Published in final edited form as:

Alcohol Clin Exp Res. 2012 May ; 36(5): 854–862. doi:10.1111/j.1530-0277.2011.01669.x.

Phosphatidylethanol (PEth) as a biomarker of alcohol consumption in HIV positives in sub-Saharan Africa

Judith A. Hahn¹, Loren M. Dobkin¹, Bernard Mayanja², Nneka I. Emenyonu³, Isaac M. Kigozi², Stephen Shiboski¹, David R. Bangsberg^{2,3}, Heike Gnann⁵, Wolfgang Weinmann^{5,6}, and Friedrich M. Wurst⁷

¹University of California, San Francisco, California, USA ²Mbarara University of Science and Technology, Mbarara, Uganda ³Massachusetts General Hospital, Harvard Medical School, Massachusetts, USA ⁵Institute of Legal Medicine, University Medical Centre, University of Freiburg, Freiburg, Germany ⁶Institute of Forensic Medicine, Forensic Chemistry and Toxicology, University of Bern, Bern, Switzerland ⁷Department of Psychiatry and Psychotherapy II, Christian-Doppler-Hospital, Paracelsus Medical University, Salzburg, Austria

Abstract

Background—Alcohol is heavily consumed in sub-Saharan Africa and affects HIV transmission and treatment but is difficult to measure. Our goal was to examine the test characteristics of a direct metabolite of alcohol consumption, phosphatidylethanol (PEth).

Methods—Persons infected with HIV were recruited from a large HIV clinic in southwestern Uganda. We conducted surveys and breath alcohol concentration (BRAC) testing at 21 daily home or drinking establishment visits and blood was collected on day 21 (n=77). PEth in whole blood was compared to prior 7-, 14-, and 21-day alcohol consumption.

Results—1) The receiver operator characteristic area under the curve (ROC-AUC) was highest for PEth versus any consumption over the prior 21 days (0.92; 95% CI: 0.86-0.97). The sensitivity for any detectable PEth was 88.0% (95% CI: 76.0-95.6%) and the specificity was 88.5% (95% CI: 69.8-97.6%). 2) The ROC-AUC of PEth versus any 21-day alcohol consumption did not vary by age, body mass index, CD4 cell count, hepatitis B virus infection and antiretroviral therapy status, but was higher for men compared to women (p=0.03). 3) PEth measurements were correlated with several measures of alcohol consumption, including number of drinking days in the prior 21 (Spearman r=0.74, p<0.001) and BRAC (r=0.75, p<0.001).

Conclusions—The data add support to the body of evidence for PEth as a useful marker of alcohol consumption with high ROC-AUC, sensitivity, and specificity. Future studies should further address the period and level of alcohol consumption for which PEth is detectable.

Keywords

Alcohol; biomarker; phosphatidylethanol; HIV; Africa

Corresponding author: Dr. Judith Hahn, Address: Box 0886, University of California, 94143-0886, USA. judy.hahn@ucsf.edu, phone: 415-597-4961.

Author contribution:

JH, DB, NE, BM, and FW were responsible for the study concept and design. JH, BM, NE, IK and LD implemented the study and collected the data. HG and WW performed the phosphatidylethanol testing. JH and FW performed the data analysis and SS assisted with data analysis and interpretation of findings. JH and FW drafted the manuscript and all authors provided critical revision of the manuscript for important intellectual content and approved the final version for publication.

Conflict of interest declaration: None

Introduction

Alcohol is currently the most widely distributed and commonly used recreational drug in Africa; even the most rural areas in Africa have reliable production and distribution systems (Parry, 2005, Parry et al., 2002, Obot, 1990, Gillis and Stone, 1977, Adomakoh, 1976, Omoluabi, 1995, Acuda and Sebit, 1997). The estimated total per capita adult (age 15 and over) consumption of pure alcohol is 7.1 liters per year in East Africa (2000, Rehm et al., 2003) and 11.9 liters per year in Uganda (2011b). Alcohol consumption has been a consistent risk factor for HIV acquisition in sub-Saharan Africa (Fisher et al., 2007), for lower antiretroviral therapy (ART) adherence worldwide (Hendershot et al., 2009), and may lead to more rapid HIV disease progression (Hahn and Samet, 2010), therefore alcohol is a significant issue among the HIV-infected in this region (Hahn et al., 2011).

However, measuring alcohol consumption in sub-Saharan Africa can be challenging. While commercially prepared alcohol is available, much of available alcohol is home-brewed or home-distilled from locally grown grains or fruits (Rehm et al., 2003, Mwesigye and Okurut, 1995), and the alcohol content may vary widely. For example, the alcohol content of locally produced maize-based brews and liquor in Kenya ranged from 2% to 7% and 18% to 53% respectively (Papass et al., 2008), while the alcohol content of banana-based and finger millet-based brew ranges from 6% to 11% and 6% to 8.5% respectively (Msesigye and Okia Okurut, 1995). It is also difficult to assess the volume of alcohol consumed because home-brewed or home-distilled forms of alcohol are obtained in varying container sizes and home-brews may be consumed using long straws shared among the people at the pot side (Msesigye and Okia Okurut, 1995).

Social desirability bias may also cause under-reporting if there is the perception of negative consequences associated with reported use (Del Boca and Darkes, 2003, Del Boca and Noll, 2000, Harrison and Hughes, 1997). Persons infected with HIV who are receiving or hoping to receive HIV ART may under-report their alcohol consumption if they fear that they will be denied ART if they report alcohol consumption. Non-oxidative direct ethanol metabolites as biomarkers of alcohol consumption can offer objective measures of the level of alcohol consumption (Hannuksela et al., 2007).

Promising metabolites of ethanol consumption that may serve as biomarkers include ethyl glucuronide (EtG) (Dahl et al., 2002, Wurst et al., 2002, Wurst and Metzger, 2002, Morini et al., 2009), ethyl sulfate (EtS) (Helander and Beck, 2005, Wurst et al., 2006), and phosphatidylethanol (PEth) (Aradottir et al., 2004a, Hartmann et al., 2007, Gunnarsson et al., 1998, Hansson et al., 1997, Varga et al., 2000, Varga et al., 1998). Each of these remains detectable for a characteristic time spectrum after the cessation of alcohol intake – EtG and EtS in urine for up to five days, PEth in whole blood for more than two weeks, and EtG in hair for months (Alt et al., 2000, Wurst et al., 2010, Wurst et al., 2002). While some studies have been conducted to validate these markers, there still is a need to further delineate their characteristics and the factors that might affect their sensitivity and specificity (Bearer et al., 2010, Freeman and Vrana, 2010, Litten et al., 2010). Most previous studies measured PEth using high-performance liquid chromatography with evaporative light scattering detection (HPLC-ELSD), however more sensitive methods using liquid chromatography with tandem mass spectrometry (LC-MS-MS) technology have recently been developed (Isaksson et al., 2011).

Indirect markers of heavy alcohol consumption such as gamma glutamyltransferase (GGT), mean corpuscular volume (MCV), aspartate transaminase (AST) and alanine transaminase (ALT) lack specificity and may be susceptible to elevations caused by hepatitis B virus

(HBV) infection, and hepatitis C virus (HCV) infection and use of ART by those infected with HIV. Carbohydrate deficient transferrin (%CDT) is approved by the Food and Drug Administration (FDA) as an indirect marker of heavy alcohol consumption which has been used in clinical practice (Anton, 2010) and clinical trials (Anton et al., 2006). While sex differences in %CDT appear to exist, more sophisticated newer and standardized assays and methods (Bergstrom and Helander, 2008, Helander et al., 2010) have overcome this issue with the current test for the percent disialo isoform of CDT (%dCDT). The sensitivity of %CDT and %dCDT is frequently low (Niemela, 2007) but may be increased in combination with GGT (Hietala et al., 2006). However, GGT may be elevated and therefore not valid for use in those infected with HIV and/or HBV.

The goal of this study was to examine the test characteristics of PEth in HIV-infected persons in Uganda by self-report and breath alcohol concentration (BRAC).

Materials and methods

Overview

We compared PEth results to self-reported alcohol consumption in 77 adults. We corroborated self-report with collateral reports of drinking and by BRAC results collected at daily home or drinking establishment visits. We determined the sensitivity, specificity, and the receiver operating characteristic area under the curve (ROC-AUC) of PEth. Individual PEth molecular species were determined using LC-MS-MS technology (Gnann et al., 2010). Because early indications are that this technique detects isolated episodes of moderate drinking (Gnann et al., 2009) and is more sensitive than previous methods (Isaksson et al., 2011), we sought to determine what pattern of alcohol consumption would be detectable in terms of quantity (any versus heavy) and frequency (ever versus frequent). We chose a 21-day period as the longest period that PEth had been previously noted to be detectable (Gunnarsson et al., 1998, Hansson et al., 1997, Wurst et al., 2010), and examined the test characteristics for alcohol consumption in the prior 7, 14, and 21 days. We additionally examined whether the test characteristics of PEth are affected by HBV infection which is common in sub-Saharan Africa, ART for HIV, age, sex, body mass index (BMI), and CD4 cell count (a measure of immunological health).

Ethical review

The Institutional Review Boards of the University of California, San Francisco, the Mbarara University of Science and Technology (MUST), and the Uganda National Council for Science and Technology approved the protocols of this study.

Sample population and recruitment

The study was conducted from 2007 to 2008 in Mbarara, Uganda. Eight-eight (88) study subjects were recruited from the MUST Regional Referral Hospital Immune Suppression Syndrome (ISS) Clinic; 77 were included in the final analysis. Potential participants were referred by ISS Clinic health counselors who were aware of the study enrollment goals. Referred participants were screened for eligibility and informed consent was obtained by the study research assistants. Study eligibility criteria were: being 18 years of age and older, fluency in either Runyakole or English, being HIV positive. Those on ART needed to have been on treatment for at least six months to avoid elevated liver enzymes that are frequently due to ART initiation, because such enzyme elevations might be incorrectly attributed to heavy drinking. We aimed to enroll equal numbers of participants by sex, ART status (on ART versus not on ART), and drinking stratum (abstainer for at least 1 year, at-risk drinker: >7 drinks/week by women; >14 drinks week by men, and moderate drinker: drank any alcohol prior year but not at-risk drinker).

Study procedures

Baseline study procedures included informed consent, a baseline study questionnaire, and collection of study specimens. The baseline study questionnaire included demographic information, and questions about alcohol consumption in the prior 90 days and when alcohol was last consumed. After baseline, we conducted visits to the participants' homes or another location, usually a drinking establishment. These visits were pre-arranged to coincide with the end of anticipated drinking (for the drinkers), and occurred daily over a 21-day period. At each daily visit, a short survey about alcohol consumed since the last visit was conducted and BRAC testing was performed. Participants also recruited a friend or relative to provide a collateral report of when that collateral reporter last observed drinking by the study participant. The interview with the collateral reported occurred during one of the daily visits. Participants visited the study offices for blood sample collection 21 days after baseline.

Sample collection and processing—Plasma and whole blood samples were collected at baseline and whole blood was collected 21 days after baseline. The plasma samples were tested at the MUST laboratory for hepatitis B surface antigen (HBsAg) to indicate HBV infection, and CD4 cell count was obtained from whole blood. We did not test for HCV because we previously found zero prevalence of HCV in persons infected with HIV in Uganda (Hahn et al., 2007). Breath alcohol concentration testing was performed at least 15 minutes since the last drink using an Intoximeter alcohol sensor (Intoximeters, Inc., St. Louis, MO). Blood specimens were collected only when the BRAC result was 0 g/l, to ensure that no phosphatidylethanol formed in vitro during processing (Aradottir et al., 2004b, Aradottir et al., 2004a). Those with results greater than 0 g/l were asked to abstain from drinking that day and return the following day. Blood was stored at -7 degrees C and tested for PEth at the Institute of Legal Medicine at the University of Freiburg, Germany. Whole blood was tested using LC-MS-MS for the PEth homologues 16:0/18:1, 18:1/18:1, and 16:0/16:0 as previously described (Gnann et al., 2010). All homologues were identified and quantified using one MRM-transition. The limit of detection was 10 ng/ml and the limit of quantification was 20 ng/ml.

Eleven of the samples underwent one freeze-thaw cycle in Uganda when the tubes containing the blood cracked or broke in the freezer in Uganda. While a previous study showed that freeze-thaw cycles could decrease the sensitivity of PEth (Marques et al., 2011), analyses excluding these samples led to virtually the same estimates of sensitivity and specificity, therefore the 11 samples were retained in all analyses.

Variables

Baseline variables included demographic variables, whether or not the participant was taking ART, typical number of days consuming alcohol in the prior 90, when the participant last consumed alcohol, and typical and maximum volumes of alcohol consumed, by beverage type (commercial and homemade wine, beer or other grain-based fermented beverage, and spirits), the Alcohol Use Disorders Identification Test (AUDIT) (Babor et al., 2001) which provides an indication of hazardous drinking in the prior year, BMI, and CD4 cell count (cells/mm³), and HBV infection. Variables collected on the daily drinking survey included beverage-specific volumes of each type of alcohol consumed. We estimated the grams of alcohol consumed assuming pure alcohol concentrations of 5% for brewed beverages, 12.5% for wines, and 40% for spirits, and converted from volume (ml) to weight (grams) using a factor of 0.7893 (Miller et al., 1991). We chose these alcohol concentrations because they were consistent with those reported by the beverage industry in Uganda (2011a) and testing of samples of both commercially and home produced beverages using the Analox Analyzer AM3 (Analox Instruments, 2003) conducted at the Alcohol Research Group, Public Health Institute, Emeryville, CA.

Dichotomous measures of alcohol consumption—We defined any alcohol consumption during the 21-day observation period as any self-reported consumption on the daily surveys or any BRAC value of >0 g/l and frequent alcohol consumption as any alcohol consumption at least three days per week, on average. We defined heavy alcohol consumption on any day as reaching or exceeding a BRAC of 0.1% or reporting consumption of more than 56 grams of alcohol (men) or 42 grams (women) (equivalent to the NIAAA 4 and 3 drink cutoffs for heavy drinking for men and women respectively (NIAAA, 2004)) on a daily survey. We defined frequent heavy alcohol consumption as meeting the criteria for heavy alcohol consumption an average of three or more times per week.

We calculated AUDIT scores at baseline and considered scores of 5 or 8 in women and men respectively as indicators of heavy or high risk drinking (Aalto et al., 2009, Neumann et al., 2004).

Continuous measures of alcohol consumption—We determined the total number of days over the 21-day observation period that any alcohol was consumed, the total number of days of heavy drinking, the average BRAC, the average estimated grams of alcohol on days that alcohol was consumed, the cumulative number of grams of alcohol consumed, the sum of all BRAC tests over the observation period, and the maximum BRAC during the 21-day observation period.

Statistical analysis

We examined the overlap between the individual PEth homologues using contingency table analysis. We used receiver operating characteristic (ROC) curve methods to compare the most common PEth homologue, PEth 16:0/18:1 (Helander and Zheng, 2009, Gnann et al., 2009) generically referred to as PEth, to any alcohol consumption, frequent alcohol consumption, any heavy alcohol consumption, and frequent heavy alcohol consumption in the prior 7, 14, and 21 days. We chose these intervals to coincide with those studied previously (Gunnarsson et al., 1998, Hansson et al., 1997, Wurst et al., 2010, Aradottir et al., 2006, Hartmann et al., 2007). We constructed ROC plots of the sensitivity versus one minus the specificity using each potential cutoff value of PEth for each dichotomous measure of alcohol consumption. The potential cutoff values for these curves were the observed PEth values, which ranged from undetectable (zero) to 2592 ng/ml. We summarized the ROCs using the area under the ROC curve, i.e. the ROC-AUC. For the measure of alcohol consumption with the highest ROC-AUC, which turned out to be “any alcohol consumption in the prior 21 days”, we determined the cutoff that yielded the highest proportion correctly classified. This cutoff was the limit of detection, and we used this to calculate the sensitivity and specificity for all the other dichotomous measures of alcohol consumption. This cutoff value was used in a recent study comparing PEth detected by HPLC-ELSD to that detected by LC-MS-MS in a sample of drivers in an alcohol ignition interlock program (Marques et al., 2011) although a higher cutoff (20 ng/ml) was used in two other recent studies (Stewart et al., 2010, Stewart et al., 2009). Therefore we additionally examine this higher cutoff. We constructed exact binomial 95% confidence intervals for each sensitivity and specificity estimate.

We calculated the ROC-AUC, sensitivity and specificity statistics and 95% confidence intervals for PEth at baseline compared to any alcohol consumption in the prior 21 days by the levels of several covariates (sex, age group, BMI, CD4 cell count, HBV infection status, whether or not the participant was on ART). We dichotomized BMI as not overweight (BMI <25) versus overweight (BMI ≥ 25); there were only 5 persons who were underweight (BMI <18.5). We dichotomized CD4 cell count as below versus above or equal to 350 cells/

mm³, the level at which ART initiation is recommended by the World Health Organization. We conducted a non-parametric test to determine whether the ROC-AUC differed between the strata defined above (DeLong et al., 1988). Lastly, we calculated Spearman correlations to summarize the relationship between the PEth values during the prior 21 days and several continuous measures of alcohol consumption.

Results

Participant characteristics

Of a total of 88 participants enrolled, one was withdrawn from the study because she did not comply with the study protocol, one participant died on day 13, and one was lost to follow up after day 10. Of the remaining 85 patients, 77 (91%) completed the 21-day blood draw, one participant did not have blood drawn due to repeatedly detectable breath alcohol, and seven failed to return to the study site for the blood draw. The median age of the 77 participants with complete data was 32 (interquartile range [IQR]: 26-38), 61% were female, the median CD4 cell count was 368 cells/mm³ (IQR: 239-536), and 39% were on ART. These characteristics were similar to those of patients entering the ISS Clinic in Mbarara during this same time period, in which 64% were female, the median age was 33, and 40% were eligible for treatment based on their symptoms (Kigozi et al., 2009). At baseline, 77% (n=59) of the participants reported that they had consumed any alcohol in the prior year and 23% (n=18) had not, while 42% of the sample (n=32) had consumed alcohol in the prior 3 days. All (100%) participants reported consuming alcohol as or more recently than reported by their collateral reporters. The majority (60%, n=46) of study participants were above the cutoff for high risk drinking by the AUDIT. Among those who had reported drinking any alcohol in the 90 days prior to baseline (75%, n=58), the median estimated number of days of alcohol consumption was 24 (IQR 8-48) and the estimated median volume of alcohol consumed each typical drinking day was 68.0 grams (IQR: 39.5-82.9). The median volume of alcohol consumed on the heaviest drinking day in the 90 days prior to baseline was 138.1 grams (IQR: 78.9-202.1).

Daily home or drinking establishment visit results

For the 77 analyzed study participants, 1623 home or drinking establishment daily visits were completed. In 337 of these visits, the participant reported consuming alcohol since the previous home visit, and the median BRAC reading for such visits was 0.03% ml/L (IQR: 0.0-0.15%) and the median self-reported grams of alcohol consumed was 63.1 (IQR: 39.5-110.5). Among the 66.2% (n=51) who drank alcohol during the 21-day period, the median of the maximum volume of alcohol consumed was 98.7 grams (IQR: 47.4-213.1). Alcohol consumption was detected by BRAC >0 g/l in 2% of the daily visits in which no alcohol consumption was reported. Among those visits in which alcohol consumption was reported, BRAC was measured a median of 48 minutes (IQR: 20 minutes-2.2 hours) after the last drink was consumed. The estimated total alcohol consumption over the 21-day period by alcohol consumption group is summarized in Table 1.

Test characteristics

We examined the overlap between PEth homologue 16:0/18:1 and PEth homologues 16:0/16:0 and 18:1/18:1 in the samples collected at study day 21. PEth 16:0/18:1 was detected in all of the samples in which PEth 16:0/16:0 and 18:1/18:1 were detected; while PEth 16:0/18:1 was detected in another 37% and 38% of the samples in which PEth 16:0/16:0 and 18:1/18:1, respectively, were not detected. Further PEth results refer to PEth 16:0/18:1 unless otherwise specified.

We calculated the ROC-AUC and the sensitivity and specificity and the corresponding 95% confidence intervals for PEth compared to the dichotomous measures of alcohol consumption (Table 2 and Figure 1). Of the four dichotomous measures of alcohol consumption over 3 different durations of look-back, the ROC-AUC was highest (0.92) when PEth 16:0/18:1 was compared to any alcohol consumption in the prior 21 days (95% CI: 0.86-0.97). This was slightly higher than the ROC-AUC for any alcohol consumption in the prior 14 days (0.90; 95% CI: 0.83-0.96) and any heavy alcohol consumption in the prior 21 days (0.89; 95% CI: 0.81-0.96). PEth 18:1/18:1 and 16:0/16:0 had consistently lower ROC-AUCs than for PEth 16:0/18:1 (data not shown).

The cutoff that maximized the percent accurately classified for any alcohol consumption in the prior 21 days was the limit of detection, i.e. 10 ng/ml, and the corresponding sensitivity for any alcohol consumption in the prior 21 days was 88.0% (95% CI: 76.1-95.6%) and the specificity was 88.5% (95% CI: 69.8-97.6%) (Table 2). When we increased the cutoff value for PEth to 20 ng/ml, the cutoff used in two recent studies, (Stewart et al., 2010, Stewart et al., 2009) the specificity for any 21-day alcohol consumption remained the same (88.5%) while the sensitivity for detecting any alcohol consumption decreased to 82.3% (95% CI: 69.1-91.6%).

When we examined the data for the three participants who reported no alcohol consumption in the 21-day period but had detectable PEth at day 21, we found that all three had reported consuming alcohol in the 90-days prior to baseline. When we extended the look-back period to additionally include the 90 days prior to baseline, the ROC-AUC for any alcohol consumption was 0.91 (95% CI: 0.86-0.96), the sensitivity was 76.3% (95% CI: 63.4-86.4%), and the specificity was 100.0% (95% CI: 81.5-100.0%).

We calculated ROC-AUCs, sensitivities, and specificities for PEth compared to any alcohol consumption in the 21-day period stratified by sex, age, BMI, CD4 cell count, HBV infection status, and ART status (Table 3). The ROC-AUCs did not differ by any of these variables except for sex, with males having a higher ROC-AUC of 0.98 (95% CI: 0.94-1.00) compared to 0.86 (95% CI: 0.77-0.96) in females ($p=0.03$).

We calculated the Spearman correlations between continuous measures of drinking and PEth, obtained from self-report on the daily surveys (corroborated with BRAC) and BRAC results (Table 4 and Figure 2). All measures were significantly correlated with PEth ($p<0.001$) and the correlation was highest for the cumulative BRAC level over the 21-day period ($r=0.75$).

Discussion

To the best of our knowledge, this is the first study assessing the potential usefulness of PEth in HIV positives. This study was also unique in that we obtained collateral report and daily breath analysis to corroborate self-reported alcohol consumption. The main findings are: 1) the ROC-AUC was highest (0.92; 95% CI: 0.86-0.97) when PEth 16:0/18:1 was compared to any alcohol consumption over 21 days. 2) The test characteristics of PEth did not significantly vary by age, BMI, CD4 cell count, ART status, or HBV infection status, but was higher in men compared to women. 3) PEth measurements were highly correlated with several measures of alcohol consumption, such as number of drinking days ($r=0.74$, $p<0.001$) and the sum of the BRAC measurements over the 21 day period ($r=0.75$, $p<0.001$). That the correlations were lower for those variables for which grams of alcohol was estimated (e.g. average grams of alcohol consumed per day) highlights the difficulties in estimating drink quantities in addition to estimating alcohol concentration in sub-Saharan Africa.

Our results are comparable to those from two prior studies of the test characteristics of PETH. In a study of alcohol dependent patients, total PETH, analyzed by HPLC-ELSD, demonstrated high (99%) sensitivity and was moderately correlated (Spearman $r=0.57$) with grams of alcohol consumed in the prior 14 days (Aradottir et al., 2006). In a study of alcohol dependent patients entering detoxification and closed-ward abstaining psychiatric patients, PETH was 94.5% sensitive and 100% specific for differentiating between the two groups, and was correlated (Spearman $r=0.80$) with alcohol consumption in the prior 7 days (Hartmann et al., 2007). The somewhat lower sensitivity and specificity in the current study are not surprising because the entire range of alcohol consumers were included in this study, as opposed to only alcohol dependent patients or abstainers as in the previous studies. This was also noted in a study of PETH in reproductive age women in which a cutoff of 45 ng/ml yielded 61% sensitivity and 95% specificity for detecting drinking more than one drink per day (Stewart et al., 2010). The authors found that some of the less heavy drinkers were also positive for PETH, making it difficult to determine a cutoff that yielded both high sensitivity and specificity.

There are some limitations to note. First, while we attempted to corroborate self-reported alcohol consumption, unreported and underreported alcohol consumption may have occurred. We used breath analysis in an attempt to corroborate daily drinking in the 21-day period and collected collateral reports, but these methods may have missed some drinking. We also cannot determine if the quantities reported were accurate. In addition, using average alcohol concentration for each beverage type was also likely to introduce error. Alcohol concentration may vary widely within beverage categories and between batches of homemade alcohol.

While we supplemented self-reported alcohol consumption with BRAC testing during the 21-day period, only daily home or drinking establishment visits were feasible and drinking occasions may have been missed. Alcohol consumption that was not reported may have decreased our estimates of specificity. We felt that covert drinking was unlikely among these study subjects because they agreed to have daily visits including BRAC tests, and there were very few occasions in which alcohol consumption was detected by BRAC testing that was not also reported. Three participants who did not report alcohol consumption in the 21-day period and had BRAC=0 for all visits had detectable PETH on day 21. They did report consuming alcohol in the 90 days prior to baseline, so it is not known whether the positive result reflects unreported alcohol consumption in the 21-day period or whether PETH is detectable for longer than 21 days. PETH detected using LC-MS-MS has a lower limit of detection and appears to be more sensitive than when detected by HPLC-ELSD (Isaksson et al., 2011). For example, a recent study reported a high correlation between PETH results measured using HPLC-ELSD versus LC-MS-MS ($r=0.93$) in paired samples, while a greater number were detectable on LC-MS-MS compared to HPLC-ELSD (Marques et al., 2011). This suggests that PETH determined using LC-MS-MS methods may be detectable for longer than the 21 days previously found in studies of PETH elimination conducted using HPLC-ELSD (Gunnarsson et al., 1998, Wurst et al., 2010).

While we did not find statistically significant differences in the test characteristics of PETH by most of the variables of importance, we were limited by small sample sizes and therefore the confidence intervals were wide. When we stratified by sex, we found increased sensitivity and specificity among males, but the sample size was small. We recommend that further studies be conducted to verify these results.

This study did not provide clear evidence of whether detection of PETH by LC-MS-MS differentiates light from heavy alcohol consumption. The ROC-AUCs for measures of any alcohol consumption and heavy alcohol consumption during the 21-day observation period

were very similar. The lack of differentiation might be because there was little light alcohol consumption, because the estimated amount of alcohol consumed per session was at least 40 grams for 75% of the visits in which alcohol consumption was reported and the total level of alcohol consumption between the “any” and the “heavy” groups were very similar. We suggest that studies that include a variety of drinking strata are needed to further delineate the drinking patterns for which PEth can be detected. In addition, we found nearly equivalent ROC-AUCs for any alcohol consumption in the prior 14 and 21 days, and more work is needed to determine the rate of elimination of PEth when determined by LC-MS-MS.

In summary, we conclude that PEth, a metabolite of alcohol consumption, may be a useful marker in research settings in which alcohol consumption may be hard to measure, such as in HIV positives in sub-Saharan Africa, where self-report is in question. We did not find as high sensitivity and specificity for heavy drinking as reported in some previous studies conducted using HPLC-ELSD methods, however the correlations with continuous measures of drinking were reassuring. While intensive laboratory methods preclude the routine use of PEth in developing countries, immunoassays may make the detection of PEth more widely accessible in the future (Nissinen et al., 2011). The use of valid biomarkers of alcohol consumption will improve research of the effect of alcohol consumption on the HIV epidemic, and markers that are available for routine use will improve individual patient care.

Acknowledgments

Funding: This study was funded by NIH/NIAAA R21 AA015897

References

- The World Health Report 2000 -- Health Systems: Improving Performance. WHO; Geneva: 2000.
- East African Breweries Limited; 2011a.
- Global Status Report on Alcohol and Health. World Health Organization; Geneva: 2011b.
- Aalto M, Alho H, Halme JT, Seppa K. AUDIT and its abbreviated versions in detecting heavy and binge drinking in a general population survey. *Drug Alcohol Depend.* 2009; 103:25–9. [PubMed: 19395203]
- Acuda SW, Sebit MB. Prevalence of psychoactive substance use among psychiatric in-patients in Harare, Zimbabwe. *Cent Afr J Med.* 1997; 43:226–9. [PubMed: 9431760]
- Adomakoh CC. Alcoholism: the African Scene. *Ann N Y Acad Sci.* 1976; 273:39–46. [PubMed: 1072366]
- Alt A, Janda I, Seidl S, Wurst FM. Determination of ethyl glucuronide in hair samples. *Alcohol Alcohol.* 2000; 35:313–4. [PubMed: 10869254]
- Anton RF. Editorial commentary: alcohol biomarker papers. *Alcohol Clin Exp Res.* 2010; 34:939–40. [PubMed: 20374210]
- Anton RF, O'malley SS, Ciraulo DA, Cisler RA, Couper D, Donovan DM, Gastfriend DR, Hosking JD, Johnson BA, Locastro JS, Longabaugh R, Mason BJ, Mattson ME, Miller WR, Pettinati HM, Randall CL, Swift R, Weiss RD, Williams LD, Zweben A. Combined pharmacotherapies and behavioral interventions for alcohol dependence: the COMBINE study: a randomized controlled trial. *Jama.* 2006; 295:2003–17. [PubMed: 16670409]
- Aradottir S, Asanovska G, Gjerss S, Hansson P, Alling C. PHosphatidylethanol (PEth) concentrations in blood are correlated to reported alcohol intake in alcohol-dependent patients. *Alcohol Alcohol.* 2006; 41:431–7. [PubMed: 16624837]
- Aradottir S, Moller K, Alling C. Phosphatidylethanol formation and degradation in human and rat blood. *Alcohol Alcohol.* 2004a; 39:8–13. [PubMed: 14691067]

- Aradottir S, Seidl S, Wurst FM, Jonsson BA, Alling C. Phosphatidylethanol in human organs and blood: a study on autopsy material and influences by storage conditions. *Alcohol Clin Exp Res*. 2004b; 28:1718–23. [PubMed: 15547459]
- Babor, T.; Higgins-Biddle, J.; Saunders, J.; Monteiro, MG. The Alcohol Use Disorders Identification Test: Guidelines for use in primary care. World Health Organization, Department of Mental Health and Substance Dependence; 2001.
- Bearer CF, Bailey SM, Hoek JB, Freeman WM, Vrana KE. Advancing alcohol biomarkers research. sFuture prospects for biomarkers of alcohol consumption and alcohol-induced disorders. *Alcohol Clin Exp Res*. 2010; 34:941–5. [PubMed: 20374221]
- Bergstrom JP, Helander A. Influence of alcohol use, ethnicity, age, gender, BMI and smoking on the serum transferrin glycoform pattern: implications for use of carbohydrate-deficient transferrin (CDT) as alcohol biomarker. *Clin Chim Acta*. 2008; 388:59–67. [PubMed: 17980706]
- Dahl H, Stephanson N, Beck O, Helander A. Comparison of urinary excretion characteristics of ethanol and ethyl glucuronide. *J Anal Toxicol*. 2002; 26:201–4. [PubMed: 12054359]
- Del Boca FK, Darkes J. The validity of self-reports of alcohol consumption: state of the science and challenges for research. *Addiction*. 2003; 98(Suppl 2):1–12. [PubMed: 14984237]
- Del Boca FK, Noll JA. Truth or consequences: the validity of self-report data in health services research on addictions. *Addiction*. 2000; 95(Suppl 3):S347–60. [PubMed: 11132362]
- DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics*. 1988; 44:837–45. [PubMed: 3203132]
- Fisher JC, Bang H, Kapiga SH. The Association Between HIV Infection and Alcohol Use: A Systematic Review and Meta-Analysis of African Studies. *Sex Transm Dis*. 2007; 34:856–863. [PubMed: 18049422]
- Freeman WM, Vrana KE. Future prospects for biomarkers of alcohol consumption and alcohol-induced disorders. *Alcohol Clin Exp Res*. 2010; 34:946–54. [PubMed: 20374220]
- Gillis LS, Stone GL. The fate of drinkers: a six-year follow-up study of a community survey. *S Afr Med J*. 1977; 51:789–91. [PubMed: 877806]
- Gnann H, Engelmann C, Skopp G, Winkler M, Auwarter V, Dresen S, Ferreiros N, Wurst FM, Weinmann W. Identification of 48 homologues of phosphatidylethanol in blood by LC-ESI-MS/MS. *Anal Bioanal Chem*. 2010; 396:2415–23. [PubMed: 20127079]
- Gnann H, Weinmann W, Engelmann C, Wurst FM, Skopp G, Winkler M, Thierauf A, Auwarter V, Dresen S, Bouzas NF. Selective detection of phosphatidylethanol homologues in blood as biomarkers for alcohol consumption by LC-ESI-MS/MS. *J Mass Spectrom*. 2009
- Gunnarsson T, Karlsson A, Hansson P, Johnson G, Alling C, Odham G. Determination of phosphatidylethanol in blood from alcoholic males using high-performance liquid chromatography and evaporative light scattering or electrospray mass spectrometric detection. *J Chromatogr B Biomed Sci Appl*. 1998; 705:243–9. [PubMed: 9521560]
- Hahn JA, Maier M, Byakika-Tusiime J, Oyugi JH, Bangsberg DR. Hepatotoxicity during nevirapine-based fixed-dose combination antiretroviral therapy in kampala, Uganda. *J Int Assoc Physicians AIDS Care*. 2007; 6:83–6.
- Hahn JA, Samet JH. Alcohol and HIV disease progression: weighing the evidence. *Curr HIV/AIDS Rep*. 2010; 7:226–33. [PubMed: 20814765]
- Hahn JA, Woolf-King SE, Muyindike W. Adding Fuel to the Fire: Alcohol's Effect on the HIV Epidemic in Sub-Saharan Africa. *Curr HIV/AIDS Rep*. 2011; 8:172–80. [PubMed: 21713433]
- Hannuksela ML, Liisanantti MK, Nissinen AE, Savolainen MJ. Biochemical markers of alcoholism. *Clin Chem Lab Med*. 2007; 45:953–61. [PubMed: 17579567]
- Hansson P, Caron M, Johnson G, Gustavsson L, Alling C. Blood phosphatidylethanol as a marker of alcohol abuse: levels in alcoholic males during withdrawal. *Alcohol Clin Exp Res*. 1997; 21:108–10. [PubMed: 9046381]
- Harrison L, Hughes A. Introduction--the validity of self-reported drug use: improving the accuracy of survey estimates. *NIDA Res Monogr*. 1997; 167:1–16. [PubMed: 9243554]
- Hartmann S, Aradottir S, Graf M, Wiesbeck G, Lesch O, Ramskogler K, Wolfersdorf M, Alling C, Wurst FM. Phosphatidylethanol as a sensitive and specific biomarker: comparison with gamma-

- glutamyl transpeptidase, mean corpuscular volume and carbohydrate-deficient transferrin. *Addict Biol.* 2007; 12:81–4. [PubMed: 17407500]
- Helander A, Beck O. Ethyl sulfate: a metabolite of ethanol in humans and a potential biomarker of acute alcohol intake. *J Anal Toxicol.* 2005; 29:270–4. [PubMed: 16105250]
- Helander A, Wielders JP, Jeppsson JO, Weykamp C, Siebelder C, Anton RF, Schellenberg F, Whitfield JB. Toward standardization of carbohydrate-deficient transferrin (CDT) measurements: II. Performance of a laboratory network running the HPLC candidate reference measurement procedure and evaluation of a candidate reference material. *Clin Chem Lab Med.* 2010; 48:1585–92. [PubMed: 21034250]
- Helander A, Zheng Y. Molecular species of the alcohol biomarker phosphatidylethanol in human blood measured by LC-MS. *Clin Chem.* 2009; 55:1395–405. [PubMed: 19423735]
- Hendershot CS, Stoner SA, Pantalone DW, Simoni JM. Alcohol use and antiretroviral adherence: review and meta-analysis. *J Acquir Immune Defic Syndr.* 2009; 52:180–202. [PubMed: 19668086]
- Hietala J, Koivisto H, Anttila P, Niemela O. Comparison of the combined marker GGT-CDT and the conventional laboratory markers of alcohol abuse in heavy drinkers, moderate drinkers and abstainers. *Alcohol Alcohol.* 2006; 41:528–33. [PubMed: 16799164]
- Isaksson A, Walther L, Hansson T, Andersson A, Alling C. Phosphatidylethanol in blood (B-PEth): a marker for alcohol use and abuse. *Drug Test Anal.* 2011; 3:195–200. [PubMed: 21438164]
- Kigozi IM, Dobkin LM, Martin JN, Geng EH, Muyindike W, Emenyonu NI, Bangsberg DR, Hahn JA. Late-disease stage at presentation to an HIV clinic in the era of free antiretroviral therapy in Sub-Saharan Africa. *J Acquir Immune Defic Syndr.* 2009; 52:280–9. [PubMed: 19521248]
- Litten RZ, Bradley AM, Moss HB. Alcohol biomarkers in applied settings: recent advances and future research opportunities. *Alcohol Clin Exp Res.* 2010; 34:955–67. [PubMed: 20374219]
- Marques P, Hansson T, Isaksson A, Walther L, Jones J, Lewis D, Jones M. Detection of phosphatidylethanol (PEth) in the blood of drivers in an alcohol ignition interlock program. *Traffic Inj Prev.* 2011; 12:136–41. [PubMed: 21469020]
- Miller WR, Heather N, Hall W. Calculating standard drink units: international comparisons. *Addiction.* 1991; 86:43–47.
- Morini L, Politi L, Poletini A. Ethyl glucuronide in hair. A sensitive and specific marker of chronic heavy drinking. *Addiction.* 2009; 104:915–20. [PubMed: 19392911]
- Msesigye PK, Okia Okurut T. A survey of the production and consumption of traditional alcoholic beverages in Uganda. *Process Biochemistry.* 1995; 30:497–501.
- Mwesigye P, Okurut T. A survey of the production and consumption of traditional alcoholic beverages in Uganda. *Process Biochemistry.* 1995; 30:497–501.
- Neumann T, Neuner B, Gentilello LM, Weiss-Gerlach E, Mentz H, Rettig JS, Schroder T, Wauer H, Muller C, Schutz M, Mann K, Siebert G, Dettling M, Muller JM, Kox WJ, Spies CD. Gender differences in the performance of a computerized version of the alcohol use disorders identification test in subcritically injured patients who are admitted to the emergency department. *Alcohol Clin Exp Res.* 2004; 28:1693–701. [PubMed: 15547456]
- NIAAA. NIAAA Council Approves Definition of Binge Drinking. NIAAA Newsletter. 2004
- Niemela O. Biomarkers in alcoholism. *Clin Chim Acta.* 2007; 377:39–49. [PubMed: 17045579]
- Nissinen AE, Laitinen LM, Kakko S, Helander A, Savolainen MJ, Horkko S. Low plasma antibodies specific for phosphatidylethanol in alcohol abusers and patients with alcoholic pancreatitis. *Addict Biol.* 2011
- Obot IS. Substance abuse, health and social welfare in Africa: an analysis of the Nigerian experience. *Soc Sci Med.* 1990; 31:699–704. [PubMed: 2237512]
- Omoluabi PF. A review of the incidence of nonprescription psychoactive substance use/misuse in Nigeria. *Int J Addict.* 1995; 30:445–58. [PubMed: 7607778]
- Papas RK, Sidle JE, Wamalwa ES, Okumu TO, Bryant KL, Goulet JL, Maisto SA, Braithwaite RS, Justice AC. Estimating Alcohol Content of Traditional Brew in Western Kenya Using Culturally Relevant Methods: The Case for Cost Over Volume. *AIDS Behav.* 2008
- Parry CD. South Africa: alcohol today. *Addiction.* 2005; 100:426–9. [PubMed: 15784051]

- Parry CD, Bhana A, Myers B, Pluddemann A, Flisher AJ, Peden MM, Morojele NK. Alcohol use in South Africa: findings from the South African Community Epidemiology Network on Drug use (SACENDU) Project. *J Stud Alcohol*. 2002; 63:430–5. [PubMed: 12160101]
- Rehm J, Rehn N, Room R, Monteiro M, Gmel G, Jernigan D, Frick U. The global distribution of average volume of alcohol consumption and patterns of drinking. *Eur Addict Res*. 2003; 9:147–56. [PubMed: 12970583]
- Stewart SH, Law TL, Randall PK, Newman R. Phosphatidylethanol and alcohol consumption in reproductive age women. *Alcohol Clin Exp Res*. 2010; 34:488–92. [PubMed: 20028353]
- Stewart SH, Reuben A, Brzezinski WA, Koch DG, Basile J, Randall PK, Miller PM. Preliminary evaluation of phosphatidylethanol and alcohol consumption in patients with liver disease and hypertension. *Alcohol Alcohol*. 2009; 44:464–7. [PubMed: 19535495]
- Varga A, Hansson P, Johnson G, Alling C. Normalization rate and cellular localization of phosphatidylethanol in whole blood from chronic alcoholics. *Clin Chim Acta*. 2000; 299:141–50. [PubMed: 10900300]
- Varga A, Hansson P, Lundqvist C, Alling C. Phosphatidylethanol in blood as a marker of ethanol consumption in healthy volunteers: comparison with other markers. *Alcohol Clin Exp Res*. 1998; 22:1832–7. [PubMed: 9835304]
- Wurst FM, Dresen S, Allen JP, Wiesbeck G, Graf M, Weinmann W. Ethyl sulphate: A direct ethanol metabolite reflecting recent alcohol consumption. *Addiction*. 2006; 101:204–11. [PubMed: 16445549]
- Wurst FM, Metzger J. The ethanol conjugate ethyl glucuronide is a useful marker of recent alcohol consumption. *Alcohol Clin Exp Res*. 2002; 26:1114–9. [PubMed: 12170122]
- Wurst FM, Seidl S, Ladewig D, Muller-Spahn F, Alt A. Ethyl glucuronide: on the time course of excretion in urine during detoxification. *Addict Biol*. 2002; 7:427–34. [PubMed: 14578020]
- Wurst FM, Thon N, Aradottir S, Hartmann S, Wiesbeck GA, Lesch O, Skala K, Wolfersdorf M, Weinmann W, Alling C. Phosphatidylethanol: normalization during detoxification, gender aspects and correlation with other biomarkers and self-reports. *Addict Biol*. 2010; 15:88–95. [PubMed: 20002024]

Figure 1.
Receiver operator receiver operating characteristic curve for phosphatidylethanol (PEth) compared to varying levels of alcohol consumption in the prior 21 days.

Figure 2. Scatterplots of phosphatidylethanol (PEth) versus continuous measures of alcohol consumption in the prior 21 days.

Table 1

Estimated total grams of alcohol consumed, 21-day observation period, by group, in Mbarara, Uganda (n=77)

Level of alcohol consumption	n	Median total grams (IQR)
All	77	98.7 (0-497.3)
Any alcohol consumption Yes No	51 26	437.3 (98.7-812.2) 0 (0-0)
Frequent alcohol consumption (≥ 3 x/week) Yes No	16 61	1374.0 (728.9-1840.6) 39.5 (0-266.0)
Any heavy * alcohol consumption Yes No	40 37	490.6 (267.2-1272.7) 0 (0 -19.7)
Frequent heavy * alcohol consumption (≥ 3 x/week) Yes No	11 66	1442.8 (918.7-1870.6) 49.3 (0-386.8)

* Any breath alcohol concentration>0.1% or daily self-reported consumption >42g (women) or >56g (men)

Table 2

ROC-AUC, sensitivity, and specificity of PEth 16:0/18:1 homologue in comparison to several measures of prior 21-day alcohol consumption, corroborated by breath alcohol testing (n=77)

Level of alcohol consumption	ROC-AUC (95% CI)	Sensitivity % (95% CI) (# PEth+/n)	Specificity % (95% CI) (# PEth-/n)
PEth versus prior 7-day alcohol consumption			
Any alcohol consumption	0.88 (0.81-0.96)	92.7 (80.1-98.5) (38/41)	72.2 (54.8-85.8) (26/36)
Frequent alcohol consumption (≥ 3x/week)	0.75 (0.64-0.86)	95.0 (75.1-99.9) (19/20)	49.1 (35.6-62.7) (28/57)
Any heavy* alcohol consumption	0.81 (71.3-90.8)	93.3 (77.9-99.2) (28/30)	57.4 (42.2-71.7) (27/47)
Frequent heavy* alcohol consumption (≥ 3x/week)	0.81 (0.71-0.96)	100.0 (75.3-100.0) (13/13)	45.3 (32.8-52.3) (29/64)
PEth versus prior 14-day alcohol consumption			
Any alcohol consumption	0.90 (0.83-0.96)	87.8 (75.2-95.4) (43/49)	82.1 (63.1-93.9) (23/28)
Frequent alcohol consumption (≥ 3x/week)	0.79 (0.68-0.89)	100.0 (79.4-100.0) (16/16)	47.5 (34.6-60.7) (29/61)
Any heavy* alcohol consumption	0.88 (0.80-0.95)	94.7 (82.2-99.4) (36/38)	69.2 (52.4-83.0) (27/39)
Frequent heavy* alcohol consumption (≥ 3x/week)	0.82 (0.72-0.92)	100.0 (71.5-100.0) (11/11)	43.9 (31.7-56.7) (29/66)
PEth versus prior 21-day alcohol consumption			
Any alcohol consumption	0.92 (0.86-0.97)	88.0 (76.1-95.6) (45/51)	88.5 (69.8-97.6) (23/26)
Frequent alcohol consumption (≥ 3x/week)	0.85 (0.77-0.94)	100.0 (79.4-100.0) (16/16)	47.5 (34.6-60.7) (29/61)
Any heavy* alcohol consumption	0.89 (0.81-0.96)	95.0 (83.0-99.9) (35/40)	73.0 (55.9-86.2) (27/37)
Frequent heavy* alcohol consumption (≥ 3x/week)	0.83 (0.77-0.93)	100.0 (71.5-100.0) (11/11)	43.9 (31.7-56.7) (29/66)

ROC-AUC: receiver operator characteristic area under the curve; PEth: phosphatidylethanol; PEth+: PEth greater than the limit of detection 10 ng/ml; PEth-: PEth undetectable

* Any breath alcohol concentration >0.1% or daily self-reported consumption >42g (women) or >56g (men)

Table 3

ROC-AUCs, sensitivity, and specificity (with 95% confidence intervals) for PEth homologue 16:0/18:1 compared to any alcohol consumption in the prior 21 days, by several covariates

	ROC-AUC (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)
Overall	0.92 (0.86-0.97)	88.0 (76.1-95.6)	88.5 (69.8-97.6)
Sex *			
Male (n=30)	0.98 (0.94 -1.00)	95.7 (78.0-99.9)	100.0 (59.0-100.0)
Female (n=47)	0.86 (0.77-0.96)	80.0 (56.3-94.3)	82.1 (60.4-96.6)
Age			
18-29 (n=29)	0.84 (0.69-0.99)	87.0 (66.4-97.2)	66.7 (22.3-95.7)
30-39 (n=33)	0.96 (0.89-1.00)	94.1 (71.3-99.9)	93.8 (69.8-99.8)
40-67 (n=15)	0.91 (0.71-1.00)	81.8 (48.2-97.7)	100.0 (39.8-100.0)
Body mass index <25 (n=62)	0.94 (0.90-0.99)	88.6 (75.4-96.2)	100.0 (81.5-100.0)
25 (n=14)	0.78 (0.52-1.00)	83.3 (35.9-99.6)	62.5 (24.5-91.5)
CD4			
<350 (n=33)	0.95 (0.89-1.00)	90.9 (70.8-91.9)	100.0 (71.5-100.0)
350 (n=42)	0.89 (0.80-0.98)	88.9 (70.8-97.6)	80.0 (51.9-95.7)
Hepatitis B virus infection status			
Positive (n=15)	0.91 (0.79-1.00)	81.8 (48.2-97.7)	100.0 (39.8-100.0)
Negative (n=59)	0.94 (0.88-0.99)	91.9 (78.1-98.3)	86.4 (65.1-97.1)
Antiretroviral therapy (ART) status			
Not on ART (n=47)	0.93 (0.87-1.00)	94.3 (80.8-99.3)	75.0 (42.8-94.5)
On ART (n=30)	0.88 (0.77-0.98)	75.0 (47.6-92.7)	100.0 (76.8-100.0)

ROC-AUC: receiver operator characteristic area under the curve; PEth: phosphatidylethanol

*
p-value for homogeneity of ROC-AUCs=0.03

Table 4

Spearman correlations ($p < 0.01$ for all) for PEth 16:0/18:1 with continuous measures of alcohol consumption in the prior 21 days ($n=77$).

Measure	Spearman r
Self-reported measures	
Number of days consumed any alcohol *	0.74
Number of days with heavy alcohol consumption *	0.68
Average grams of alcohol consumed ** per drinking day	0.60
Maximum grams of alcohol consumed ** per drinking day	0.65
Total grams of alcohol consumed	0.67
BRAC measures	
Average BRAC ** per drinking day	0.74
Maximum BRAC ** per drinking day	0.74
Cumulative BRAC over study period	0.75

PEth: phosphatidylethanol; BRAC: Breath alcohol concentration

* Corroborated by breath analysis

** Set to zero for those abstaining the entire period