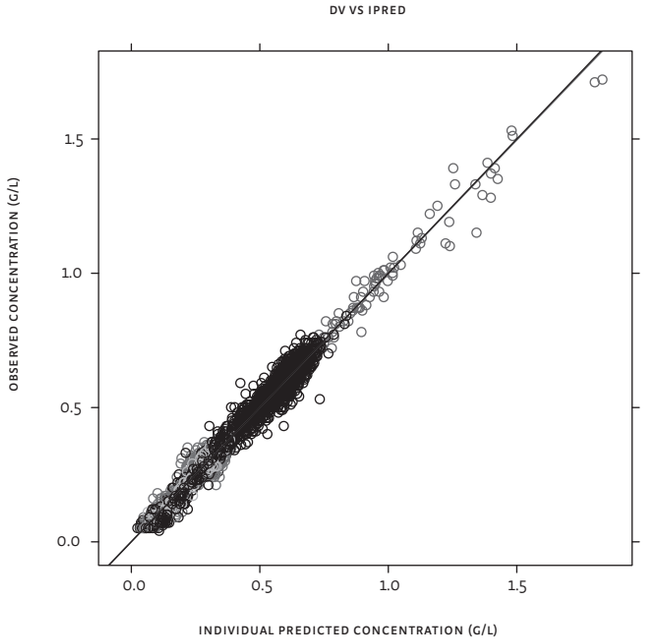


FIGURE 5 Goodness of fit plots of final PK model with Loess curve. Conditional Weighted Residuals (CWRESI) vs. individual predicted concentrations (IPRED) (upper panel); CWRESI vs. Time (lower panel).

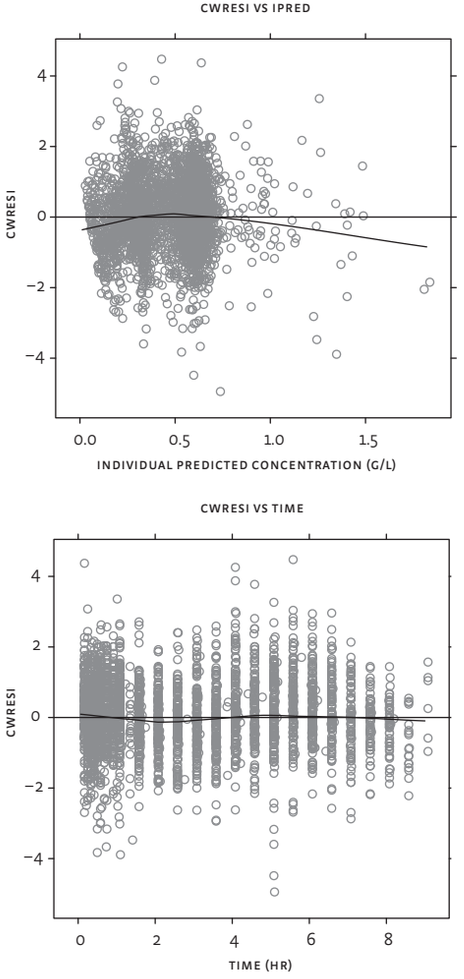


FIGURE 6 Distribution of ETAs. The continuous line represents a normal distribution with the same mean and standard deviation as the ETA. Corresponding parameters are: ETA 1: Q, ETA 2: Vm, ETA 3: V2 and ETA 4: V3.

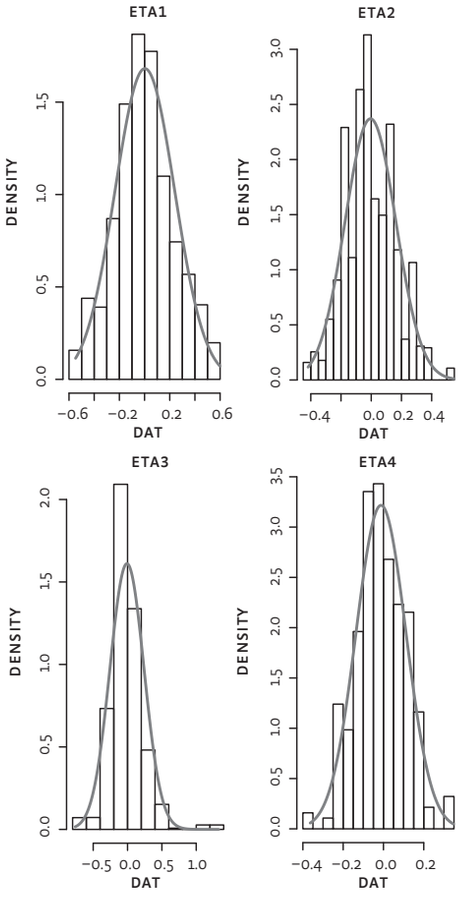


FIGURE 7 Individual predicted smooth pursuit performance (IPRED) versus observations (DV). A linear regression of IPRED vs. DV is plotted as a continuous line.

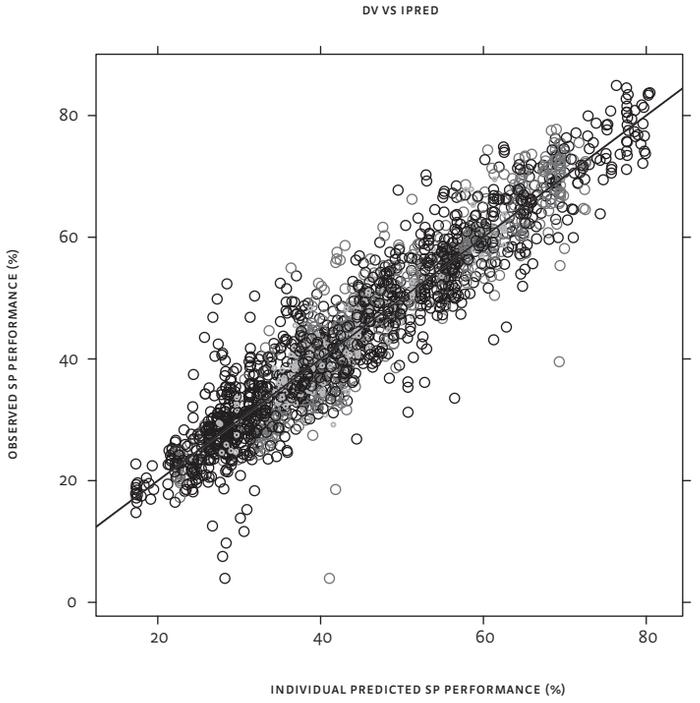


FIGURE 8 Goodness of fit plots of final PK/PD model for Smooth pursuit performance. Shown are conditional weighted residuals (CWRESI) versus IPRED (upper), time (middle) and ethanol concentration (lower panel).

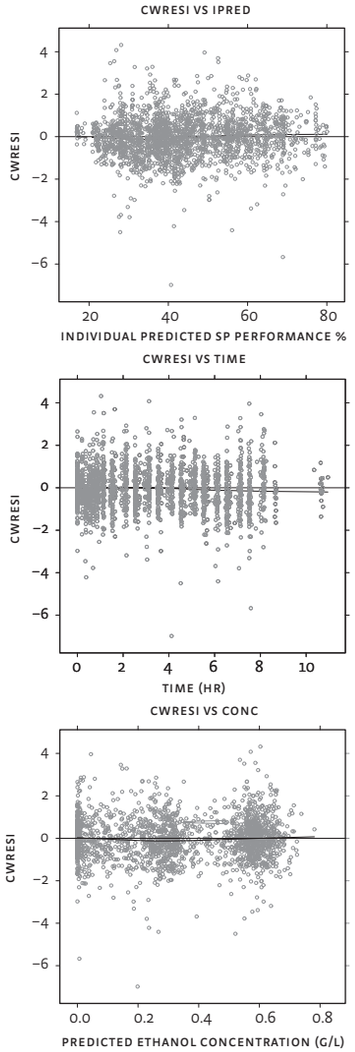


FIGURE 9 Distribution of ETAS. The continuous line represents a normal distribution with the same mean and standard deviation as the ETA. Corresponding parameters are: ETA 1: BL, ETA 2: KE.

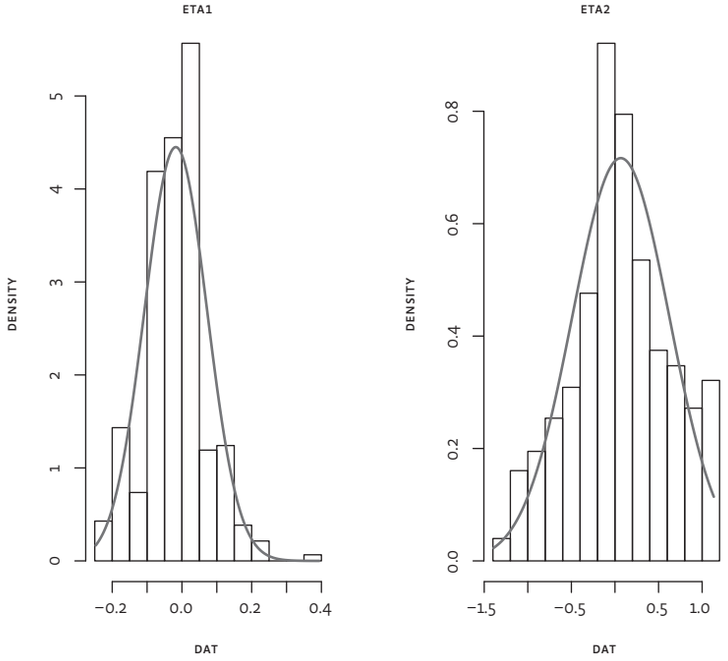


FIGURE 10 PD CWRESI over PK CWRESI plots, per study. PK data was matched to the PD by closest time point (open circles). A Loess curve was drawn through the data points (solid black line). Studies: 502=CHDR0502[29], 714= CHDR 0714[32], 1011= CHDR 1011 (adolescent study), 1214= CHDR 1214[35], 313= CHDR 0313[28].

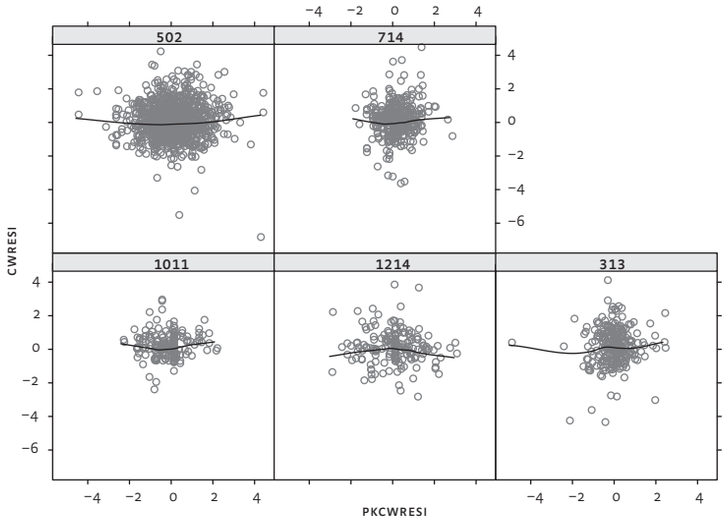


FIGURE 11 Individual predicted VAS Alertness score (IPRED) versus observations (DV). A linear regression of IPRED vs. DV is plotted as a solid black line.

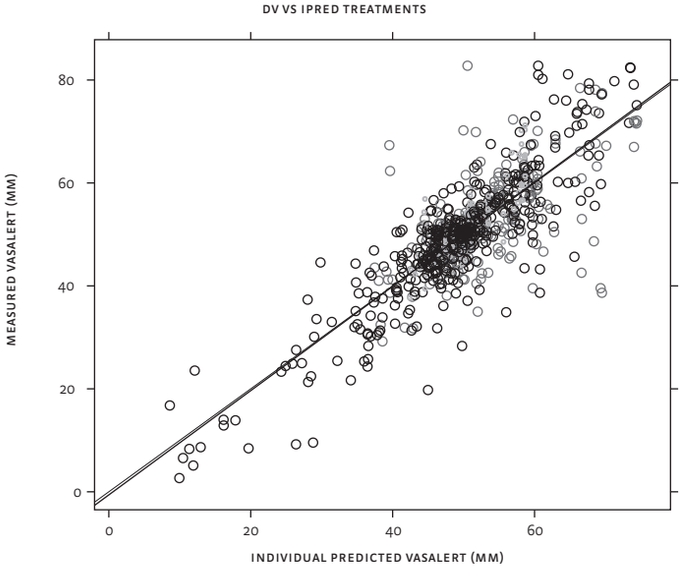


FIGURE 12 Goodness of fit plots of VAS Alertness PK/PD model. Shown are conditional weighted residuals (CWRESI) versus IPRED (upper), time (middle) and ethanol concentration (lower). A Loess curve (solid black line) was drawn through the data points (open circles).

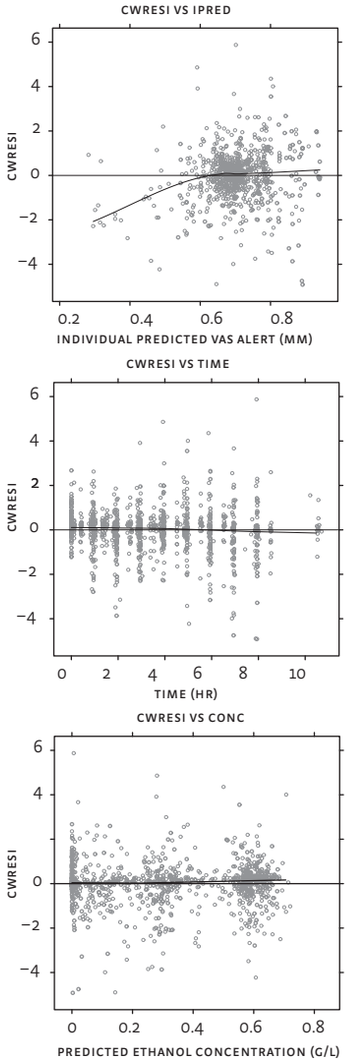
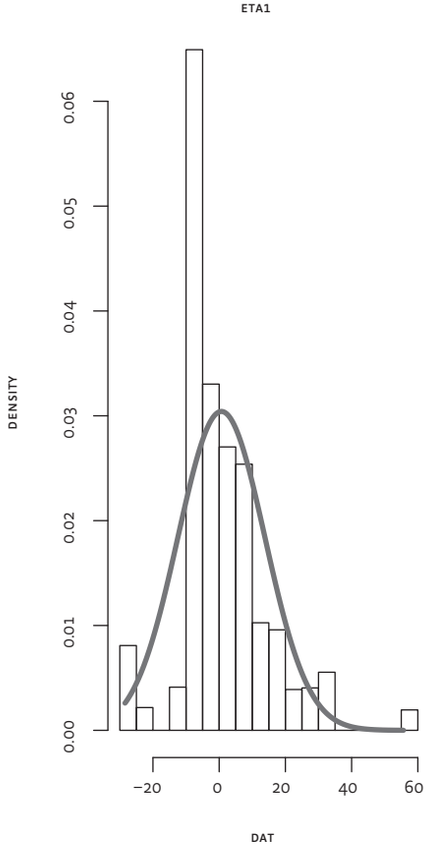


FIGURE 13 ETA distribution. The black line shows a normal distribution with the same mean and standard deviation as the ETA. Corresponding parameter of ETA 1 is KE.



SUPPLEMENTARY DATA

FIGURE 1 **Smooth pursuit LSMs change from baseline profile with 95% CI as error bars.**

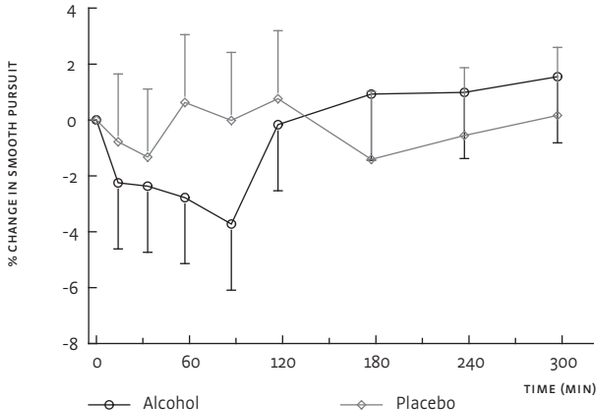


FIGURE 2 **VAS alertness LSMs change from baseline profile with 95% CI as error bars.**

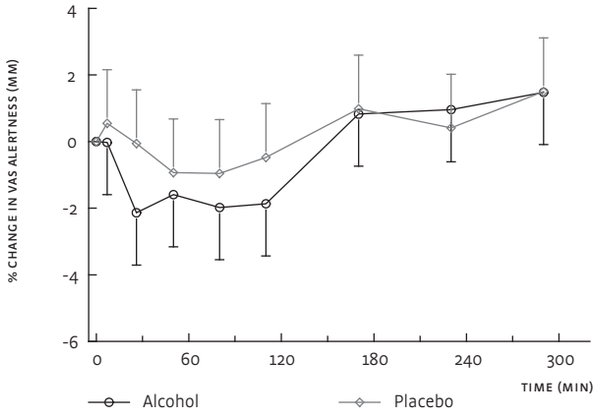


FIGURE 3 **VAS alcohol effect LSMs change from baseline with 95% CI error bars.**

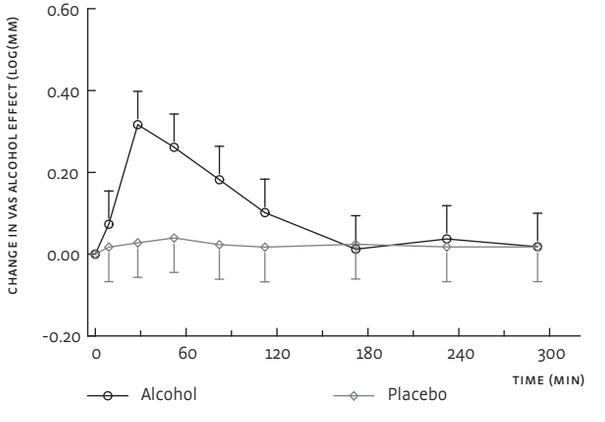


FIGURE 4 **Body sway LSMs change from baseline with 95% CI error bars.**

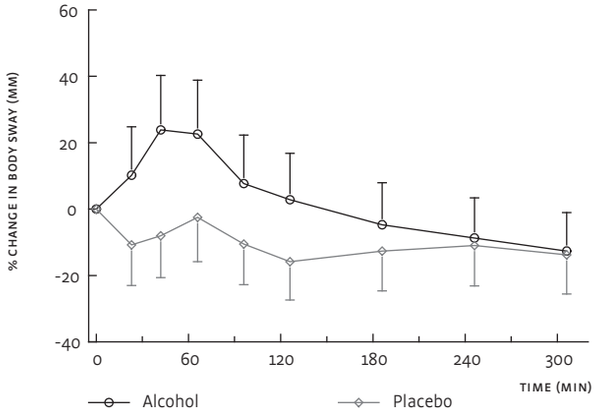


FIGURE 5 **Heart rate LSMs change from baseline with 95% CI error bars.**

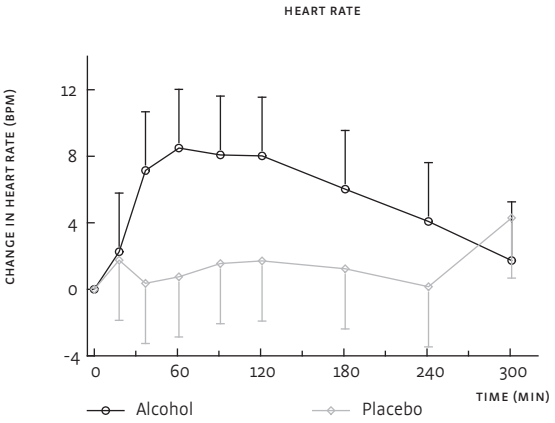


FIGURE 6 **Systolic blood pressure LSMs change from baseline with 95% CI error bars.**