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Characterization of the Pharmacokinetics of Phosphatidylethanol 16:0/18:1 and 16:0/18:2 in Human Whole Blood After Alcohol Consumption in a Clinical Lab Study

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Abstract

Background—The purpose of this study was to characterize the pharmacokinetics of two homologues of phosphatidylethanol (PEth) and their combined total in uncoagulated, whole blood samples taken from participants in a human clinical lab study after consumption of low doses of ethanol.

Methods—As part of a larger study, 14 male and 13 female participants received either 0.25 or 0.50 g/kg oral doses of ethanol during a 15 min period. Blood samples were collected before and throughout 6 h after each ethanol dose on the day of consumption, and then every 3 days during the next 14 days. PEth 16:0/18:1 and PEth 16:0/18:2 levels were quantified in blood samples by HPLC/MS/MS and reported separately or as their combined total (combined PEth). Breath alcohol concentrations (BrAC) were measured concurrently with each blood collection. Transdermal alcohol concentrations (TAC) were measured every 30 min during the entire 22 day study to confirm the absence of drinking during a 7 day period before and the 14 day period after ethanol consumption.

Results—(1) Single doses of 0.25 and 0.50 g ethanol/kg produced proportional increases in BrAC and combined PEth levels of all participants; (2) the areas under the curve (AUC) for each participant's BrAC levels during the 6 h period after ethanol administration were correlated with AUCs of cPEth (calculated as the AUC of the increase above baseline for combined PEth); (3) the mean half-life of combined PEth, determined during the 14 day period after ethanol consumption, was 4.6 ± 3.5 (SD) days (range: 1.0 to 13.1 days).

Conclusions—Combined PEth is a sensitive biomarker for the identification of relatively low levels of ethanol consumption. The measurement of these two homologues may provide additional sensitivity to identify low levels of drinking.

Keywords

phosphatidylethanol; pharmacokinetics; blood; HPLC/MS/MS; ethanol

INTRODUCTION

Biological markers for the objective identification and quantification of potentially harmful ethanol consumption are needed for medical and forensic purposes. Objective biomarkers to assess either any drinking or the level and time frame from last drinking are necessary for a thorough evaluation of alcohol consumption in medical and forensic applications.

Phosphatidylethanol (PEth), a metabolite of ethanol, is a promising candidate as a stand-alone direct biomarker and for inclusion with a short list of indirect (serum GGT and CDT) and direct (urinary EtG/EtS) biomarkers for ethanol consumption because of its several day half-life in red blood cells and its potential sensitivity for even low levels of drinking.

Less than 0.5% of consumed ethanol is metabolized to PEth (Maenhout et al., 2013), but high pressure liquid chromatography combined with tandem mass spectroscopic detection (HPLC/MS/MS) provides adequate sensitivity for the precise quantification of several PEth homologues at low ng/mL concentrations in uncoagulated, whole blood samples (Zheng et al., 2011). Several reports highlight the value of PEth for identification of harmful drinking (Aradottir et al., 2006, Hahn et al., 2010, Hansson et al., 1997, Miller et al., 2005, Stewart et al., 2010, Hahn et al., 2012), monitoring consumption during treatment (Kip et al., 2008), and various forensic applications (Marques et al., 2011, Marques et al., 2010). Yet a more complete characterization of the synthesis and elimination of PEth would provide more accurate estimations of the amount and time frame of ethanol consumption. Differences in the pharmacokinetic parameters of specific, individual PEth homologues may provide useful diagnostic information, e.g., one homologue may be synthesized and eliminated at faster or slower rates than other homologues (Gnann et al., 2014).

PEth is formed in various tissues such as brain, liver, platelets, lymphocytes, and red blood cells (Viel et al., 2012). PEth's *in vivo* precursor, phosphatidylcholine, is normally converted to phosphatidic acid by a chemical reaction catalyzed by phospholipase D. When ethanol is present, however, this reaction strongly favors the covalent incorporation of ethanol to produce PEth (Alling et al., 1984, Gustavsson and Alling, 1987, Kobayashi and Kanfer, 1987). The wide variety of fatty acid pairs that occurs at the sn1 and sn2 positions of phosphatidylcholine species accounts for 48 isoforms of PEth identified in red blood cells (Gnann et al., 2010). Unlike other tissues, red blood cells lack the enzyme phosphatidylcholine phospholipase C, which catalyzes PEth metabolism, so PEth can accumulate (Viel et al., 2012). Of the 48 known homologues of PEth, two appear to contribute significantly to the total PEth level in whole blood samples, PEth 16:0/18:1 (38%) and PEth 16:0/18:2 (24%) (Helander and Zheng, 2009, Nalesso et al., 2011). The individual levels of PEth 16:0/18:1 and PEth 16:0/18:2 and the combined total (combined PEth) were determined for this study.

The purpose of this study was to characterize the synthesis and elimination of PEth 16:0/18:1 and PEth 16:0/18:2 and their combined total (combined PEth) during a clinical lab

study of human participants who consumed low doses of ethanol (either 0.25 or 0.50 g/kg). The concentration-time curves of PEth during 6 hours after ethanol doses and the elimination of PEth during the next 14 days was studied and compared against the concentration-time curves of BrAC, which was measured at each blood collection time point.

MATERIALS AND METHODS

Subjects

Twenty-seven healthy men ($n = 14$) and women ($n = 13$) aged 21 to 52 (mean = 28.3 years) were recruited from the community through internet advertisements and were part of a larger clinical laboratory study in which ethanol was consumed. Exclusion criteria were: body mass index $>30 \text{ kg/m}^2$, a current Axis I psychiatric disorder, pregnancy, a current medical health condition, or a positive urine-drug test for the metabolites of drugs of abuse (cocaine, opiates, methamphetamines, barbiturates, benzodiazepines) during study entry or leading up to the day of alcohol administration in the laboratory, evidence of alcohol withdrawal (Clinical Institute Withdrawal Assessment for Alcohol scores > 10 ; Sullivan et al., 1989) pending criminal charges related to alcohol abuse, jail time or a court mandate to not consume alcohol. The Institutional Review Board at The University of Texas Health Science Center at San Antonio reviewed and approved the experimental protocol, and written informed consent was obtained prior to study participation.

Recruitment

Participants responded to community advertisements and initial eligibility was determined via a phone screen. Individuals who met basic criteria were invited to the laboratory for an in-person interview and to give consent. Additional screening included a detailed substance abuse history, a history of ethanol consumed within the last 28 days using the Timeline Follow-back interview method (Sobell and Sobell, 1992), a psychiatric screening using the Structured Clinical Interview for DSM-IV-TR Axis I Disorders: Research Version, Non-Patient Edition (First et al., 2001), urine drug and pregnancy tests, and a medical history and physical examination by a physician's assistant.

Experimental Chronology

Participants were required to abstain from alcohol outside of the laboratory, as verified by negative transdermal alcohol concentration (TAC) readings on an ankle monitor worn continuously for the 22 days of the study. The ankle monitor was fitted on the second day of participation. After one week of negative TAC readings, participants reported to the laboratory for the alcohol administration day. Participants were randomly assigned to receive 0.25 or 0.50 g/kg ethanol. Randomization was stratified on sex (M vs. F), body weight (i.e., above or below 140 lbs for females or 185 lbs for males), and drinking level (i.e., heavy vs non-heavy defined as drinking >3 drinks per drinking day and >7 drinks per week for females or >4 drinks per drinking day and >14 drinks per week for males. Participants were instructed to begin fasting at 12 AM on the alcohol consumption day.

Upon arrival on the ethanol consumption day, BrAC levels of zero g/dL were verified, and negative pregnancy and urine toxicology tests were obtained. Also, data from the transdermal ankle monitor (see below) were downloaded to confirm abstinence during the previous 7 day period. Participants were excluded from the study if any positive TAC readings occurred. Prior to alcohol administration, an intravenous catheter was placed in the antecubital fossa of the participant and a baseline blood sample was obtained prior to ethanol consumption. Participants then consumed the randomly assigned alcohol dose mixed with tonic water. BrAC was measured repeatedly throughout the day and blood samples were collected at specified intervals. A meal was provided 4 h post-ethanol consumption and participants remained in the laboratory until their BrAC was 0.030 g/dL. Laboratory follow-up visits occurred at 2, 4, 7, 11 and 14 days post-alcohol consumption during which participants were expected to remain abstinent (as verified by negative TAC readings) outside the laboratory. BrAC levels and TAC measurements were used at follow up visits to monitor drinking and blood samples were collected for the quantification of PEth.

Participants received \$10 for each screening day, \$75 for the alcohol consumption day, \$25 for each laboratory follow-up, and \$10 per day for wearing the transdermal ankle monitor. Also, a bonus of \$300 also was awarded for successfully maintaining negative TAC readings during the 22 days of participation.

Ethanol Administration Day

Participants were randomly assigned to receive either 0.25 g/kg or 0.5 g/kg of ethanol (Everclear; Luxco, Inc., St. Louis, Missouri) which was divided into 3 cups with tonic water (8 ounces per cup). The 0.25 g/kg dose provided between 1 and 2 standard ethanol drinks and the 0.50 g/kg dose provided between 2 and 3 standard ethanol drinks, depending on the weight of the participant. Participants completed each cup within 5 minutes as monitored by research staff so that the entire dose of alcohol was consumed during a 15 min period. Blood samples were collected pre-consumption (baseline) and at 15, 30, 45, 60, 90, 120 and 360 min post-consumption. Blood samples were collected into 4 mL vacutainer tubes containing 7.2 mg of K₂EDTA and immediately stored away from light at 4°C. Within 24 h, blood samples were aliquoted into 1.7 mL cryotubes and stored at -80°C until analysis for PEth. During each follow-up visit, a single blood sample was collected and stored as described above.

Measurement of Breath Alcohol (BrAC)

Dräger Alcotest 6810 portable, hand-held breathalyzers (Dräger Safety Diagnostics Inc., Irving, TX) were used during the study to measure BrAC. The Dräger breathalyzer uses an electrochemical sensor that reacts specifically to alcohol. The breathalyzer has a 365 day “Calibration Test lockout” feature that ensures that each breathalyzer is sent to the manufacturer for calibration. Each breath sample reading was acquired using a new disposable mouthpiece and participants rinsed their mouths with water twice to prevent residual alcohol contamination. Results were displayed on the device as estimated % blood alcohol concentration (BAC) and recorded by study personnel at 15, 30, 45, 60, 90, 120 and 360 min post-alcohol consumption.

Measurement of Transdermal Alcohol Concentrations (TAC)

SCRAM-CAMTM (Alcohol Monitoring Systems Inc., Highlands Ranch, CO) transdermal ankle monitors were used to promote abstinence during the entire 22 day study period. This device measures TAC levels every 30 minutes continuously (Dougherty et al., 2015a, Dougherty et al., 2015b, Marques and McKnight, 2009, Sakai et al., 2006). Our lab has used it in previous research to monitor low, moderate, and heavy drinking patterns ((Dougherty et al., 2015a, Dougherty et al., 2015b), and have demonstrated more than 60% sensitivity to detect one drink, more than 95% sensitivity to detect two drinks, and 100% sensitivity to detect three or more drinks (Roache et al., 2015). The device records infrared signals and skin temperature to ensure no device disruption occurs. TAC monitoring results were downloaded at the laboratory on alcohol consumption day and during each of the 5 follow-up visits, using SCRAM Direct ConnectTM. Newly recalibrated monitors were used for each participant.

Measurement of PEth 16:0/18:1 and 16:0/18:2 in Whole Blood

PEth 16:0/18:1 and PEth 16:0/18:2 were quantified in uncoagulated, whole blood using HPLC with mass spectroscopic detection. All solvents and reagents were HPLC grade and purchased from either Fischer or Sigma Chemical. Milli-Q Plus water was used to prepare solutions used in the experiments. 1-palmitoyl-2-oleoyl-phosphatidylethanol (PEth-16:0/18:1), 1-palmitoyl-2-linoleoyl-phosphatidylethanol (PEth-16:0/18:2), and deuterated 1-palmitoyl-2-oleoyl-phosphatidylethanol (PEth-16:0/18:1) were purchased from Avanti Polar Lipids (Alabaster, USA) and Echelon (Salt Lake City, UT). EDTA vacutainer tubes (Becton Dickinson Vacutainer Franklin Lakes, NJ USA) were used for all blood collection. PEth-16:0/18:1 and PEth 16:0/18:2, which are 2 of the predominant forms of PEth, were quantified in uncoagulated, whole blood samples using HPLC and tandem mass spectroscopic detection according to minor modifications of recently published methods (Zheng et al., 2011, Helander et al., 2012). The pharmacokinetics of PEth-16:0/18:1, PEth 16:0/18:2, and the total of the two homologues (combined PEth) were studied. On the day of the assay for PEth, samples were thawed at room temperature, then 300 µl of each sample was added with constant mixing into 600 µL isopropanol and 5 µL of the internal standard solution (deuterated PEth-16:0/18:1). The samples were vortexed for 1 min and 1 mL of hexane was added with mixing followed by shaking for 15 min. The samples were centrifuged for 20 min at 3200 g at 4°C. The clear supernatants were transferred to new tubes and evaporated to residues with a gentle stream of nitrogen at 30°C. The residues were dissolved in 100 µL mobile phase A (20% 10 mM ammonium acetate dissolved in water and 80% acetonitrile), and then transferred to microfilterfuge tubes and centrifuged at 1000 g for 10 min. These final samples were transferred to 300 µL glass autosampler tubes and then 10 µL were injected into the HPLC/MS/MS system. The ratio of peak areas of PEth-16:0/18:1 and PEth 16:0/18:2 to dPEth-16:0/18:1 were compared against a linear regression of ratios of calibrators from 0 to 4000 ng/mL. PEth-16:0/18:1, PEth 16:0/18:2, and cPEth concentrations were expressed in ng/mL. The lower limit of detection was estimated to be 5 ng/mL as determined using product ions m/z 281 (PEth-16:0/18:1) and m/z 279 (PEth-16:0/18:2). Imprecision of quantification ranged between 6 and 11% for control samples spiked with 42 and 187 ng/mL.

The HPLC system comprised a Shimadzu SCL controller, two LC-20AD pumps with a DGU20A degassing unit and mixing chamber, SIL-20AHT autosampler, and an AB Sciex API 4000 Q-trap mass spectrometer with turbo ion spray. The analytical column was a HyPurity C4 column (Thermo Scientific). For the gradient elution of PEth isoforms, mobile phase A was 2 mM ammonium acetate in water/acetonitrile (20:80) and mobile phase B was 100% isopropanol. The gradient was: 25% B for 2 min, linear gradient to 50% B from 2 to 3 min, linear gradient to 100% B from 3 to 6 min, 100% B from 6 to 7 min, linear gradient to 25% B from 7 to 8 min, then equilibration of the column at 25% B for 10 min before the next injection. The flow rate was 200 μ L/min and the injection volume 10 μ L. Mass spectrometer parameters: ESI interface operated in negative ion mode using selected reaction monitoring to detect the major product ions from the deprotonated molecules of PEth-16:0/18:1 (m/z 701.7 $\rightarrow$ 281.5) PEth 16:0/18:2 (m/z 699.7 $\rightarrow$ 279) and dPEth-16:0/18:1 (m/z 732.7 $\rightarrow$ 286.5). The MS conditions were: curtain gas 15 psi, collision gas (CAD) 8 psi, ion spray voltage -4500 V, temperature 550°C , nebulizer gas 15 psi, auxiliary gas 40 psi, declustering potential -61 V, focusing potential -350 V, entrance potential -10 V, and collision energy -40 V.

Statistics

Owing to the potentially high levels and long half-life of PEth, and despite 7 days of verified negative TAC readings, we observed detectable levels of PEth in the majority of subjects prior to any alcohol administration. Therefore, we used the difference from time zero (baseline) in combined PEth levels to calculate the areas under the concentration-time curves (AUCs) during 360 min after ethanol doses. AUCs were quantified using the equation in Prism6 software. Two-tailed Mann Whitney tests were used to compare pharmacokinetic AUCs between dosage pairs of BrAC and combined PEth. Correlations and their statistical significance were determined using the equations in Prism6 software. Half-lives of combined PEth levels were determined with data from the 360 min time point on ethanol administration day through the end of the 2 week abstinence period, 6 time points in all. Student's t-tests and Fishers exact tests analyses were used to identify significant differences between the groups receiving different doses at baseline for continuous and categorical variables, respectively.

RESULTS

The demographics of research participants are shown in Table 1 and the chronological design of the experiment is shown in Fig. 1. Twenty-seven participants were recruited into the study. All reported a regular pattern of drinking alcohol before they entered the study, but were able to provide negative TAC readings throughout the study indicating compliance with our abstinence requirement. After the first 7 days of negative TAC readings, blood samples collected the morning of the alcohol consumption day showed that combined PEth levels were still detectable for 22 of 27 participants (baseline levels ranged from 12.0 to 347 ng/mL).

BrAC and Combined PEth Levels

After 0.25 and 0.5 g/kg doses of ethanol were consumed, increases in BrAC and combined PEth levels were observed (Fig. 2A, 2B). These mean changes are reflective of the positive increases in BrAC and PEth observed in every participant. After alcohol consumption, combined PEth levels rose immediately and reached a maximum at about 90–120 min (Figs. 2B), which was an approximately 75 min lag of time behind the increase of BrAC levels. The mean AUC for BrAC was higher at the 0.50 than at the 0.25 g/kg dose ($p = 0.0002$) with no overlap between the 2 dosage groups (Figs. 3A). The mean of combined PEth AUCs was higher at the 0.50 than the 0.25 g/kg dose ($p = 0.0172$), but there was substantial overlap between the 2 dosage groups (Fig. 3A). Despite between-subject variability within the dosage groups, and overlap of PEth levels between groups, the AUCs for BrAC and the AUCs for combined PEth were correlated as expected (Fig. 3B; $p = 0.0027$). However, the r^2 value for the correlation indicated that only about 31% (confidence interval = 5.2% to 59.9%) of the variability among AUCs of combined PEth was accounted for by the variability among BrAC AUCs.

Synthesis and Elimination of Individual PEth Homologues

The pharmacokinetic profiles of PEth 16:0/18:1 and PEth 16:0/18:2, and combined PEth, during and after ethanol consumption are shown in Fig. 4. The data on the graph are PEth levels not adjusted for baseline levels and thus there are positive values at the zero time point. Also, data at both doses of ethanol are combined, which is justified by the overlap in PEth between the dosage groups (i.e., Fig. 3A). Mean concentrations of PEth 16:0/18:1, PEth 16:0/18:2, and combined PEth increased to a maximum at about 90–120 min after alcohol administration and then decreased during the next 14 days of abstinence. PEth 16:0/18:1 and PEth 16:0/18:2 appear to have different rates of synthesis and elimination (Fig. 4) as observed in another recent report (Gnann et al., 2014).

Half-life of Combined PEth

The half-life of combined PEth was determined using data collected at the 360 min time point on alcohol administration day and during the 14 day period of abstinence after the ethanol administration day for 24 of 27 participants (Fig. 4). The mean half-life of combined PEth was 4.6 ± 3.5 (SD) days with a range of 1.0 to 13.1 days. We were unable to determine half-lives for the other 3 participants, who received the 0.25 g/kg ethanol dose and whose combined PEth levels at baseline and at the 360 min time point were near the limit of detection of the assay and were undetectable two days later. All participants appeared to have complied with the abstinent requirement as verified by continuously negative TAC readings throughout the study and by the continuous and regular decrease in combined PEth levels observed during the 2 week period after the alcohol administration day.

Sensitivity of Combined PEth Versus Individual Homologues

Most samples that were collected in this study had both PEth 16:0/18:1 and PEth 16:0/18:2 values that were above the limits of detection (> 4 ng/mL) for individual homologues. All participants consumed ethanol during the days and weeks before study entry according to self-report. Of 741 samples tested, PEth 16:0/18:1 and PEth 16:0/18:2 were both below the

limit of detection (<4 ng/mL) in 20 samples, PEth 16:0/18:1 was detectable and PEth 16:0/18:2 was not in 27 samples, and, conversely, PEth 16:0/18:2 was detectable and PEth 16:0/18:1 was not in 16 samples. This suggests that quantification of both PEth homologues might provide greater sensitivity to identify drinking. That is, 2% of participants had detectable PEth of only a single homologue.

DISCUSSION

The results of this clinical lab study showed: (1) administration of single doses of 0.25 and 0.50 g/kg of alcohol produced increases in BrAC and combined PEth levels in every participant; (2) the areas under the concentration-time curve (AUC) for BrAC levels after alcohol administration correlated with the AUCs of combined PEth (calculated as increased 6-hr AUC of change from baseline combined PEth); (3) the mean half-life of combined PEth, determined during the 14 day period after ethanol consumption, was 4.6 ± 3.5 (SD) days (range: 1.0 to 13.1 days).

The alcohol doses used in this study (0.25 and 0.50 g/kg) were similar to social or moderate drinking, i.e., 1–2 standard drinks of 14 grams ethanol at the low dose and 3–4 standard drinks at the high dose, depending on the weight of the participant. An increase in combined PEth levels after ethanol was observed in every participant indicating a good sensitivity of this biomarker to detect even acute low doses of alcohol exposure. The BrAC AUCs, and by assumption blood levels of ethanol, were different in the two dose groups as expected, and with no overlap (Figs. 1A, 2A; $p = 0.0002$). Because the presence of ethanol is required for the synthesis of PEth in red blood cells, the time frame of synthesis and elimination for combined PEth was similar, but with about a 75 min lag in the rise to peak levels of PEth. The mean combined PEth AUCs after ethanol administration were higher for the 0.5 g/kg than the 0.25 g/kg dose (Fig. 2B, 3A), but the variability of combined PEth AUCs was higher than that seen with BrAC at both doses (Fig. 3A) and variability in BrAC only explained 31% (confidence interval = 5.2% – 59.9%) of the variance in PEth. This suggests that other factors regulating the synthesis and elimination of PEth likely explain the between-subject variance among the participants. Additional factors that might account for these differences include between-subject variability in (1) phospholipase D levels in red blood cells among human subjects, (2) phosphatidylcholine homologues, and (3) PEth elimination rates. Other studies have shown that similar consumption of ethanol produced widely varied levels of PEth. In a recently reported study, eleven research participants drank ethanol to reach an ethanol blood concentration of about 1 g/L, yet whole blood PEth 16:0/18:1 levels varied as much as 3-fold in the group (Gnann et al., 2012).

The development of a HPLC-tandem mass spectroscopy method for the quantification of individual homologues of PEth has provided greater sensitivity to identify alcohol consumption than the measurement of only a single homologue. Our results indicate that one or the other of the two homologues were not detectable in 5.8% of the samples - though measuring more than one homologue provided a positive test of drinking in all of those samples. However, the more pertinent statistic might be the 2% increase in detection achieved by going from testing one homologue (PEth 16:0/18:1 being the one in commercial

testing) to using the 2 homologues. Clearly, the remarkable sensitivity provided by HPLC/MS/MS allows for the detection of small amounts of multiple homologues of PEth.

Importantly, recent reports suggest that different homologues of PEth appear to be eliminated at different rates during abstinence (Helander and Zheng, 2009, Gnan et al., 2014). The data from this relatively small study appear to confirm that increase of mean PEth 16:0/18:2 was greater than the mean increase of PEth 16:0/18:1 immediately after alcohol consumption. Also, it appears that PEth 16:0/18:2 had a shorter half-life than PEth 16:0/18:1 during the 2 week abstinence period. If this observation can be confirmed with further study, then it may be possible to use the ratio of PEth 16:0/18:1 to PEth 16:0/18:2 to estimate of the time since the last previous drink.

Certain limitations existed in the design of this study. First, sample size was limited and prevented comparisons among groups of participants such as males and females, heavy versus light drinkers, and age categories. Second, though the negative TAC level criterion was stringent and suggests participants complied with our abstinence criteria, we previously reported that the SCRAM ankle monitor would not detect a single drink in as many as 40% of participants though it would detect 2 beers in 95% and 3 or more beers in 100% of participants (Roache et al., 2015). Third, the low doses used in the study limited our ability to acquire half lives in three study participants. There also were 14 participants whose levels were low when they entered the study became undetectable during the 2 week period hindered a precise calculation of their PEth half-lives. Nevertheless, PEth half-lives determined with or without these participants did not change the results, so we have confidence in these findings. Future studies with a larger sample and higher doses of ethanol will be necessary for a more comprehensive assessment of these pharmacokinetic parameters. In summary, this and other recent studies continue to strongly support the measurement of whole blood PEth levels as a sensitive biomarker for ethanol consumption. Low doses of ethanol, similar to the consumption of 1 to 3 standard ethanol drinks, produced immediate increases in blood levels of PEth 16:0/18:1 and PEth 16:0/18:2 homologues and combined PEth levels correlated with the dose and absorption of ethanol. The half-life of combined PEth was 4.6 days, which enabled combined PEth levels to be detectable even after two weeks of confirmed abstinence in most participants. Finally, the differential synthesis and elimination of PEth 16:0/18:1 and PEth 16:0/18:2 observed in this and previous studies suggests that useful parameters may be discovered such as estimating the time from the last drink.

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Figure 1.
Chronology of Study

Figure 2.

Concentration-Time Curves for BrAC and Combined PEth After Doses of Ethanol. BrAC and combined PEth levels after consumption of 0.25 and 0.50 g/kg doses of ethanol are plotted. These biomarker levels were determined in breath and blood samples collected at 0, 15, 30, 45, 60, 90, 120, and 360 min after ethanol. (A) Symbols represent the mean and SEM of BrAC levels. Baseline levels of BrAC were zero before the administration of ethanol. (B) Symbols represent the mean and SEM of combined PEth levels. The plot reflects the combined PEth levels expressed as a change above the baseline levels observed before alcohol was administered. Baseline levels ranged from 12 to 347 ng/mL in 22 of the 27 subjects.

Figure 3.

AUCs of BrAC and Combined PEth after 0.25 and 0.50 g/kg doses of Ethanol. Levels of statistical significance are listed on the graphs. (A) the AUCs for BrAC and change in combined PEth during the 6 h period after ethanol. BrAC AUCs are plotted on the left y axis and combined PEth AUCs are plotted on the right y axis. Also, the mean $\pm$ SEM error bars are shown for each group. (B) Scatterplot of the relationship between AUCs for BrAC and cPEth after administration of ethanol (linear regression, $y = 1277x + 5627$).

Figure 4. Concentration-Time Curves for PEth 16:0/18:1, PEth 16:0/18:2, and Combined PEth During Abstinence After Ethanol. Symbols represent individual and combined PEth levels for both dosage groups combined. The values of PEth at time zero are the baseline levels in blood samples collected before ethanol doses.

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Table 1

Demographics Characteristics

Characteristic	0.25 g/kg dose (<i>n</i> = 16)		0.50 g/kg dose (<i>n</i> = 11)		<i>p</i>
	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	
Age (Years)	29.7	9.5	26.7	4.5	.785
Weight (kg)	72.7	10.3	71.8	11.9	.711
Standard Drinks/week	9.8	5.6	11.1	16.6	.227
Male/Female	8/8		6/5		1.00
Race (AA/C/O)*	3/11/2		1/9/1		1.00
Ethnicity [†] (H/N)	8/8		6/5		0.83

Note.

* Race is represented as the frequency of individuals in each group identifying as African-American (AA), Caucasian (C), or Other (O).

[†] Ethnicity is represented as the frequency of individuals in each group identifying as Hispanic (H) or Non-Hispanic (N).