Published in final edited form as:
Traffic Inj Prev. 2011 April ; 12(2): 136–141. doi:10.1080/15389588.2010.544048.

Detection of phosphatidylethanol (PEth) in the blood of drivers in an alcohol ignition interlock program

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Abstract

Objective—The rate of failed interlock blood alcohol concentration (BAC) tests is a strong predictor of recidivism post-interlock and a partial proxy for alcohol use. Alcohol biomarkers measured at the start of an interlock program are known to correlate well with rates of failed BAC tests over months of interlock use. This study evaluates two methods of measuring low blood levels of the biomarker PEth (phosphatidylethanol). PEth is a 100% alcohol specific biomarker and strongly intercorrelated with several independent indicators of drinking driving risk, including 8 other biomarkers, 3 psychometric assessments, and the rate of failed interlock BAC tests during many months of interlock use. Does a more sensitive method of measuring PEth at program entry detect drinking even among those who subsequently log no failed interlock tests?

Methods—In a sample of 281 driver blood samples, PEth was measured by both high-performance liquid chromatography (HPLC) and liquid chromatography tandem mass spectrometry (LCMSMS) in order to compare sensitivity and accuracy. The average rate of failed interlock BAC tests was the criterion measure for marker sensitivity. LCMSMS, calibrated to detect low levels of drinking as a possible measure of abstinence violation, was judged relative to the standard HPLC assay for PEth measured up to 4 μmol/L.

Results—The two methods showed a good quantitative relationship (r^2>.86). LCMSMS detected positive PEth levels in samples that were below the limit of detection of the HPLC method. PEth measured by LCMSMS was positive for a higher proportion of DUI offenders who logged zero failed interlock BAC tests than were detected by HPLC.

Conclusion—Although HPLC is the widely used standard for measuring PEth in clinical alcoholism samples, the LCMSMS method, when calibrated to detect trace amounts of the major component of PEth, can detect abstinence levels of alcohol near zero intake and still correlate strongly with other indicators related to alcohol use and road safety.

Keywords

phosphatidylethanol; HPLC; LCMSMS; DUI drivers; alcohol; interlocks; abstinence

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INTRODUCTION

A multinational collaborative research project studied alcohol biomarker of drivers convicted of driving under the influence (DUI) of alcohol; all participants were enrolled in the alcohol ignition interlock program in Alberta, Canada (Marques et al., 2010). Although an interlock can prevent a car from starting if the driver has been drinking alcohol, an important aspect of interlocks – beyond simply locking out the ignition – is the record of BAC tests these devices log each time a driver attempts a start. Only this aspect of the interlock is the topic of this paper. The interlock requires a breath sample below some cutoff level before a car can be started. In Alberta, BAC levels at or above .04 g/dL lock out the ignition; in the United States, .025 g/dL is the most common lock out setting. Studies have documented that the average DUI offender, who must pass an interlock BAC test before a car can start, logs 1000–1500 start attempts per year and more subsequent "rolling retests" after start up. The rate of failed BAC tests to total tests is a high sensitivity predictor of future recidivism as has been shown in Alberta, Quebec, and New Mexico (Marques et al., 2001; Marques et al., 2003b; Marques et al., 2010).

Alcohol biomarkers are often enzymes, or products of minor alcohol metabolism pathways. These enzymes or products can be measured in a variety of tissue types, persist for days after alcohol has cleared from circulation, and can serve as extended time indicators of alcohol level and use. They are often used in clinical decision-making about alcoholism but less often as an aid for driver licensing decisions (Bjerre et al., 2007). In the Marques et al. (2010) biomarker study, cooperating participants from the interlock program gave informed consent and provided specimens of blood, urine, hair, and also participated in psychometric assessments. It was found that those with the highest rates of failed BAC tests logged over an average of 8 months of interlock use (approximately 2,800 BAC tests), also had the highest levels of baseline alcohol biomarkers at the beginning of the interlock program. Knowledge of entry-level alcohol biomarkers proved to be predictive of future failed BAC tests, and failed BAC tests are known, based on studies in 3 different states and provinces, to predict future DUI recidivism.

In the United States, some interlock programs are managed by the courts and others by the licensing authorities. Court-based programs often advise the DUI offender that no drinking is permitted, and judges that give such directives sometimes like to use the interlock BAC test record as a means of documenting compliance or noncompliance with that directive. There are several ways for offenders to confound that plan, not the least of which is just not driving the interlock car. Unlike interlock BAC test records, alcohol biomarkers are specific to an individual (not a car), and some markers are sensitive enough to detect abstinence violations. The purpose of this study, using driver data, is to assess the sensitivity of a method for detection of low levels of phosphatidylethanol (PEth), an alcohol biomarker that is 100% specific to ethanol and that might serve the needs of courts that desire an analytic method to confirm abstinence from alcohol. We use drivers' rates of failed interlock BAC tests accumulated over an average of 8 months to serve as the criterion indicator of drinking.

But what is PEth? PEth is an abnormal phospholipid formed in mammalian cell membranes uniquely in the presence of ethanol and phospholipase D, a ubiquitous enzyme found in all cell membranes. From 1991 until 2010, there have been 40 research papers published on the use of PEth as a marker of excessive alcohol intake (Montisci et al., 2010). Studies have estimated it to be 100% specific (Wurst et al., 2010). The circulatory half life varies with alcohol intake; Hansson et al. (1997) reported it to be measurable in chronic alcoholics for 14 days after sobriety following entry into a rehabilitation clinic. PEth cannot be measured in people who do not drink alcohol.

The alcohol biomarkers GGT (gamma glutamyltransferase) and CDT (carbohydrate deficient transferrin) are sometimes used in treatment centers today as indicators of alcohol dependence levels of drinking, CDT more so than GGT. Both are well correlated to the rates of failed BAC tests (Marques et al., 2010) and other alcohol risk indicators, such as recidivism (Appenzeller et al., 2005; Gjerde et al., 1986). Of the biomarkers studied in the Alberta interlock study, PEth was the one with the most consistent and strongly significant association with other indicators of alcohol-related risk, including 9 of 9 other alcohol biomarkers tested, 16 of 19 scales of psychometric assessments, and many months of alcohol interlock BAC test results. The probability that the relationship between PEth levels at program entry and the rate of failed interlock BAC tests was just a chance association is $P<10^{-15}$.

Alcoholism Clinic versus Road Safety Samples

The PEth levels in the blood of DUI drivers entering an interlock program is much lower than values published for clinical alcoholism samples when measured at the commencement of treatment (Hartmann et al., 2006; Wurst et al., 2010). This is not surprising as PEth is a direct marker of recent consumption, and someone intending to drive an interlock-equipped car must keep drinking somewhat controlled in order to start the car. PEth levels in clinical samples of alcohol-dependent patients measured by high-performance liquid chromatography (HPLC) have been reported in one study as mean ± standard deviation of 2.47 ± 2.2 μmol/L (Hartmann et al., 2006) and, in another study, as 3.4 ± 2.6 μmol/L for outpatients with up to 7.7 μmol/L mean for inpatients (Aradottir et al., 2006). The range of PEth values in the DUI sample were well below those published for the alcoholism clinics; even the high alcohol risk subsample of 58 interlock drivers was reported as 1.45 ± 1.17 (20% of the total sample); (Marques et al., 2010).

Some of the drivers who log BAC test failures have undetectable PEth when measured by HPLC with an evaporative light scattering detector (ELSD). HPLC measurement of PEth is the most widely used method of measurement. Because some interlock users who failed BAC tests had undetectable levels of PEth measured by HPLC, we wanted to determine two things: (1) if an alternative method of analysis (LCMSMS – liquid chromatography tandem mass spectrometry), when tuned to detect one molecular species of PEth at low concentrations, would better discriminate drivers at the lower end of the interlock BAC risk continuum (sensitivity), and (2) how the quantification of PEth results compare across the two PEth analytic methods (accuracy). The HPLC method detects all molecular species of PEth, whereas the LCMSMS method detects only the most prevalent molecular species (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylethanol; PEth 16:0/18:1). Is there evidence that PEth measurement tuned to very low levels of alcohol use via this LCMSMS procedure has a potential for use by the courts or treatment programs to detect abstinence violations?

This report is an effort to ask the latter question as an extension of the Marques et al. (2010) study that has already documented multiple biomarker and psychometric profiles of interlock stipulated offenders. This subset report of the larger study includes new analyses.

METHOD

Blood, urine, and hair samples were collected from DUI offenders in Edmonton, Alberta, who gave consent to participate in a research study. Samples were shipped to different laboratories for analysis. A subset of 281 blood samples collected were measured for PEth by two different laboratories using two different methods: one in Sweden, and one in the United States. In August 2008, the final year of data collection, the last and largest batch of blood samples for PEth measurement (n=281) was sent to the Department of Clinical Chemistry, University Hospital, Lund, Sweden, and then later these were sent on to the

United States Drug Testing Laboratory (USDTL) in Des Plaines, Illinois, USA. All samples for PEth measurement were collected in heparinized tubes, stored and shipped at −80°C. PEth was measured in the 281 samples by both analytic methods; 56% were program entry samples and 43% program exit samples. The measured levels of both the HPLC and LCMSMS are reported with the same units (μmol/L). To do this, the LCMSMS laboratory reported values for PEth levels, originally reported in ng/ml, were converted to μmol/L by dividing by 703, the molecular weight of the target PEth species (16.0/18.1).

The procedure for the measurement of PEth by HPLC at the Lund laboratory has been described in several research papers (Varga et al., 2000; Varga et al., 1998). USDTL measured PEth by LCMSMS in accordance with a procedure modified from the published protocol of Helander and Zhang (2009). The LCMSMS-method is specific for the most prevalent molecular species (PEth 16.0/18.1). The limit of detection for the HPLC method of PEth measurement is 0.25 μmol/L. The limit of detection for the LCMSMS method in μmol/L is .005.

Measuring PEth first by HPLC and second by LCMSMS imposes a potential confound on the results if there was some degradation in the sample between the first and second measurements. Because the samples were frozen and thawed for the HPLC analyses and then subsequently frozen and thawed for measurement of PEth by LCMSMS, we examined whether multiple freeze thaw cycles affect PEth measurement by LCMSMS. To evaluate this, 5 PEth negative blood samples were spiked with PEth at .07, .14, and .28 μmol/L (equivalent to 50, 100, 200 ng/ml) and then subjected to three freeze thaw cycles to evaluate stability of the measured values.

Other Biomarkers

The 4 traditional biomarkers—gamma glutamyltransferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and mean corpuscular volume (MCV)—were measured locally in Alberta at Dynacare Kasper Laboratories; other specimens for biomarker measurement were batched and shipped to laboratories with special expertise. Serum was sent to the laboratory of Dr. Martin Javors at the University of Texas in San Antonio, USA, for measurement of percent carbohydrate deficient transferrin (%CDT) using Axis-Shield (Oslo, Norway) kits. Results from GGT and CDT were evaluated separately and also combined into γCDT (gamma CDT), a log transformed combination of CDT and GGT that is a single composite marker first reported by Finnish researchers (Sillanaukee and Olsson, 2001). Alcohol biomarkers in urine and hair samples were also measured in selected subsamples, but those analyses are not germane to this report because they were not run on the same subsample reported here.

Interlock Data

Among the 147 unique individuals with both interlock data and program entry biomarker results including both types of PEth, the interlocks logged 5 (median) or 6 (mean) start attempts per day during the full study period (at 6 months, a mean of 1115 and a median of 900 start tests were performed). The target for prediction is the rate of failed BAC tests (ignition lockouts relative to engine starts over time), not the BAC levels attained on tests or the post-start running retest results. Three subsets of drivers defined by the rate of failed interlock BAC tests were identified: the 27% with all zero failed BAC tests, the 20% with the highest rate (mean fail rate=3.4%), and the 53% between those extremes having a middle rate of failed tests (mean fail rate =0.5%). Defining the zero fails group was straightforward. Setting a split between the high and middle risk groups was done by inspection of the continuous series; the split was set at a gap in the sequence of failure rates. We used these 3 risk subgroups to evaluate alcohol biomarkers.

Statistical Analysis

All data analyses were computed with SPSS version 18 IBM Corporation 2010, Route 100, Somers, NY 10589. Methods include analysis of variance, Kruskal-Wallis nonparametric analyses, and linear regression; graphing was done in Microsoft Excel. In addition to the use of nonparametric statistics, the raw interval data were subjected to natural log transformation to limit the chance that skewed distributions and outliers unduly influenced conclusions. Those log transformation results are not reported here, but they confirmed and improved upon the initial analyses of group differences based on the raw data that are reported here.

RESULTS

Comparison of Methods within the DUI Sample

Analyses of the DUI driver blood samples for PEth levels found that the two analytic methods showed a strong relationship (r^2=.86), 86% of the variance is shared as can be seen in the scatter plot of Figure 1. Results below the limits of detection are set to zero for both methods. Not shown separately is the test and retest of individuals tested by both methods at two time points an average of 8 months apart. The initial (program entry) and followup (program exit) scatter plots of PEth by the HPLC and LCMSMS for those participants were very close with r^2 > .85 and .87, respectively. The coefficient in the equation in Figure 1 suggests that the molecular species of PEth 16:0/18:1 on average represents approximately 45 % of total PEth, which is consistent with the findings of Helander and Zheng (2009). This is why the scatter plot of the LCMSMS results are (on a molar basis) less than half the values found with HPLC method.

In Figure 1, it is also evident that there are a substantial number of zero values (< 0.25 μmol/L) on the HPLC (X)-axis that have positive measured values on the LCMSMS (Y)-axis. This apparent difference in low-end measured values suggests that the LCMSMS method is detecting alcohol use more sensitively.

Other estimates of driver alcohol use (other biomarkers as well as the rate of failed BAC tests from the interlock) were available in this data set as well. Even though entry samples of alcohol biomarkers reflect drinking just days to weeks before measurement, they were strongly related to the rate of failed BAC tests during the mean 8 months of alcohol interlock use. The three subgroups of drivers defined by rates of failed BAC tests (zero, low, high) are shown in Table 1, with the mean, standard deviation and median values of different alcohol biomarkers for each subgroup. In addition to the results from the two methods of measuring PEth, other markers (MCV, AST, ALT, GGT, %CDT), and the calculated combination of GGT and %CDT, γCDT (Sillanaukee and Olsson, 2001;Portman et al., 2010) are also shown segregated by a subset (listwise selection) of the interlock-defined risk subgroups. For each marker, the chance probability that these subgroups did not differ is shown for both the ANOVA F ratio and Kruskal Wallis k statistics.

All markers, except ALT, are significantly different by both parametric and nonparametric statistics, and in all cases, post-hoc analyses showed the unique subgroup is one with high alcohol risk. Raw values in Table 1 show that by clinical standards, only the high alcohol risk group has alcohol biomarkers that are above, or approaching, the cutoff levels often used for clinical samples. The high-risk alcohol group as defined by the interlock BAC test fail rates had approximately 8 times more failed BAC tests than the low-risk group. The mean GGT (127 U/L) and %CDT (3.7%) of the high fail risk group are both above widely used clinical cutoff levels for problem drinking of 75 and 2.7, respectively. The median GGT of 50 for the high-risk group is somewhat below clinical cutoff, and the median %CDT is well above the clinical cutoff. There is no recognized clinical reference level or cutoff value for PEth, but as noted before, the PEth mean ± s.d. for outpatient clinic samples

reported by Hartmann et al. (2006) was 2.47 ± 2.2 μmol/L. The mean HPLC PEth in this DUI subsample is 1.41, about one s.d. below the mean of the Hartmann et al. clinic sample also measured by HPLC.

Can LCMSMS PEth analysis detect drinking that is not detectable by HPLC PEth measurement? Figure 2 compares the baseline PEth of the two methods against the three interlock BAC fail groups. The Y axis shows the proportion of the group that had any detectable PEth by each method. In the high fail group, detection of positive values of PEth was similar by both methods with PEth found in nearly 100% of the group members. The two lower-risk groups had lower rates of failed BAC tests (by definition), but the LCMSMS method of PEth measurement detected a higher proportion than HPLC of those at these lower-risk levels who nonetheless consume alcohol. For the zero BAC group, it should be noted that zero interlock BAC fails does not mean zero drinking – it means zero drinking-driving logged on the interlock car, so it is not diagnostic of baseline drinking levels, but it is a behavioral correlate. ANOVA showed that both analytic methods easily discriminated PEth levels by interlock BAC alcohol risk group (F ratio = 13.3 by HPLC, F ratio = 16.1 by LCMSMS; both $P<.0005$) and both methods found the high-risk group to be uniquely different (Scheffé procedure) from the two lower-risk groups.

In a crosstabs of PEth values measured jointly by both methods (not shown), 17 were detected as zero (below the limit of detection) by both methods, and 27 had measureable levels of PEth by LCMSMS that were undetectable by HPLC (below the limit of detection). One measured as undetectable by LCMSMS was positive by HPLC. Overall, 88.5% were positive for PEth by LCMSMS; 71.2% were positive by HPLC.

Freeze Thaw Levels of PEth by LCMSMS

Five blood samples spiked with known quantities of PEth and then subjected to three freeze thaw cycles revealed that the refrozen samples ranged from 9% less to 26% less in the post-thaw measurements; all 5 samples varied in a narrow range at each concentration of spiked PEth. This suggests that there may be some loss of measureable PEth following freezing. However, because the LCMSMS measurements (which we have determined to be more sensitive than HPLC in this study) followed the freezing of samples measured by HPLC, it is unlikely that the apparently higher sensitivity reported for LCMSMS relative to HPLC is a secondary consequence of the additional freeze-thaw cycle.

DISCUSSION

The blood marker results from the DUI samples reflect the drinking levels at the commencement of an interlock program in Alberta, Canada. In Alberta, like the United States, the “legal limit” is set at .08 g/dL. The average BAC of a driver arrested for alcohol in North America is in the range of .15–.16 g/dL. Accordingly, those who are arrested for DUI and later enter interlock programs are known to be significant drinkers who have to learn how to adjust their drinking habits in order to participate in an interlock program. Drivers who want to use their interlock cars must limit their overall levels of drinking, or else use the car more sparingly, in order to avoid ignition lockouts as alcohol interlock devices prevent engine starts when the driver’s BAC is over .04 g/dL (in Alberta). U.S. states and other Canadian provinces lock out engine starts when the driver’s BAC is in the range of .02 to .051 g/dL. Failed BAC tests that lead to lockouts are an established proxy for alcohol-related driving risk and strongly predict future DUI recidivism.

In an Expert Panel comprised of judges, researchers, government officials, victim advocates, and industry representatives, it was learned that judges often impose an expectation of abstinence on the DUI offenders who come before them in the court (Marques and Voas,

2010). Although the interlock is not well suited to serve as an abstinence-monitoring device, other methods can accomplish this task. Among these methods are daily BAC measurements, alcohol-monitoring bracelets, or occasional measurement of alcohol biomarkers. The periodic use of alcohol biomarkers would render abstinence monitoring for alcohol as similar to abstinence monitoring for drugs of abuse.

By re-measurement of blood PEth with a different technology, this sub-study has provided evidence that a narrowly tuned LCMSMS method for detecting one specific molecular species of the alcohol consumption marker PEth, can identify more non-abstinent DUI offenders than can the wide spectrum HPLC method. The HPLC method used is the best choice for measuring PEth in clinical studies of alcohol consumption in which patients may have high rates of consumption and high levels of PEth. While measurements in the current study were made on interlock program entry samples, the findings have broad implications on the availability of a targeted method for detecting low levels of drinking at any time. The LCMSMS method targeting the single dominant molecular form of PEth and the HPLC method that measures all PEth have a strong relationship (r^2>.86) when levels are less than 4 μmol/L. Figure 2 shows that the LCMSMS PEth measurement detects drinking in the lowest risk subset of drivers (defined by failed interlock BAC tests) that the HPLC PEth method could not detect. At program entry, PEth was detectable in 88.5% of the offenders with LCMSMS, whereas 71.2% had detectable PEth by HPLC. This suggests LCMSMS was able to detect more alcohol use.

Studies that have analyzed the interlock BAC test results have shown that most interlock stipulated drivers continue to drink, at least initially (Marques et al., 2001; Marques et al., 2003a), even when ordered to no longer drink at all. Those who choose not to drive the interlock car when under a stipulation to only drive that way can potentially confound courts or other monitoring agencies that expect the interlock record of BAC tests to always be an adequate way to document drinking. As more states require a period of interlock-controlled driving as a condition of license reinstatement (e.g., Florida, Arizona, Washington, and other states), there may be more DUI offenders who find an incentive to avoid the interlock car due to the restrictions it places on their drinking. In such cases, alcohol biomarkers that can sensitively detect consumption could be deployed to address the question of compliance. Although complete abstinence from alcohol is not a necessary target goal for all DUI offenders, courts that impose an abstinence requirement will need better methods than interlock BAC tests.

For safety-related alcohol use countermeasures, the cost, speed, accuracy, and availability of analytic methods are important considerations in the selection of detection methods. Many alcohol remediation programs, especially court-ordered programs, are less concerned about levels of drinking, and prefer to know if any drinking at all has occurred. In the medical clinic, actual drinking levels are important to determine problem magnitude and treatment progress. Accordingly, different methods are appropriate for different questions about drinking. Linking improved monitoring to better treatment might one day reduce the cycle of catch and release of DUI offenders.

Acknowledgments

This work was supported by two grants from the National Institute on Alcohol Abuse and Alcoholism: one to study Alcohol Interlocks and Biomarkers (US NIH/NIAAA 5 R01 AA014206) and the other for an Impaired Driving Center (US NIH/NIAAA P20 AA017831).

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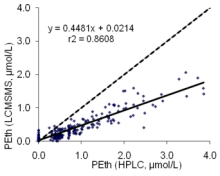

Fig 1.

Scatter plot of PEth from DUI drivers in an alcohol interlock program. PEth 16:0/18:1by LCMSMS (Y axis) is about 45% of Total PEth by HPLC (X axis).

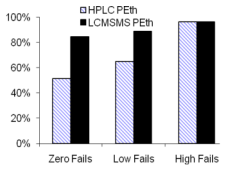

Fig. 2.

Bars represent percent of the initial blood samples of DUI offenders with detectable PEth by HPLC and LCMSMS for the three alcohol risk groups defined by rate of failed interlock BAC tests.

Traffic Inj Prev. Author manuscript; available in PMC 2012 April 1.

Table 1

Program entry alcohol biomarkers by interlock risk group, subset of 147 with complete values

Report									
Interlock Fail Rate Bins		**MCV[1,4] fL**	**ALT[3] U/L**	**AST[1,4] U/L**	**GGT[1,4] U/L**	**CDT[1,4] %**	**gamma[1,2] CDT**	**PEth[1,2] HPLC µmol/L**	**PEth[1,2] LCMSMS µmol/L**
None n=39	Mean	90.5	28.0	24.8	28.3	2.58	3.74	.51	.23
	Std. Dev	3.5	15.0	13.2	20.6	.859	.54	.66	.29
	Median	91.0	24.0	21.0	22.0	2.4	3.62	.42	.11
Low n=80	Mean	90.7	32.0	26.2	41.2	2.75	4.01	.63	.32
	Std. Dev	3.8	20.1	8.1	37.7	1.19	.69	.67	.34
	Median	91.0	25.5	24.0	29.5	2.55	3.97	.48	.23
High n=28	Mean	93.6	49.5	38.7	127.9	3.70	4.83	1.41	.74
	Std. Dev	4.5	49.2	30.6	217.6	1.87	.977	1.11	.59
	Median	93.5	34.5	26.0	50.0	3.14	4.73	.84	.47
Total n=147	Mean	91.2	34.27	28.23	54.3	2.89	4.10	.75	.38
	Std. Dev	4.0	28.0	16.8	104.6	1.33	.80	.83	.42
	Median	91.0	26.0	24.0	26.0	2.59	3.99	.50	.26
	N	147	147	147	147	147	147	147	147

[1] P<.001 Analysis of Variance

[2] P<.001 Kruskal Wallis non-parametric

[3] P<.01 ANOVA

[4] P<.01 Kruskal Wallis non-parametric