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ORIGINAL ARTICLE

Phosphatidylethanol (PEth) detected in blood for 3 to 12 days after single consumption of alcohol—a drinking study with 16 volunteers

Alexandra Schröck[1] · **Annette Thierauf-Emberger**[2] · **Stefan Schürch**[3] · **Wolfgang Weinmann**[1]

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Abstract In most studies, the alcohol marker phosphatidylethanol (PEth) was used to differentiate social drinking from alcohol abuse. This study investigates PEth's potential in abstinence monitoring by performing a drinking study to assess the detection window of PEth after ingesting a defined amount of alcohol. After 2 weeks of abstinence, 16 volunteers ingested a single dose of alcohol, leading to an estimated blood alcohol concentration (BAC) of 1 g/kg. In the week after drinking, blood and urine samples were taken daily; in the second week, samples were taken every other day. PEth 16:0/18:1 and 16:0/18:2 were analyzed in blood by online-SPE-LC-MS/MS. Ethyl glucuronide and ethyl sulfate were determined in urine for abstinence monitoring. Prior to start of drinking, PEth 16:0/18:1 exceeded 30 ng/mL in blood samples of five volunteers despite the requested abstinence period. Positive PEth values resulted from drinking events prior to this abstinence period. After the start of drinking, maximum BACs were reached after 2 h with a mean of 0.80 ± 0.13 g/kg (range: 0.61–1.11 g/kg). PEth 16:0/18:1 increased within 8 h to maximum concentrations (mean: 88.8 ± 47.0 ng/mL, range: 37.2–208 ng/mL). After this event, PEth was detectable for 3 to 12 days with a mean half-life time of approximately 3 days. PEth has a potential in abstinence monitoring, since PEth could be detected for up to 12 days after a single drinking event. Further investigations are necessary, to establish cut-off levels for PEth as diagnostic marker for the determination of drinking habits like abstinence, social drinking, or risky alcohol consumption.

Keywords Phosphatidylethanol (PEth) · Drinking study · Alcohol marker · Online-SPE-LC-MS/MS · Abstinence monitoring

Introduction

Problematic alcohol consumption is a global health problem: to the drinker through negative consequences of alcohol dependence like liver diseases, cancer, and injuries; and to others through drunken driving and violence [1]. To reveal problematic drinking habits and alcohol-related disorders at an early stage, alcohol biomarkers are highly valuable [2]. Recent alcohol exposure can be detected via blood alcohol concentration (BAC), or ethyl glucuronide (EtG) and ethyl sulfate (EtS) in blood and urine [3, 4]. These direct markers are highly specific as they are only formed if alcohol was consumed [5, 6].

For the detection of prolonged excessive alcohol consumption, the traditional indirect markers carbohydrate deficient transferrin (CDT) and γ-glutamyltransferase (GGT) are used [6, 7]. Since recently, also the direct marker EtG in hair (hEtG) is routinely used for the detection of excessive alcohol consumption and for abstinence monitoring as well [8].

However, an additional direct marker in this field is phosphatidylethanol (PEth) representing a group of phospholipids present in cell membranes [9–11]. The occurrence of these different PEth homolgues may be influenced by nutrition [12]. PEth is formed directly after alcohol intake [10] via the enzyme phospholipase D (PL D) from phosphatidylcholine (PC), as long as ethanol is present [13]. The elimination

✉ Alexandra Schröck
Alexandra.schroeck@irm.unibe.ch

[1] Institute of Forensic Medicine, University of Bern, Bühlstrasse 20, 3012 Bern, Switzerland

[2] Institute of Forensic Medicine, University Medical Center Freiburg, Albertstraße 9, 79104 Freiburg, Germany

[3] Department of Chemistry and Biochemistry, University of Bern, Freie Strasse 3, 3012 Bern, Switzerland

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half-life time of PEth is about 4 days [14], but can vary strongly between individuals [10]. As a result, PEth is accumulated after repeated alcohol consumption and can be used to observe long-term drinking behavior [15–17]. PEth concentrations correlate with the amount of ingested alcohol in alcohol-dependent persons [18]. Therefore, PEth is a promising new biomarker for the detection of alcohol abuse [15, 19].

However, PEth also has a potential in abstinence monitoring, which was shown in a pilot drinking study: two volunteers ingested a single dose of ethanol which led to an estimated BAC of 1 g/kg after an abstinence period of 2 weeks. PEth 16:0/18:1 formation was observed over several hours, as long as ethanol was present in blood, then PEth 16:0/18:1 slowly decreased, being detectable in blood for 9–10 days [2, 20].

The aim of this drinking study with 16 volunteers was to further specify the detection window of PEth 16:0/18:1 after drinking an amount of alcohol leading to an estimated BAC of 1 g/kg to investigate the potential of PEth in abstinence monitoring.

Material and methods

Chemicals and materials

PEth 16:0/18:1 and PEth 16:0/18:2 were purchased from Avanti Polar Lipids (Alabaster, USA). Ammonium acetate was provided by Merck (Darmstadt, Germany). Acetonitrile was supplied by Agros Organics (New Jersey, USA), 2-propanol was obtained from Fisher Scientific (Loughborough, UK), and formic acid (HCOOH) was from Sigma Aldrich (Buchs, Switzerland). Deionized H_2O was produced in-house with a Milli-Q water system from Millipore (Billerica, USA). Deuterated standards were synthesized in our laboratory from phosphatidylcholine 16:0/18:1 and phosphatidylcholine 16:0/18:2 and D_6-ethanol catalyzed by phospholipase D [21–23].

Heparin S-Monovettes (volume 9 mL) were obtained from Sarstedt (Nümbrecht, Germany). Lithium heparinized whole blood, which was used as blank blood, was obtained from volunteers who were abstinent from alcohol for at least 4 weeks.

Study design

Sixteen volunteers (7 female and 9 male) ingested a single dose of alcohol (vodka mixed with a soft drink), which should lead to the targeted BAC of 1 g/kg. The alcohol doses were calculated for each test person by the Widmark formula. Blank urine and blood samples were obtained from all subjects before starting the experiment, which was performed at the Institute of Forensic Medicine, University Medical Center, Freiburg, Germany. An hour after drinking the individual amounts of alcohol, the first urine and blood samples were taken, then samples were collected every 2 h on this day (day 1) until 8 h after the alcohol intake. In the first week, after this drinking experiment, urine and blood samples were taken daily (days 2, 3, 4, 5, 6, and 7). In the second week, urine and blood samples were taken on day 8, 10, and 12. To prevent post-sampling formation of PEth in the ethanol containing blood samples (samples of day 1), the samples were stored at 4 °C prior to analysis and were analyzed promptly after sampling to ensure the overall PEth stability [24–26]. EtG and EtS were analyzed in the urine samples to monitor the abstinence of the volunteers after the drinking event. EtG in hair (hEtG) was analyzed for some volunteers (2, 4, 6, 9, and 12) to get further insight in their drinking behavior of the last months. The study has been approved by the Cantonal Ethics Commission Bern (064/13) on March 03, 2014 and by the Ethics Committee of the University of Freiburg (285/09).

Determination of alcohol biomarkers

BAC and PEth were determined in lithium-heparinized blood samples. For abstinence monitoring, EtG and EtS were analyzed in urine, and in some volunteers, EtG was also analyzed in hair.

Determination of BAC

The ethanol concentration was determined by a validated headspace gas chromatography method with flame ionization detection (HS-GC-FID) in the blood samples of the day of drinking of all volunteers. According to forensic guidelines, samples were analyzed with two GC-FID systems, twice on each system [27].

Determination of EtG and EtS

EtG and EtS were determined in urine by a validated LC-MS/MS method [28], which is used for routine analysis at the Institute of Forensic Medicine Bern for samples from withdrawal therapy to monitor abstinence.

HEtG was determined by a validated online-SPE-LC-MS/MS method according to forensic guidelines [29] using the cut-offs recommended by the SOHT (hEtG ≥30 pg/mg: excessive alcohol consumption; 7 pg/mg ≤ hEtG <30 pg/mg: moderate alcohol consumption) [30, 31].

Determination of PEth

For analyzing PEth 16:0/18:1 and PEth 16:0/18:2, 200 μL of whole blood, 10 μL of internal standard (D_5-PEth 16:0/18:1, D_5-PEth 16:0/18:2), 100 μL of 2-propanol and 600 μL of acetonitrile were pipetted into a micro tube and mixed for 10 min. Afterwards, the samples were centrifuged for 10 min at 16,000 g. The supernatant was transferred to a vial

and evaporated to dryness under a stream of nitrogen at 50 °C. The residue was redissolved in 300 μL of mobile phase A (ammonium acetate (10 mM)/acetonitrile (30:70, *v*/*v*)). An aliquot of 80 μL was injected into the online-SPE-LC-MS/MS system.

Method validation

The determination of PEth in whole blood was validated according to FDA guidelines [32, 33] for the most abundant PEth homologues PEth 16:0/18:1 and PEth 16:0/18:2 in human blood [16, 21]. Selectivity, linearity, limit of quantification (LOQ), limit of detection (LOD), precision, accuracy, and carry-over were investigated.

Selectivity was determined by testing six blank samples of blood from "social alcohol consumers", who were abstinent over a period of ≥4 weeks, to test for disturbances from endogenous matrix components or metabolites, which could interfere the signals of PEth homologues and corresponding internal standards.

For linearity testing, working solutions (0.50, 2.50, 6.25, 12.5, 25.0, and 50.0 μg/mL) for the calibration samples of PEth 16:0/18:1 and PEth 16:0/18:2 were prepared in ammonium acetate (10 mM)/acetonitrile solution (30:70, *v*/*v*). Ten microliters of each were spiked into 240-μL aliquots of blank blood. This resulted in the following concentrations for the calibrators: 20, 100, 250, 500, 1000, and 2000 ng/mL. Six-point calibration curves of PEth 16:0/18:1 and PEth 16:0/18:2 were measured twice on three different days.

Precision and accuracy were investigated by preparing blood samples (quality control samples, QC) spiked at different PEth concentration levels: 20 ng/mL (QC_{LOQ}), 60 ng/mL (QC_{low}), and 1500 ng/mL (QC_{high}).

Carry-over was examined by injecting the highest calibrator (2000 ng/mL) three times; next to that, a blank blood sample was injected twice to test if substances of the previous injections are carried over to the next measurement.

Possible matrix effects have been compensated by the use of authentic deuterated internal standards.

Instrumentation

The online-SPE-LC-MS/MS system was composed of a CTC PAL autosampler (CTC Analytics, Zwingen, Switzerland), an Agilent 1200 series HPLC (Agilent, Waldbronn, Germany), a Hewlett Packard 1100 HPLC (Agilent, Waldbronn, Germany), and a QTrap 3200 mass spectrometer (Sciex, Toronto, Canada) controlled by Analyst 1.5.1 software.

Chromatographic separation was conducted with a Luna RP-C5 column, 50 mm × 2 mm, 5 μm (Phenomenex, Brechbühler, Schlieren, Switzerland) heated to 50 °C with a flow rate of 0.25 mL/min. The trapping column was a Synergi Polar-RP, 20 × 2 mm, 5 μm (Phenomenex, Brechbühler, Schlieren, Switzerland). Mobile phase A consisted of ammonium acetate (10 mM)/acetonitrile (30:70, *v*/*v*) and mobile phase B was 2-propanol. The mobile phase A for the trapping column consisted of 0.1 % HCOOH and acetonitrile (70:30, *v*/*v*); here, mobile phase B was also 2-propanol. PEth 16:0/18:1 and PEth 16:0/18:2 were separated with the following 12 min gradient: 0 to 2 min, 10 % B; 2 to 3.5 min, 10 to 99 % B linear; 3.5 to 6 min, 99 % B; 6 to 7.5 min, 99 to 10 % B linear; and 7.5 to 12 min, 10 % B with a retention time of 6.42 min for PEth 16:0/18:1 and 6.29 min for PEth 16:0/18:2.

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

Results

Method validation

Regarding selectivity, there were no disturbances in any of the tested blank samples for the PEth homologues 16:0/18:1 and 16:0/18:2.

Concerning linearity, all calibrators were well within range of the defined limits, and a linear calibration model with weighting 1/x was used in the range of 20–2000 ng/mL with correlation coefficients >0.99. The concentration of the lowest calibrator (20 ng/mL) was adopted as the LOQ with a signal-to-noise (S/N) ratio of 11.3 for PEth 16:0/18:1 and an S/N ratio of 12.0 for PEth 16:0/18:2. The LOD was 10 ng/mL with S/N ratios >3 for PEth 16:0/18:1 and for PEth 16:0/18:2.

Precision and accuracy were in acceptable ranges (PEth 16:0/18:1: precision: 6.3–11.4 CV%, accuracy: 95.0–101.6 %; PEth 16:0/18:2: precision: 6.9–12.3 CV%, accuracy: 89.0–97.3 %). Carry-over was not observed.

Drinking study

The group of participating volunteers was rather homogenous in age and body mass index (BMI) with a mean age of 26.1 ± 3.0 years (range: 23–33 years) and a BMI of 21.6 ± 2.2 kg/m^2 (range: 16.2–24.8 kg/m^2). All collected blood samples prior to drinking were negative for ethanol, EtG, and EtS.

In some test persons, there was a notable distinction between the estimated and the measured BAC values. Concerning this study set-up, maximum BACs (BAC_{max}) were measured after 2 h after the start of drinking with a mean

observed BAC_{max} of 0.80 ± 0.13 g/kg (range: 0.61–1.11 g/kg). Table 1 contains data of the participating volunteers, such as age, BMI, measured BAC_{max}, measured maximum PEth 16:0/18:1 concentrations (PEth 16:0/18:1_{max}), measured maximum PEth 16:0/18:2 concentrations, time when $PEth_{max}$ was measured (t_{max}), amounts of consumed alcohol and stated time of abstinence prior to the drinking experiment.

After drinking the calculated amounts of vodka, the measured PEth 16:0/18:1_{max} of all 16 volunteers ranged between 37.2 and 208 ng/mL (mean: 88.8 ± 47.0 ng/mL) concerning the used study schedule. PEth formation started after alcohol ingestion and increased to a measured maximum (8 h after the start of the experiment) during day 1 (Fig. 1), as long as ethanol was present in the human body [10]. After the drinking event, the test persons had to remain abstinent for the rest of the trial period and PEth values decreased slowly during 1–2 weeks.

The formation and degradation of the PEth homologue 16:0/18:2 was detectable as well in all volunteers, but in lower concentrations as PEth 16:0/18:1. Maximum observed PEth 16:0/18:2 concentrations ranged between 21.0 and 130 ng/mL (mean: 63.5 ± 33.3 ng/mL).

Despite of an abstinence period of 2 weeks prior to the drinking experiment, PEth 16:0/18:1 concentrations exceeded 30 ng/mL in the blood samples of five test persons prior to start of drinking, which had been supposed to be "blank" samples.

Test persons 2 and 12 showed the highest PEth 16:0/18.1 concentrations in these samples with 145 and 129 ng/mL, respectively. Test person 4 had a PEth 16:0/18.1 value of 76.4 ng/mL, and test persons 6 and 9 had not only positive PEth 16:0/18:1 values (81.2 and 72.5 ng/mL, respectively), but also positive EtG values (0.30 and 0.17 mg/L, respectively) in the corresponding urine samples. These volunteers were therefore not taken into account for the further evaluation of this drinking study.

As the above mentioned, test persons (2, 4, 6, 9, and 12) had elevated PEth 16:0/18:1 concentrations in blood after the required abstinence period of 2 weeks, additionally hEtG was determined in a proximal hair segment of 3 cm to get an insight in their drinking behavior of the last months. Hair analysis revealed moderate alcohol consumption habits for test persons 2, 4, and 9 with hEtG concentrations of 10.4, 9.2, and 21.6 pg/mg, respectively, and excessive alcohol consumption habits for test persons 6 (45.7 pg/mg) and 12 (37.5 pg/mg) according to SOHT guidelines [30, 31].

Of the other 11 test persons, five persons had PEth 16:0/18:1 concentrations of 20–30 ng/mL in the blood samples taken prior to drinking and the PEth 16:0/18:1 concentrations for the other six test persons were below 10 ng/mL (LOD). No hair analysis was performed for these 11 persons.

As test persons 10 and 15 (PEth 16:0/18:1 < 10 ng/mL) showed positive EtG values during the requested abstinence period after the day of drinking, they were also excluded from further evaluation of this study. Therefore, only nine test persons, who had PEth 16:0/18:1 values ≤30 ng/mL in the sample prior to the start of drinking and who stayed abstinent (no EtG and EtS were found in urine samples) over the trial period after the single drinking event, were taken into account for the evaluation of this drinking study (Fig. 2).

With an LOD of 10 ng/mL PEth 16:0/18:1 was detectable in blood for 3 days (test person 7) and up to 12 days (test persons 1, 3, and 5) after the drinking event. The mean detection time of PEth 16:0/18:1 calculated from the results of these 9 test persons was 9.3 ± 3.0 days. Maximum PEth 16:0/18:1 concentrations in these nine test persons are greatly variable: test person 1 had a PEth 16:0/18:1_{max} of 122 ng/mL, whereas for test person 3 a PEth 16:0/18:1_{max} of 82.1 ng/mL and for test person 11 a PEth 16:0/18:1_{max} of 37.2 ng/mL were found.

The second measured PEth homologue, PEth 16:0/18:2 (LOD of 10 ng/mL), was detectable in blood for 1 day (test person 7 and 16) and up to 8 days (test persons 1) after the drinking event. The mean detection time of PEth 16:0/18:2 calculated from the results of these nine test persons was 4.4 ± 2.2 days. Maximum PEth 16:0/18:2 concentrations in these nine test persons are greatly variable: test person 1 had a PEth 16:0/18:2_{max} of 88.6 ng/mL, whereas for test person 3 a PEth 16:0/18:2_{max} of 52.8 ng/mL and for test person 11 a PEth 16:0/18:2_{max} of 36.0 ng/mL were found (Fig. 3).

Discussion

This drinking experiment with social drinkers was performed to get information about the detection window of PEth with the newly developed online-SPE-LC-MS/MS method with an LOD of 10 ng/mL.

Seven test persons (test persons 2, 4, 6, 9, 10, 12, and 15) were excluded from the evaluation of the detection window of PEth after single ingestion of a defined amount of alcohol leading to an estimated BAC of 1 g/kg.

In case of test persons 2 and 12, the time frame of abstinence prior to the experiment was definitely too short and/or the elimination time of PEth too long, as both test persons declared their abstinence of 2 weeks as it was required. Both volunteers admitted that they had consumed several drinks per day prior to the 2 weeks of abstinence during Carnival events. However, both volunteers sticked to the requested abstinence period (no positive EtG values in urine) after the day of drinking during the whole trial period (12 days). Test persons 6 and 9 did not stick to the expected abstinence period of 2 weeks prior to the drinking event (positive EtG values in urine), although both had declared their abstinence. Test person 9 sticked to the

Table 1 Data of the participating volunteers (−: Data not available; t_{max}: time after which $PEth_{max}$ 16:0/18:1 and $PEth_{max}$ 16:0/18:2 were measured); BAC_{max}, $PEth_{max}$ 16:0/18:1 and $PEth_{max}$ 16:0/18:2 depending on the sampling schedule during this study

No	Sex	Age (years)	Height (m)	Weight (kg)	BMI (kg/m^2)	Measured BAC_{max} (g/kg)	Measured $PEth_{max}$ 16:0/18:1 (ng/mL)	Measured $PEth_{max}$ 16:0/18:2 (ng/mL)	t_{max} (h)	Amount of vodka 40 % (mL)	Stated time of abstinence prior to drinking study (days)
1	M	25	1.87	85	24.3	0.9	122	88.6	8	210	15
2	M	26	1.84	78	23.0	0.77	208	94.9	6	200	14
3	F	33	1.71	67	22.9	0.64	82.1	52.8	8	140	15
4	F	23	1.71	59	20.2	0.72	110	64.4	4	130	15
5	F	23	1.60	54	21.1	0.81	83.5	44.4	8	120	16
6	M	25	1.87	69	19.7	0.72	98.6	35.5	8	170	15
7	F	25	1.64	61	22.7	0.61	45.2	21.0	6	130	15
8	F	24	1.79	52	16.2	0.84	91.9	99.7	8	110	21
9	M	27	1.78	78.5	24.8	1.11	172	130	8	210	14
10	F	30	1.58	53	21.2	0.75	43.6	31.9	8	150	-
11	M	32	1.81	68	20.8	0.73	37.2	36.0	8	170	74
12	M	26	1.89	71	19.9	0.85	165	121	8	180	15
13	M	25	1.89	72	20.2	0.62	54.1	42.1	8	180	16
14	F	24	1.68	67	23.7	0.96	113	65.2	8	180	14
15	M	25	1.89	86	24.1	0.87	40.8	45.4	8	230	15
16	M	25	1.87	73	20.9	0.83	53.8	43.6	8	190	14

required abstinence period after the day of drinking (no positive EtG values in urine). Although test person 6 claimed to be abstinent, positive EtG values were found at days 7, 8, and 10 after the day of drinking. Together with the EtG result in hair (hEtG: 45.7 pg/mg), which is above the suggested cut-off for excessive alcohol consumption, these findings reveal problematic alcohol consumption.

Test person 4 also showed relatively high amounts of PEth 16:0/18:1 despite the declared abstinence period prior to the day of drinking. Hair analysis showed moderate consumption habits and test person 4 sticked to the required abstinence period after drinking to the estimated BAC of 1 g/kg.

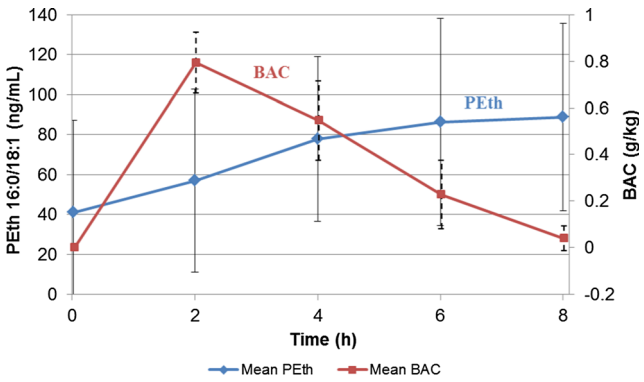

Fig. 1 Mean values of measured BAC and PEth 16:0/18:1 at the day of drinking to the targeted BAC of 1 g/kg: PEth 16:0/18:1 formation starts after alcohol ingestion and seems to be only formed as long as BAC is still positive in the sample

PEth formation and elimination kinetics varied individually and were not influenced by higher PEth concentrations in the "blank sample".

The detection window of PEth 16:0/18:1 after single ingestion of a defined amount of alcohol leading to an estimated BAC of 1 g/kg was evaluated out of the results of nine test persons, who had PEth 16:0/18:1 values ≤30 ng/mL in the blood sample prior to drinking, and furthermore, did stick to the required abstinence periods concerning this study set-up. Under these conditions, PEth 16:0/18:1 was detectable for 3 up to 12 days in blood of social drinkers. The PEth homologue 16:0/18:2 was formed in lower concentrations in most of the test persons and was eliminated faster. Therefore, PEth 16:0/18:2 showed a smaller detection window of 1 up to 8 days compared to the most abundant PEth homologue 16:0/18:1.

Comparing the results of this drinking study (PEth 16:0/$18:1_{max}$ concentrations ranging between 37.2 and 122 ng/mL) to other drinking studies with an alcohol consumption up to 1 g/kg each on five and ten subsequent days with maximum PEth 16:0/18:1 concentrations up to 237 ng/mL on day 5 [10] and up to 512 ng/mL on day 10 [17], there is a correlation between measured PEth concentrations and degree of alcohol consumption.

These studies help to define cut-offs for PEth to differentiate between abstinence, moderate alcohol consumption, and excessive alcohol consumption. The wide range of PEth levels in alcoholics and the PEth levels obtained in drinking studies led to several propositions of PEth thresholds to differentiate moderate alcohol consumption from excessive alcohol

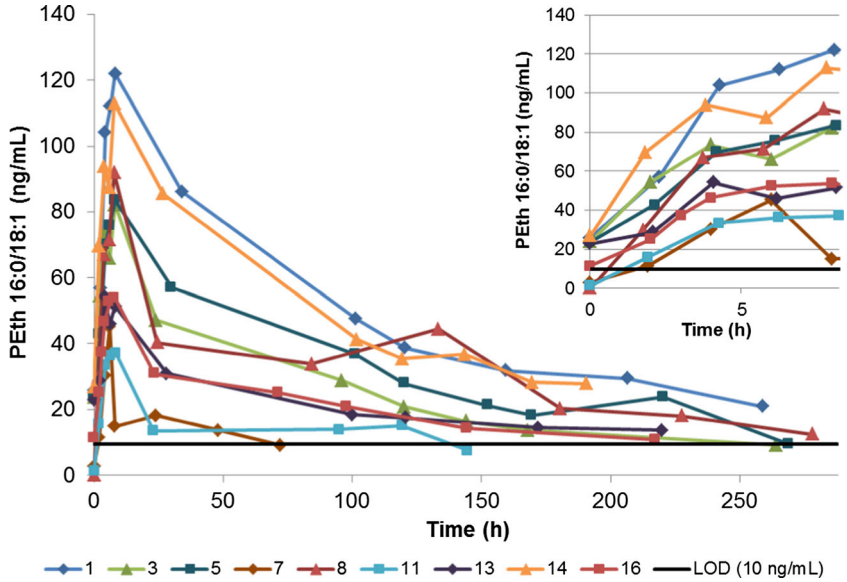

Fig. 2 Nine test persons with PEth 16:0/18:1 values ≤30 ng/mL in the blank sample and abstinence throughout the days after day of drinking (showing no positive EtG and EtS values in urine) On the right: PEth 16:0/18:1 formation over the first 8 h

consumption: 210 ng/mL [34], 800 ng/mL [17], or 700 ng/mL [23]. Reasons for these differences in PEth thresholds are different definitions of "excessive" alcohol consumption, dependent on amounts of consumed alcohol and time period of abuse, and lack of data differentiating PEth formation and elimination kinetics between male and female, young, and old test persons.

The aim of application (diagnosis of problematic alcohol consumption habits prior to withdrawal, abstinence monitoring in maintenance therapy, diagnosis of drinking habits prior to organ transplantation aiming for total abstinence, or forensic questions) also plays a role in the decision process of PEth thresholds. For forensic purposes with legal consequences, safety margins might be higher than for clinical abstinence monitoring, where a patient might have "a second chance".

Kechagias et al. showed the potential of PEth to detect moderate alcohol consumption and to distinguish between moderate alcohol consumption and abstinence [35]. However, there is still a lack of data of "typical" PEth concentrations for persons with moderate alcohol consumption and for abstinent persons. That means, there is no defined PEth level to differentiate moderate alcohol consumption from abstinence, whereby ideally PEth should not be detectable in abstinent persons.

Another important finding of this study are the inter-individual differences in PEth 16:0/18:1 and PEth 16:0/18:2 formation, which was shown by varying $PEth_{max}$ levels (Fig. 2 and Fig. 3). This might be due to inter-individual differences in phospholipase D activity, or other factors which have not yet been investigated.

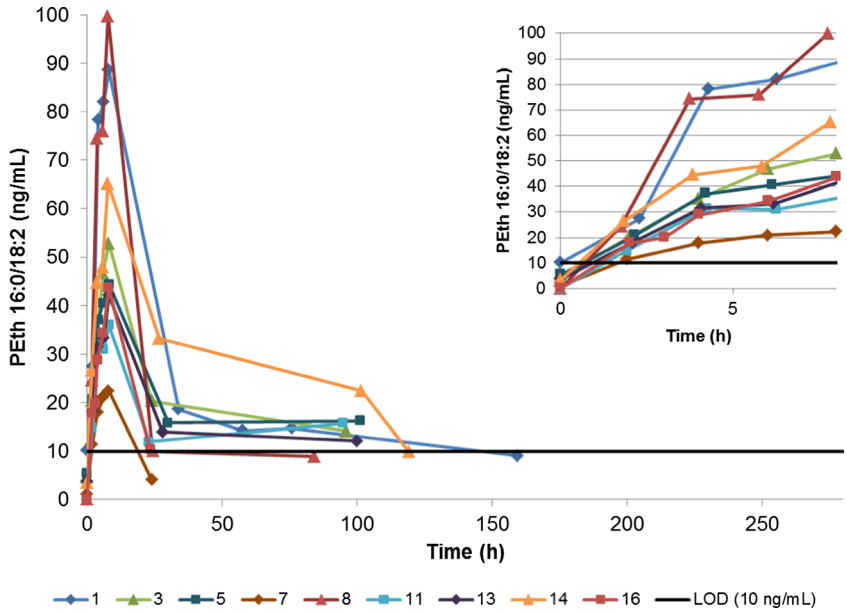

Fig. 3 Nine test persons with PEth 16:0/18:2 values ≤15 ng/mL in the blank sample and abstinence throughout the days after day of drinking (showing no positive EtG and EtS values in urine) On the right: PEth 16:0/18:2 formation over the first 8 h

Conclusion

This drinking study shows that PEth cannot only be used to differentiate moderate alcohol consumption from excessive alcohol consumption, but also has the potential of being used in abstinence monitoring to uncover single alcohol consumption several days later, depending on the amount of ingested alcohol. This is of interest in many settings, such as in alcohol withdrawal, in driving aptitude assessment, in patients with alcohol induced liver cirrhosis pre- and post-liver transplantation.

Challenges in future research on PEth are the factors influencing PEth formation, such as the inter-individual variations in PL D activity, the availability of different homologues of the precursor phosphatidylcholines due to nutrition, and the activity of other phospholipases, such as phospholipases A, B, and C, which could influence the formation and degradation of PEth as well.

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Compliance with ethical standards

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