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ORIGINAL ARTICLE

Phosphatidylethanol (PEth) in blood samples from "driving under the influence" cases as indicator for prolonged excessive alcohol consumption

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Abstract Phosphatidylethanol (PEth) is considered as specific biomarker of alcohol consumption. Due to accumulation after repeated drinking, PEth is suitable to monitor long-term drinking behavior. To examine the applicability of PEth in "driving under the influence of alcohol" cases, 142 blood samples with blood alcohol concentrations (BAC) ranging from 0.0–3.12‰ were analyzed for the presence of PEth homologues 16:0/18:1 (889±878 ng/mL; range <LOQ to 5400 ng/mL) and 16:0/18:2 (355±315 ng/mL; range <LOQ to 1440 ng/mL) by LC-MS/MS. With receiver operating characteristic analysis, PEth thresholds were evaluated to differentiate moderate and excessive alcohol consumption with acceptable sensitivity and specificity in accordance with the 1.6‰ BAC limit. With a threshold of 700 ng/mL for PEth 16:0/18:1, prolonged excessive alcohol consumption was detected in 65.9 % of drunk drivers with a BAC≥1.6‰ and in 31.6 % of the samples with a BAC<1.6‰. Similar results were obtained for PEth 16:0/18:2 with a threshold of 300 ng/mL. Both criteria, PEth 16:0/18:1 and PEth 16:0/18:2, were conform in the evaluation of drinking habits in 88.7 % of blood samples. These results show the possibility to detect prolonged excessive alcohol consumption, even if the BAC is below the legal threshold of 1.6‰ for driving aptitude assessment. As a consequence, concentrations of PEth 16:0/18:1≥700 ng/mL and of PEth 16:0/18:2≥300 ng/mL may be considered as indicators for the necessity of driving aptitude assessment in addition to BAC.

Keywords Phosphatidylethanol · Alcohol biomarker · LC-MS/MS · Driving under the influence (DUI)

Introduction

The abuse of alcohol is a worldwide social and public health problem. To reveal problematic drinking habits and alcohol misuse, especially concerning the risks of driving under the influence of alcohol (DUI), there are several direct (blood alcohol concentration (BAC), ethyl glucuronide (EtG), ethyl sulfate (EtS), phosphatidylethanol (PEth)), and indirect alcohol biomarkers (carbohydrate deficient transferrin (CDT), γ-glutamyltranspeptidase (GGT), etc.) detectable in body fluids.

BAC, EtG, and EtS reveal recent alcohol exposure [1, 2]. CDT and GGT are used to detect prolonged risky alcohol consumption. However, CDT and GGT are not only influenced by alcohol intake alone: gender, age, smoking, and various diseases can increase these markers as well [3].

A new and more specific alcohol biomarker in this field is PEth, as it is formed directly after alcohol intake [4, 5]. PEth is a direct alcohol metabolite present in cell membranes with a half-life of four days [6]. It represents a group of phospholipid homologues which have a glycerol structure, esterified with two fatty acids in sn-1 and sn-2 position—containing 14 to 22 carbon atoms with zero to six double bonds—and with ethyl phosphoric acid in sn-3 position. So far, 48 different PEth homologues have been identified, due to the variations of fatty acids [7]. PEth was first discovered in rats [8] and is biosynthesized from phosphatidylcholine (PC) catalyzed by the enzyme phospholipase D (PLD), as long as ethanol is present in the organism [9]. After blood sampling, the formation of PEth continues during storage at room temperature and at −20 °C, if ethanol is present in the sample. In contrast,

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formation of PEth is not observed during storage at +4 °C for up to 3 weeks [10, 11].

Due to its high specificity for the detection of heavy and prolonged alcohol consumption, PEth is regarded as advantageous compared to the indirect markers CDT and GGT [12], and various PEth thresholds for the differentiation of moderate from prolonged excessive alcohol consumption have been proposed [12–14].

In Switzerland, administrative regulations for traffic cases use a limit of BAC of 1.6‰ as a threshold for driving aptitude assessment, as prolonged excessive alcohol misuse is suspected above this limit. Drinking habits cannot be derived from the BAC alone, as about 30–40 % of motorists with problematic alcohol consumption remain undetected because of BAC levels below 1.6‰ at the time of control. Therefore, already 21 years ago, Iffland [15] suggested to use other markers such as GGT, CDT, methanol, and the sum of acetone and 2-propanol in addition to BAC to diagnose problematic alcohol consumption with higher sensitivity. The direct alcohol biomarker PEth might be a new option for this purpose.

For this study, the two most abundant PEth homologues in human blood PEth 16:0/18:1 and PEth 16:0/18:2 [16, 17] were determined in blood samples from DUI cases. The sum of these two PEth homologues matches with approx. 62 % of "total PEth" [17].

With receiver operating characteristic (ROC) analysis of PEth and BAC in DUI cases, we determined thresholds of PEth homologues, which—besides the threshold of BAC≥1.6‰—can be considered as indicator for prolonged excessive alcohol consumption and the necessity of driving aptitude assessment.

Material and methods

Chemicals and materials

PEth 16:0/18:1, PEth 16:0/18:2, PC 16:0/18:1, and PC 16:0/18:2 were obtained from Avanti Polar Lipids (Alabaster, USA). PLD and n-hexane were from Sigma-Aldrich (Buchs, Switzerland). Acetone, ammonium acetate, chloroform ($CHCl_3$), D_6-ethanol, diethyl ether, $CaCl_2$, and Na_2HPO_4 were obtained from Merck (Darmstadt, Germany). 2-Propanol was provided by Fisher Scientific (Loughborough, UK), methanol (MeOH) was from Biosolve BV (Valkenswaard, the Netherlands), and acetonitrile (MeCN) was supplied by Agros Organics (New Jersey, USA). Deionized water was produced in-house with a Milli-Q water system from Millipore (Billerica, USA). Blank blood was obtained from the blood donation center in Bern, Switzerland.

Synthesis of deuterated PEth homologues

The deuterated standards D_5-PEth 16:0/18:1 and D_5-PEth 16:0/18:2 were synthesized in our laboratory from PC 16:0/18:1 and PC 16:0/18:2, respectively, and D_6-ethanol catalyzed by PLD.

Ten milligrams of PC 16:0/18:1 and PC 16:0/18:2, respectively, were dissolved in 1.5 mL diethyl ether. Then, 2.25 mL of buffer solution (100 mM ammonium acetate with 100 mM $CaCl_2$, pH 5.9) and 200 μL D_6-ethanol were added. With stirring, the mixture was heated to 34–35 °C. To start the reaction, 150 μL of enzyme solution (1.0 mg/mL; PLD dissolved in deionized H_2O) were added to the mixture of PC and D_6 ethanol. During the entire reaction time, the temperature was held at 34–35 °C, as this is the optimal temperature for PLD activity. After 2 and 4 h, 10 μL of the organic phase were analyzed to monitor the reaction progress. The reaction was stopped after 4 h, when no more PC was detectable in the reaction mixture. After cooling, the organic phase was transferred to a micro-tube and the aqueous phase was washed three times with 1.5 mL of diethyl ether. Then, all organic phases were combined and evaporated to dryness (under a stream of nitrogen at 50 °C). The white residue was dissolved in 5 mL of $CHCl_3$/MeOH (5:8, *v*/*v*) and filtrated. The clear solution was evaporated and finally dissolved in 1 mL $CHCl_3$.

This synthesis was performed as described in [17, 18] with small modifications.

Determination of BAC

The ethanol concentration in lithium-heparinized blood samples from DUI cases was determined by a validated headspace gas chromatography method with flame ionization detection (HS-GC-FID). Samples were analyzed according to Swiss forensic guidelines with two GC-FID systems, in duplicate on each system [19].

Determination of PEth

For analyzing PEth 16:0/18:1 and PEth 16:0/18:2, 100 μL of lithium-heparinized whole blood, 10 μL of internal standard (D_5-PEth 16:0/18:1, D_5-PEth 16:0/18:2), and 50 μL of phosphate buffer (pH 9) were pipetted into a micro-tube and vortex-mixed. Then, 400 μL of 2-propanol was added. After 10 min of agitation, 600 μL of hexane was added and vortex-mixed again for 10 min and afterward centrifuged for 10 min at 16,000*g*. Five hundred microliters of the hexane phase were separated, evaporated to dryness under a stream of nitrogen at 50 °C and redissolved in 100 μL of mobile phase A. An aliquot of 10 μL was injected into the LC-MS/MS system.

To ensure the stability of PEth and to prevent post-sampling formation of PEth, blood samples were stored at +4 °C. The analysis was performed within 1 week after blood sampling.

Method validation

Method validation for the determination of PEth in whole blood was performed according to the guidelines of the FDA [20, 21]. The investigated parameters were selectivity, linearity, limit of quantification (LOQ), limit of detection (LOD), precision (expressed as the relative standard deviation (RSD%)), accuracy (expressed as the mean relative error (RE%)), matrix effects, and carryover.

To determine the selectivity of the developed PEth method, six blank samples of blood from alcohol abstinent people were analyzed to test for interferences from endogenous matrix components or metabolites, which could disturb the signals of PEth homologues or internal standards.

For the calibration samples, working solutions (2.50, 6.25, 12.5, 25.0, 50.0, and100 μg/mL) for PEth 16:0/18:1 and PEth 16:0/18:2 were prepared in ammonium acetate (2 mM)/acetonitrile solution (20:80, *v*/*v*), and 10 μL of each was spiked into 240 μL aliquots of blank blood. Six-point calibration curves of PEth 16:0/18:1 and PEth 16:0/18:2 were prepared in duplicate on three different days. The calibrators had the following concentrations: 100, 250, 500, 1000, 2000, and 4000 ng/mL.

Precision and accuracy were determined by preparing blood samples (quality control samples, QC) spiked at different PEth concentration levels: 300 ng/mL (QC_{low}), 800 ng/mL (QC_{medium}), and 1600 ng/mL (QC_{high}). A higher QC concentration was not used, since the relevant decision limit of 700 ng/mL chosen for this study was well within the range covered by the QC controls.

The matrix effect was studied qualitatively by measuring extracts of blank blood samples from six different sources with simultaneous post-column infusion (10 μL/min) of solutions of PEth in mobile phase A at concentrations of 500 and 2000 ng/mL, respectively, into the eluent before entering the electrospray ion source. In this way, any substance from the blank blood sample eluting from the column and causing a deviation in electrospray ionization (ESI) response is seen as a suppression or enhancement of the infused PEth [22–24].

Carryover was investigated by injecting the highest calibrator (4000 ng/mL) three times in a row followed by a blank blood sample in duplicate to test if substances of the previous injections are carried over to the next measurement.

Instrumentation

The LC-MS/MS system consisted of a CTC PAL autosampler (CTC Analytics, Zwingen, Switzerland), an Agilent 1200 series HPLC (Agilent, Waldbronn, Germany) and a 3200 QTrap mass spectrometer (Sciex, Toronto, Canada) controlled by Analyst™ software (version 1.5.1).

Analytical separation was performed by a Luna RP-C5 column, 50 mm×2 mm, 5 μm (Phenomenex, Brechbühler, Schlieren, Switzerland) heated to 50 °C with a flow rate of 0.3 mL/min. Mobile phase A consisted of ammonium acetate (2 mM)/acetonitrile (30:70, *v*/*v*) solution, and mobile phase B was 2-propanol. The following 10-min gradient was used: 0 to 1.5 min, 10 % B; 1.5 to 2.5 min, 10 to 40 % B linear; 2.5 to 3.5 min, 40 to 100 % B linear; 3.5 to 4.5 min, 100 % B; 4.5 to 6 min, 100 to 10 % B linear; and 6 to 10 min, 10 % B. Post-column infusion of 2-propanol (0.3 mL/min) was used to increase the signal intensity.

The mass spectrometer was operated in negative ESI MRM mode, with an ion-spray voltage of −4250 V and a source temperature of 650 °C with the following transitions for PEth 16:0/18:1: m/z 701.5/255.1 (quantifier), m/z 701.5/281.1 and m/z 701.5/437.2 (qualifiers), and m/z 706.5/281.1 (D_5-PEth 16:0/18:1). For PEth 16:0/18:2, the transitions were the following: m/z 699.5/255.2 (quantifier), m/z 699.5/279.2 and m/z 699.5/437.2 (qualifiers), and m/z 704.5/279.4 (D_5-PEth 16:0/18:2).

Statistic evaluation

ROC analysis was performed with GraphPad Prism 6 by classifying the results into two groups depending on the BAC.

Ethical permission

The analysis of PEth in blood samples from traffic control cases has been approved by the Cantonal Ethics Commission Bern (064/13) on March 03, 2014.

Results

Method validation

Concerning selectivity, no interferences could be observed in any of the tested blank samples. A linear calibration model with weighting 1/x was used in the range of 100–4000 ng/mL with correlation coefficients>0.99. For the purpose of this study, we used the lowest calibrator (100 ng/mL) as the limit of quantification (LOQ). The LOQ had a signal-to-noise (S/N) ratio>15 for PEth 16:0/18:1 and PEth 16:0/18:2, respectively. The limit of detection (LOD) was 40 ng/mL with an S/N ratio of 4.3 for PEth 16:0/18:1 and an S/N ratio of 7.6 for PEth 16:0/18:2, respectively.

Precision (6.4–17.5 RSD%) and accuracy (9.0–16.2 RE%) were in acceptable ranges.

By post-column infusion experiments, only minor suppression effects were detected at the retention times of both PEth homologues. Signal intensities of the analytes as well as those of the internal standards showed minor variations in different samples within several series. These effects—due to variation of extraction efficiency and due to variability of matrix effects—were compensated by addition of the corresponding

deuterated internal standards prior to extraction. Since these internal standards are co-eluting with the analytes and have the same chemical properties, they compensate these effects. Carryover was not detected.

Blood alcohol and PEth determination

One hundred forty-two blood samples from traffic control cases, containing 104 traffic controls and 38 traffic accidents, were analyzed. The controlled persons were between 16 and 82 years old (40±14 years) with a mean BAC of 1.40±0.59‰ (range 0.0–3.12‰). Twenty-two persons were female and 120 male. The mean time between control and blood sampling was 69±50 min (range 10 min to 5 h).

With a retention time of 5.25 min, the mean concentration of PEth 16:0/18:1 was 889±878 ng/mL (range <LOQ to 5400 ng/mL). The sample with 5400 ng/mL was the only one, which exceeded the calibration range. It was reanalyzed by use of less blood (50 μL instead of 100 μL) to fit in the calibration range. For PEth 16:0/18:2 with a retention time of 4.8 min, the mean concentration was 355±315 ng/mL (range <LOQ to 1440 ng/mL). A typical chromatogram of a DUI sample with positive PEth results is shown in Fig. 1, and detailed PEth and BAC results are listed in Table 1.

For PEth 16:0/18:1, a threshold to differentiate moderate from prolonged excessive alcohol consumption was evaluated by ROC analysis. Using the software GraphPad Prism 6, the PEth results were classified into two groups depending on the BAC (Fig. 2). All test persons with a BAC above 1.6‰ were assumed to have prolonged excessive drinking habits; however, no other parameters as indicator for consumption habits have been tested upon these persons.

The best compromise for sensitivity and specificity was reached by using a threshold of 700 ng/mL: For PEth 16:0/18:1, a sensitivity of 65.9 % and a specificity of 68.4 % were calculated. A higher threshold (800 ng/mL) leads to a higher specificity (72.5 %) but less sensitivity (56.8 %) for differentiation of prolonged excessive drinking and social drinking habits (with a decision limit of BAC 1.6‰). With a lower threshold (e.g., 600 ng/mL) the method would be more sensitive (72.7 %) but less specific (64.3 %) compared to a threshold of 700 ng/mL.

For PEth 16:0/18:2, ROC analysis resulted in a threshold of 300 ng/mL with a sensitivity of 61.4 % and a specificity of 66.3 % (Fig. 3).

In Fig. 4, the results of PEth 16:0/18:1 are compared to BAC. BAC was grouped in two categories (social drinking< 1.6‰ and (prolonged) excessive drinking≥1.6‰) following the legal situation in Switzerland with BAC 1.6‰ as decision limit for driving aptitude assessment.

In 65.9 % of the tested blood samples with a BAC≥1.6‰, the concentration of PEth 16:0/18:1≥700 ng/mL indicated prolonged excessive drinking habits. Below BAC 1.6‰, still 31.6 % of the samples showed PEth concentrations ≥700 ng/mL. In 140 of 142 samples, PEth 16:0/18:1 concentrations exceeded the PEth 16:0/18:2 concentrations. With a threshold of 300 ng/mL for PEth 16:0/18:2 in comparison to BAC, similar results were obtained for specificity and sensitivity as for PEth 16:0/18:1 with a decision limit of 700 ng/mL (Fig. 5). In 61.4 % of the samples with BAC≥1.6‰, concentrations of PEth 16:0/18:2 were above this threshold. Below BAC 1.6‰, still 33.7 % of the samples had PEth 16:0/18:2≥300 ng/mL. When comparing both thresholds, 700 ng/mL for PEth 16:0/18:1 and 300 ng/mL for PEth 16:0/18:2, 36.6 % of the samples indicate prolonged excessive drinking habits for both homologues. In 52.1 % of the cases, both PEth homologues indicate social drinking habits. Results were conformed for both homologues in 88.7 % of all cases (Fig. 6).

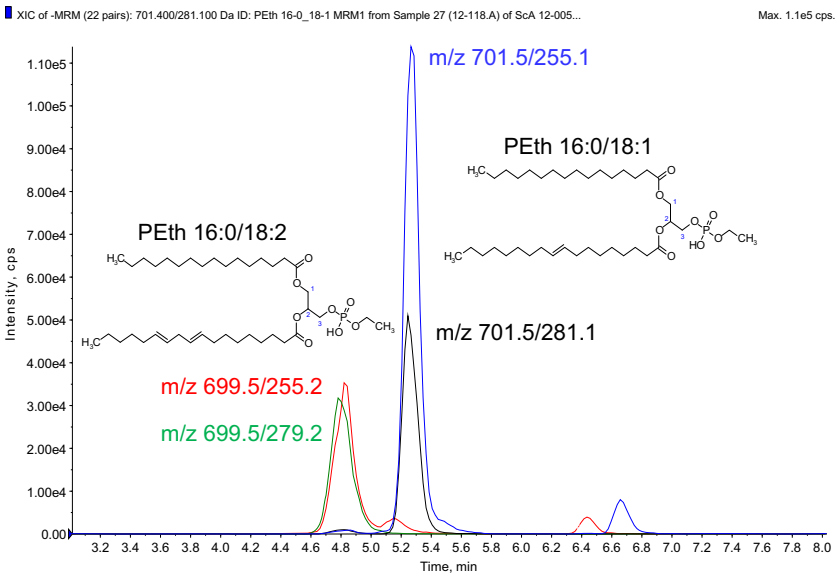

Fig. 1 Typical chromatogram of a DUI sample with positive PEth results: PEth 16:0/18:2 1035 ng/mL, retention time 4.8 min; PEth 16:0/18:1 2280 ng/mL, retention time 5.25 min; BAC 1.24‰. The structural formulas of both homologues are shown (PEth 16:0/18:2 (*left*) and PEth 16:0/18:1 (*right*))

Table 1 Mean, minimum, and maximum BAC and PEth values with standard deviations (SD) and mean, minimum, and maximum times between control and blood sampling with standard deviations (SD) of 142 samples from traffic control cases

	BAC (‰)<1.6	BAC (‰)≥1.6
Number of samples	98	44
Mean BAC and SD (‰)	1.10±0.40	2.07±0.36
Minimum BAC (‰)	0	1.64
Maximum BAC (‰)	1.58	3.12
Mean time between control and blood sampling with SD (min)	63±45	80±60
Minimum of time between control and blood sampling (min)	15	10
Maximum of time between control and blood sampling (min)	285	305
Number of samples with PEth 16:0/18:1<700 ng/mL	67	15
Number of samples with PEth 16:0/18:1≥700 ng/mL	31 (31.6 %)	29 (65.9 %)
Mean PEth 16:0/18:1 and SD (ng/mL)	716±834	1274±860
Minimum PEth 16:0/18:1 (ng/mL)	–[a]	226
Maximum PEth 16:0/18:1 (ng/mL)	5400	3260
Number of samples with PEth 16:0/18:2<300 ng/mL	65	17
Number of samples with PEth 16:0/18:2≥300 ng/mL	33 (33.7 %)	27 (61.4 %)
Mean PEth 16:0/18:2 and SD (ng/mL)	288±289	505±321
Minimum PEth 16:0/18:2 (ng/mL)	–[a]	82.2
Maximum PEth 16:0/18:2 (ng/mL)	1440	1410

[a] PEth was below LOD

Discussion

In comparison to the BAC, which only shows recent alcohol consumption, PEth reveals long-term drinking behavior. Based on ROC analysis, we propose in this study thresholds for PEth 16:0/18:1 and PEth 16:0/18:2 homologues (700 and 300 ng/mL, respectively) to determine prolonged excessive alcohol consumption in drunk drivers.

This suggested threshold for PEth 16:0/18:1 is more than three times higher than the threshold of 210 ng/mL (0.3 μmol/L), which was proposed in 2013 in a Swedish study [14] to differentiate between moderate drinking and alcohol misuse based on clinical data.

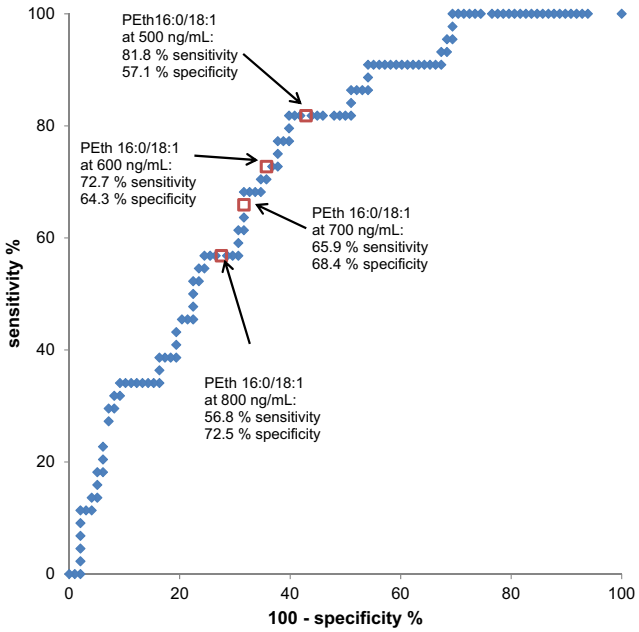

Fig. 2 ROC analysis for PEth 16:0/18:1: The samples were classified into two groups depending on the BAC: BAC≥1.6‰, excessive alcohol consumption; and BAC<1.6‰, moderate alcohol consumption. With a threshold of 700 ng/mL for PEth 16:0/18:1, a sensitivity of 65.9 % and a specificity of 68.4 % were determined

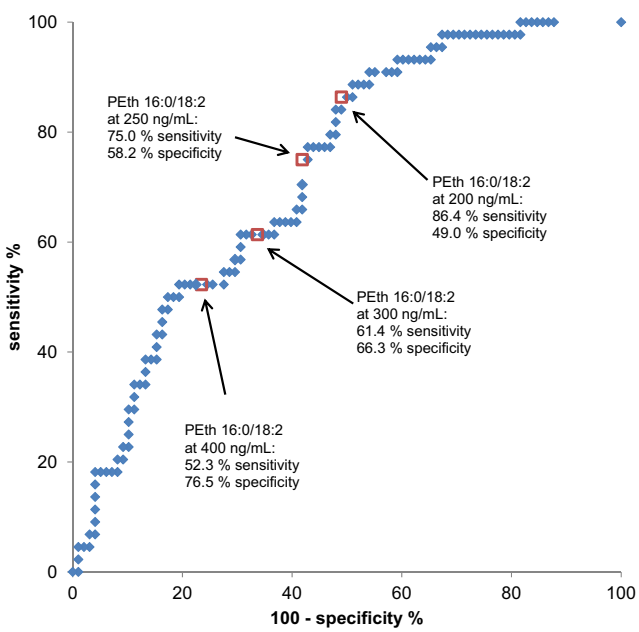

Fig. 3 ROC analysis for PEth 16:0/18:2: The samples were classified into two groups depending on the BAC: BAC≥1.6‰, excessive alcohol consumption; and BAC<1.6‰ moderate alcohol consumption. With a threshold of 300 ng/mL for PEth 16:0/18:2, a sensitivity of 61.4 % and a specificity of 66.3 % were determined

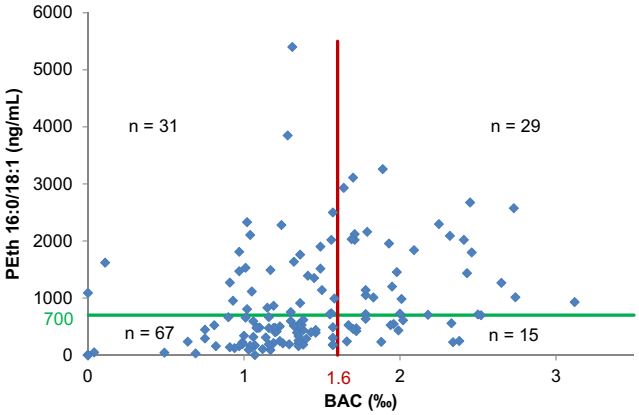

Fig. 4 PEth 16:0/18:1 results compared to the BAC of 142 heparinized blood samples from DUI cases: Of 44 samples with a BAC≥1.6‰, 65.9 % (n=29) have PEth 16:0/18:1≥700 ng/mL; of 98 samples with a BAC<1.6‰, 31.6 % (n=31) have PEth 16:0/18:1≥700 ng/mL

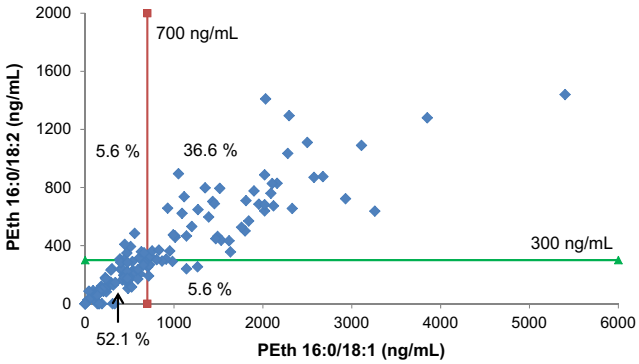

Fig. 6 PEth 16:0/18:1 compared to PEth 16:0/18:2 in 142 heparinized blood samples from DUI cases: 36.6 % of the samples have both PEth 16:0/18:1 and PEth 16:0/18:2 values above the suggested thresholds (700 ng/mL for PEth 16:0/18:1; 300 ng/mL for PEth 16:0/18:2). In 88.7 % of the tested samples, PEth 16:0/18:1 and PEth 16:0/18:2 are conform in the evaluation of drinking habits

However, Gnann [5, 13] performed drinking studies and proposed a threshold for PEth 16:0/18:1 of 800 ng/mL (1.1 μmol/L) to differentiate between (i) moderate to single (or up to five times repeated) excessive drinking and (ii) prolonged excessive drinking. During these drinking experiments with an alcohol consumption of up to BAC 1.0‰ each on five or ten consecutive days, it was possible to reach PEth 16:0/18:1 values of up to 237 ng/mL (0.34 μmol/L) on day 5 [5] and 512 ng/mL (0.73 μmol/L) on day 10 [13], respectively. These reached PEth concentrations clearly exceed the suggested threshold of the Swedish study [14], but our proposed threshold of 700 ng/mL (1 μmol/L) could not be reached by controlled ethanol consumption during drinking studies.

By comparing the BAC and PEth results of this study, prolonged excessive drinking can be uncovered in 65.9 % of drunk drivers with BAC≥1.6‰. Furthermore, it is possible to detect prolonged alcohol misuse in 31.6 % of the cases, where the BAC has already decreased below the legal limit of 1.6‰. These findings are in agreement with the suggestion of Iffland et al. [15] to use other alcohol markers in addition to BAC to diagnose problematic alcohol consumption with higher specificity, as about 30–40 % of drivers with problematic alcohol consumption remain undetected because of BAC levels below 1.6‰.

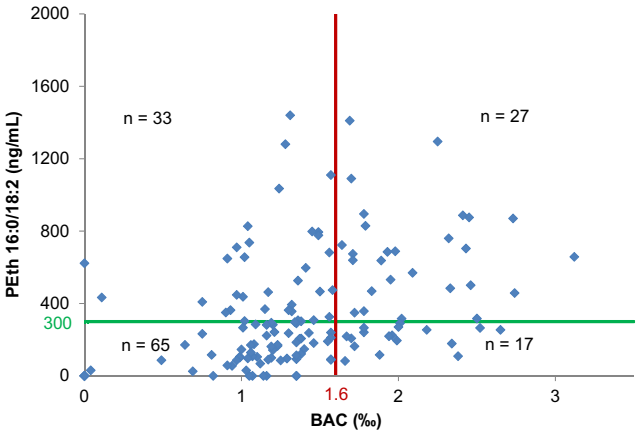

Fig. 5 PEth 16:0/18:2 results compared to the BAC of 142 heparinized blood samples from DUI cases: Of 44 samples with a BAC≥1.6‰, 61.4 % (n=27) have PEth 16:0/18:2≥300 ng/mL; of 98 samples with a BAC<1.6‰, 33.7 % (n=33) have PEth 16:0/18:2≥300 ng/mL

Time difference between incident and blood sampling was not taken into account. Despite BAC elimination during this time period, there is PEth formation, as long as there is alcohol in the blood—which was shown during drinking studies [5, 25]. With a maximum time span of five hours between incident and blood sampling and an assumed alcohol elimination rate of 0.1–0.2‰/h, there is a possible (maximum) degradation of BAC of 1‰. We recently could demonstrate that a single consumption of ethanol up to a BAC of approximately 1.0‰ yielded maximum PEth 16:0/18:1 concentrations of 120 ng/mL (0.17 μmol/L) [25]. If taking this possible increase into account, we can back-calculate the measured PEth results to the time of incident by subtraction of approximately 120 ng/mL from the measured PEth concentration. However, this would not influence the above shown classification of the PEth results in comparison to BAC. The proposed threshold of 700 ng/mL cannot be reached by a single drinking event. PEth can only be accumulated to this high level over a longer time period by prolonged excessive alcohol consumption.

In addition, we also propose a threshold of 300 ng/mL for PEth 16:0/18:2—for which no thresholds have been proposed before. In 88.7 % of all cases, the results obtained with this threshold match with the results for the 700 ng/mL threshold for PEth 16:0/18:1.

Conclusion

This study shows that PEth analysis in blood samples from DUI cases allows differentiation between social drinking behavior (PEth 16:0/18:1<700 ng/mL) and excessive alcohol

consumption (PEth 16:0/18:1≥700 ng/mL)—even when due to fast elimination of ethanol medium blood alcohol concentrations were detected. PEth increases the sensitivity for uncovering prolonged excessive alcohol consumption by 31.6 % (in blood samples with BAC<1.6‰) in comparison to only using the BAC threshold of 1.6‰. Therefore, we strongly recommend to include the most abundant PEth homologue in human blood PEth 16:0/18:1 in routine analysis for the detection of prolonged excessive alcohol consumption in DUI cases, as results of blood analysis in DUI cases with elevated PEth 16:0/18:1 values above a 700 ng/mL threshold would also justify a withdrawal of driving licenses.

At the moment, there is no legislation which allows or requires PEth determination in cases of drunk driving. If blood samples are available in traffic controls—which is not the case in all countries—especially after changing to "evidential breath alcohol testing", PEth analysis could offer the possibility to obtain information about long-term alcohol misuse even if BAC is below 1.6‰. This would require a blood sampling shortly after the elimination of ethanol in cases of suspected prolonged alcohol misuse—which might be a perspective for a strategy in the future. For further improvement of PEth analysis, it is of advantage to introduce a stabilizer for PEth in blood samples or to use dried blood spots (DBS) for analysis, which would guarantee longer post-sampling stability of PEth.

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References

1. Halter CC, Dresen S, Auwaerter V, Wurst FM, Weinmann W (2008) Kinetics in serum and urinary excretion of ethyl sulfate and ethyl glucuronide after medium dose ethanol intake. Int J Legal Med 122(2):123–128
2. Dresen S, Weinmann W, Wurst FM (2004) Forensic confirmatory analysis of ethyl sulfate–a new marker for alcohol consumption–by liquid-chromatography/electrospray ionization/tandem mass spectrometry. J Am Soc Mass Spectrom 15(11):1644–1648
3. Hannuksela ML, Liisanantti MK, Nissinen AE, Savolainen MJ (2007) Biochemical markers of alcoholism. Clin Chem Lab Med 45(8):953–961
4. Gnann H, Weinmann W, Engelmann C, Wurst FM, Skopp G, Winkler M, Thierauf A, Auwarter V, Dresen S, Ferreiros Bouzas N (2009) Selective detection of phosphatidylethanol homologues in blood as biomarkers for alcohol consumption by LC-ESI-MS/MS. J Mass Spectrom 44(9):1293–1299
5. Gnann H, Weinmann W, Thierauf A (2012) Formation of phosphatidylethanol and its subsequent elimination during an extensive drinking experiment over 5 days. Alcohol Clin Exp Res 36(9):1507–1511
6. Varga A, Hansson P, Johnson G, Alling C (2000) Normalization rate and cellular localization of phosphatidylethanol in whole blood from chronic alcoholics. Clin Chim Acta 299(1-2):141–150
7. Gnann H, Engelmann C, Skopp G, Winkler M, Auwarter V, Dresen S, Ferreiros N, Wurst FM, Weinmann W (2010) Identification of 48 homologues of phosphatidylethanol in blood by LC-ESI-MS/MS. Anal Bioanal Chem 396(7):2415–2423
8. Alling C, Gustavsson L, Anggard E (1983) An abnormal phospholipid in rat organs after ethanol treatment. FEBS Lett 152(1):24–28
9. Kobayashi M, Kanfer JN (1987) Phosphatidylethanol formation via transphosphatidylation by rat brain synaptosomal phospholipase D. J Neurochem 48(5):1597–1603
10. Aradóttir S, Seidl S, Wurst FM, Jönsson BAG, Alling C (2004) Phosphatidylethanol in human organs and blood: a study on autopsy material and influences by storage conditions. Alcohol Clin Exp Res 28(11):1718–1723
11. Aradottir S, Olsson BL (2005) Methodological modifications on quantification of phosphatidylethanol in blood from humans abusing alcohol, using high-performance liquid chromatography and evaporative light scattering detection. BMC Biochem 6:18
12. Isaksson A, Walther L, Hansson T, Andersson A, Alling C (2011) Phosphatidylethanol in blood (B-PEth): a marker for alcohol use and abuse. Drug Test Anal 3(4):195–200
13. Gnann H (2011) Phosphatidylethanol - Ein Alkoholkonsummarker auf dem Weg in die klinisch-forensische Routinediagnostik. Dissertation, University of Freiburg, Germany. http://www.freidok.uni-freiburg.de/volltexte/8428/pdf/Diss_Heike_Gnann.pdf. Accessed 15 April 2014: p. 101
14. Helander A, Hansson T (2013) National harmonization of the alcohol biomarker PEth. Lakartidningen 110(39-40):1747–1748
15. Iffland R, Balling MP, Borsch G, Herold C, Kaschade W, Loffler T, Schmidtmann U, Stettner J (1994) Evaluation of an increased blood level of GGT, CDT, methanol, acetone and isopropanol in alcohol intoxicated automobile drivers. Alcoholism indicators instead of medical-psychological examination. Blutalkohol 31(5):273–314
16. Helander A, Zheng Y (2009) Molecular species of the alcohol biomarker phosphatidylethanol in human blood measured by LC-MS. Clin Chem 55(7):1395–1405
17. Zheng Y, Beck O, Helander A (2011) Method development for routine liquid chromatography–mass spectrometry measurement of the alcohol biomarker phosphatidylethanol (PEth) in blood. Clin Chim Acta 412(15–16):1428–1435
18. Omodeo Sale MF, Cestaro B, Mascherpa A, Monti D, Masserini M (1989) Enzymatic synthesis and thermotropic behaviour of phosphatidylethanol. Chem Phys Lipids 50(2):135–142
19. Aderjan R, Daldrup T, Käferstein H, Krause D, Mußhoff F, Paul LD, Peters F, Rochholz G, Schmitt G, Skopp G (2011) Richtlinien zur Bestimmung der Blutalkoholkonzentration (BAK) für forensische Zwecke. Blutalkohol 48(3):137–143
20. U.S. Department of Health and Human Services FDA. Rockville U (2001) Guidance for Industry: Bioanalytical Method Validation. http://www.fda.gov/downloads/Drugs/Guidances/ucm070107.pdf. Accessed 24 September 2011
21. U.S. Department of Health and Human Services FDA. Rockville U (2013) Guidance for Industry: Bioanalytical Method Validation (DRAFT GUIDANCE). http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm368107.pdf Accessed 24 November 2015
22. Bonfiglio R, King RC, Olah TV, Merkle K (1999) The effects of sample preparation methods on the variability of the electrospray ionization response for model drug compounds. Rapid Commun Mass Spectrom 13(12):1175–1185
23. Dams R, Huestis MA, Lambert WE, Murphy CM (2003) Matrix effect in bio-analysis of illicit drugs with LC-MS/MS: influence of ionization type, sample preparation, and biofluid. J Am Soc Mass Spectrom 14(11):1290–1294
24. Muller C, Schafer P, Stortzel M, Vogt S, Weinmann W (2002) Ion suppression effects in liquid chromatography-electrospray-ionisation transport-region collision induced

dissociation mass spectrometry with different serum extraction methods for systematic toxicological analysis with mass spectra libraries. J Chromatogr B Analyt Technol Biomed Life Sci 773(1):47–52

25. Schröck A, Thierauf A, Weinmann W (2014) Phosphatidylethanol—pilot study: detection after single alcohol consumption. http://www.irm.unibe.ch/unibe/medizin/irm/content/e7670/e491786/Phosphatidylethanol-Pilot-Study_ger.pdf. Accessed 10 July 2015