

Article

Elimination Characteristics of the Alcohol Biomarker Phosphatidylethanol (PEth) in Blood during Alcohol Detoxification

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Abstract

Aims: The study documented elimination characteristics of three phosphatidylethanol (PEth) homologs in serially collected blood samples from 47 heavy drinkers during ~2 weeks of alcohol detoxification at hospital.

Methods: Venous whole blood and urine samples were collected every 1–2 days during treatment. Concentrations of PEth, and of urinary ethyl glucuronide (EtG) and ethyl sulfate (EtS) to detect relapse drinking, were measured using liquid chromatography-tandem mass spectrometry.

Results: When included in the study, negative or decreasing breath ethanol concentrations demonstrated that the patients were in the elimination phase. The EtG and EtS measurements further confirmed alcohol abstinence during the study, with three exceptions. On admission, all patients tested positive for PEth, the total concentration ranging 0.82–11.7 (mean 6.35, median 5.88) $\mu\text{mol/l}$. PEth 16:0/18:1, 16:0/18:2 and 16:0/20:4 accounted for on average ~42%, ~26% and ~9%, respectively, of total PEth in these samples. There were good correlations between total PEth and individual homologs ($P < 0.0001$). There was no significant difference in PEth values between male and female subjects. During abstinence, the elimination half-life values ranged 3.5–9.8 days for total PEth, 3.7–10.4 days for PEth 16:0/18:1, 2.7–8.5 days for PEth 16:0/18:2 and 2.3–8.4 days for PEth 16:0/20:4.

Conclusions: The results demonstrated a very high sensitivity (100%) of PEth as alcohol biomarker for recent heavy drinking, but considerable differences in the elimination rates between individuals and between different PEth forms. This indicates that it is possible to make only approximate estimates of the quantity and recency of alcohol intake based on a single PEth value.

INTRODUCTION

High alcohol consumption constitutes a major global health burden on society (GBD 2016 Alcohol Collaborators, 2018). Identification of individuals engaged in harmful drinking is problematic, however, due to often vague and unspecific early clinical signs and symptoms, and because many people tend not to acknowledge their true

drinking habits through self-report or questionnaires (Helander *et al.*, 1999; Helander and Eriksson, 2002; Whitford *et al.*, 2009). For this reason, different laboratory biomarkers have been proposed and evaluated as supportive, objective tests to indicate heavy drinking, confirm sobriety and for monitoring patients during treatment (Niemelä, 2016). An important drawback is that many of those

have shown limited specificity for alcohol-related effects (e.g. liver enzyme activities), implying uncertainties and risk of making incorrect conclusions.

The first alcohol-specific routine laboratory test to reveal chronic heavy drinking was carbohydrate-deficient transferrin (CDT), a temporary change in the glycosylation pattern of the iron-transport protein transferrin in serum that occurs in response to chronic high alcohol consumption (Stibler, 1991; Bergström and Helander, 2008a). Following development of analytical methods targeting the relative amount (%) of CDT to total transferrin, and international standardization of measurement and reference interval including focusing on disialotransferrin (Helander *et al.*, 2016; Schellenberg *et al.*, 2017), the principal of two alcohol-induced transferrin glycoforms (Bergström and Helander, 2008b), the reliability and utility of CDT as alcohol biomarker with important clinical and medico-legal applications was further improved (Helander *et al.*, 2016; Niemelä, 2016; Bortolotti *et al.*, 2018). A limitation is that not all individuals respond to heavy drinking with a marked increase in the CDT level (Helander *et al.*, 1996).

Later, measurement of phosphatidylethanol (PEth), a phospholipid formed from ethanol and phosphatidylcholine in cell membranes by the enzyme phospholipase D (Gustavsson, 1995), in whole blood samples was introduced as a direct, specific alcohol biomarker (i.e. ethanol metabolite) (Isaksson *et al.*, 2011; Viel *et al.*, 2012). PEth is not a single molecule but a large group of structural variants comprising a common glycerophosphoethanol head and two fatty acids of variable carbon chain length and degree of saturation (Helander and Zheng, 2009; Gnanu *et al.*, 2010), reflecting the composition of the corresponding phosphatidylcholines. As was the case with CDT, improvement of analytical methods suitable for routine use (e.g. liquid chromatography-mass spectrometry (LC-MS)) (Zheng *et al.*, 2011) was necessary, which also meant that PEth testing changed from measuring the total amount to only one or a few predominant homologs, usually the form containing one palmitic acid and one oleic acid (PEth 16:0/18:1) making up ~40% of, and correlating well with, the total amount (Helander and Zheng, 2009; Nalesso *et al.*, 2011). In addition, use of LC-MS methods with higher analytical sensitivity meant that the PEth test became more clinically sensitive, allowing detection of not only prolonged heavy drinking (Varga *et al.*, 1998) but even a single high intake (Helander *et al.*, 2012; Javors *et al.*, 2016; Schrock *et al.*, 2017). The risk of PEth formation *in vitro* constitutes the main drawback, for use as an alcohol biomarker (Aradottir *et al.*, 2004; Helander and Zheng, 2009).

Due to an increasing routine use of the PEth test (e.g. in Sweden, the number of PEth tests today roughly equals that of CDT), guidelines for the interpretation of test results to distinguish between different drinking levels (estimates of the level and duration of drinking) have been suggested and introduced (Helander and Hansson, 2013; Kummer *et al.*, 2016; Ulwelling and Smith, 2018). Nevertheless, although clinicians usually ask for simple, dichotomous outcomes (e.g. a positive or negative test result), both the dose-response effect and detection window for PEth have been reported to vary between individuals (Aradottir *et al.*, 2006; Viel *et al.*, 2012; Ulwelling and Smith, 2018).

The present investigation was undertaken to study further the elimination characteristics of PEth homologs in human blood, with focus on the predominant 16:0/18:1, 16:0/18:2 and 16:0/20:4 forms (Helander and Zheng, 2009), based on measurement of serially collected blood samples from heavy drinkers during ~2 weeks of alcohol detoxification.

METHODS

Patients and samples

Consecutive alcohol dependent subjects (meeting DSM IV criteria) who voluntarily entered a psychiatric ward for recovery from acute alcohol intoxication and for alcohol rehabilitation services were invited to participate in the study. On admission, all subjects included tested negative for ethanol in blood, based on a breath alcohol test, or showed a markedly lower ethanol concentration at the second of two subsequent tests (performed later the same day or next day), confirming they were in the elimination phase. During the ~2-week study period, the patients were hospitalized but the ward was not locked. Two weeks is the typical treatment time at the ward, and treatment was not changed due to the study. The original number of study participants was 49, 37 of which were men aged 23–72 (mean 49.5) years and 12 women aged 30–66 (mean 48.7) years.

The collection of blood and urine samples typically started (i.e. study Day 1) on the day after admission to hospital (69% of cases), but with some later in the admission day (14%) or not until after 2 days (16%). According to the study protocol, urine sampling should be carried out daily during the entire observation period, whereas blood was to be sampled once daily over 5 days and then every second day. On each sampling occasion, a breath alcohol test was done in parallel.

Venous whole blood for measurement of PEth homologs was collected in EDTA vacutainer tubes. Urine specimens for measurement of ethyl glucuronide (EtG) and ethyl sulfate (EtS), two conjugated, minor ethanol metabolites and direct alcohol biomarkers used to detect any alcohol intake over the past ~1–2 days (Helander and Beck, 2005; Helander *et al.*, 2009), were collected supervised in plastic Urine Monovettes without preservative (Sarstedt AG, Germany). Urine samples were stored at –20°C, and blood samples at –80°C (to reduce the risk for post-sampling formation of PEth in samples containing ethanol) (Helander and Zheng, 2009), until taken for analysis.

The study was approved by the ethics committee at the University of Mainz, Germany (Nr 837.277.10(7286)). All participants provided written informed consent and they could terminate the study at any time without affecting their treatment.

Phosphatidylethanol measurement in whole blood samples

Analysis of nine PEth homologs (the sum corresponding to ‘total PEth’) (Helander and Zheng, 2009) in whole blood specimens (i.e. essentially erythrocyte membranes) (Varga *et al.*, 2000) was done as previously described (Zheng *et al.*, 2011). In brief, 100 µl whole blood was mixed with 50 µl internal standard solution (PEth 16:0/18:1-d₅ and 16:0/18:2-d₅), 75 µl acetonitrile and 150 µl acetone. The mixture was gently shaken for 20 min at room temperature, centrifuged at 4000 g for 20 min, the supernatant transferred to a new vial and finally centrifuged again for 10 min. LC-MS-MS quantification of PEth homologs was done by comparison with a calibration curve covering 0–14.2 µmol/l prepared similarly in PEth negative blood spiked with known amounts of PEth 16:0/18:1 and PEth 16:0/18:2. Low and high PEth quality control samples were prepared in the same way. The limit of detection and the lower limit of quantitation (LLOQ) of the method was ~0.01 µmol/l and ~0.03 µmol/l, respectively (Zheng *et al.*, 2011).

Ethyl glucuronide and ethyl sulfate measurement in urine samples

Urinary EtG and EtS were determined using an accredited (ISO 17025) routine LC-MS-MS method, essentially as previously

described (Helander *et al.*, 2009). In brief, 25 μl of urine was diluted 20 times with an internal standard solution (475 μl of 0.5 $\mu\text{g}/\text{ml}$ EtG-d5 and 0.1 $\mu\text{g}/\text{ml}$ EtS-d5 in 0.1% formic acid) and 10 μl was injected into the LC system. The product ions monitored were m/z 221 \rightarrow 75, 85, 113 for EtG, m/z 226 \rightarrow 75, 85, 113 for EtG-d5, m/z 125 \rightarrow 97, 80 for EtS and m/z 139 \rightarrow 98, 80 for EtS-d5. The concentrations were calculated from the peak-area ratio to the internal standard, with reference to calibration curves. The measuring range for EtG was 0.05–10 $\mu\text{g}/\text{ml}$ (LLOQ 0.1 $\mu\text{g}/\text{ml}$) and 0.025–10 $\mu\text{g}/\text{ml}$ for EtS (LLOQ 0.05 $\mu\text{g}/\text{ml}$). Samples exceeding the measuring range were reanalyzed after 100-fold dilution with 0.9% NaCl. The total analytical imprecision for EtG was <9%, according to quality control samples. Method accuracy was documented by participation in the GFTCh proficiency program (www.gftch.org) with no deficiencies. The presence of EtS was required, to confirm a positive EtG finding.

RESULTS

Ethanol concentration

On admission to hospital, the blood ethanol concentration, based on a breath alcohol test, ranged 0–5.6 (mean 1.9, median 1.9; $n = 47$) g/l. There was no statistically significant difference between the values for male (range 0–5.6, mean 1.8, median 1.7 g/l; $n = 37$) and female (range 0–4.3, mean 2.3, median 2.6 g/l; $n = 10$) subjects ($P = 0.176$; Mann–Whitney test).

On the first day of blood and urine sampling (study Day 1), 16 participants still showed a positive breath alcohol test (blood ethanol concentration range 0.1–2.6, mean 0.89, median 0.78 g/l), 6 of which (range 0.6–2.6 g/l) were included in the study on the day of hospitalization. From Day 2 and during the rest of the study period, however, no more positive breath alcohol tests occurred.

Urinary ethyl glucuronide and ethyl sulfate concentrations

Testing for urinary EtG and EtS was used to confirm abstinence from alcohol during the detoxification period (EtS was employed as

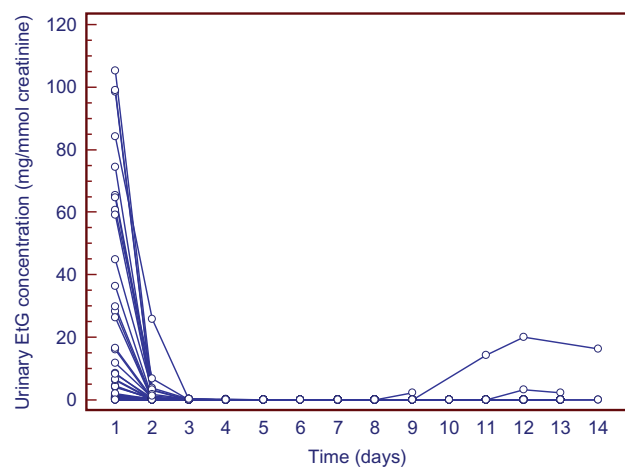


Fig. 1. Individual dot-and-line graphs for urinary EtG values (EtG concentrations normalized to the urinary creatinine concentration) over time in 47 patients during alcohol detoxification. At the end of the study period, elevated EtG values revealed that three subjects had been drinking alcohol on the day before sampling.

a qualifier to confirm a positive EtG results; data not shown). On Day 1, the urinary EtG concentrations ranged 0.17–1890 (mean 257, median 20.2; $n = 48$) mg/l, or, when expressed as a ratio

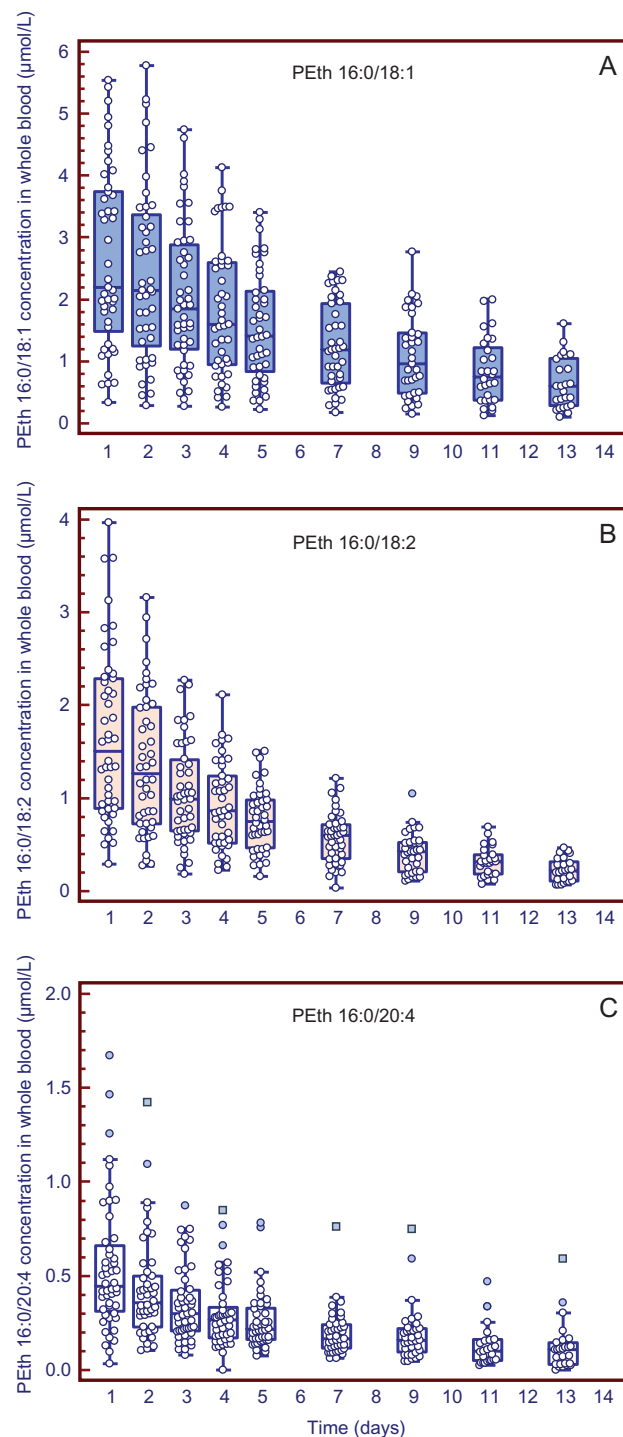


Fig. 2. Whole blood (A) PEth 16:0/18:1, (B) 16:0/18:2 and (C) 16:0/20:4 concentrations over time during alcohol detoxification. Data are individual values and Box-and-Whisker plots where the central box represents the 25–75 percentile, the middle line the median and the horizontal lines the minimum to the maximum value; statistical outliers are indicated by filled circles and far our values by filled squares. The values for a few patients that were instead sampled on Day 6, 8, 10, 12 and 14 are not shown.

to urinary creatinine to compensate for variations in urine dilution (Dahl *et al.*, 2002), 0.04–105 (mean 20.4, median 3.04) mg EtG/mmol creatinine (Fig. 1). Thereafter, the urinary EtG concentration declined in all subjects to range 0–25.7 (mean 1.13, median 0.14) mg/mmol on Day 2, and further down to 0–0.22 (mean 0.06, median 0.04) mg/mmol on Day 3 (Fig. 1). On Day 4–8, there were no indications of alcohol intake, according to the EtG test.

On Day 9, however, one participant showed an elevated urinary EtG test and so did two other subjects on Days 11 and 12 onwards, respectively (Fig. 1), indicating alcohol intake in the previous day. In these three subjects, the blood samples collected after indications of alcohol intake were excluded from the PEth half-life calculations.

Whole blood phosphatidylethanol concentration at start

On Day 1, a whole blood sample was obtained from all but one participant, and all tested positive for PEth (i.e. 100% sensitivity). The total PEth concentration ranged 0.82–11.7 (mean 6.35, median 5.88; $n = 48$) $\mu\text{mol/l}$, compared with 0.33–5.54 (mean 2.66, median 2.20) $\mu\text{mol/l}$ for PEth 16:0/18:1, 0.29–3.97 (mean 1.66, median 1.50) $\mu\text{mol/l}$ for PEth 16:0/18:2 and 0.04–1.67 (mean 0.54, median 0.46) $\mu\text{mol/l}$ for PEth 16:0/20:4 (Fig. 2). Overall PEth 16:0/18:1, 16:0/18:2 and 16:0/20:4 accounted for on average ~42%, ~26% and ~9%, respectively, of total PEth in these samples. There were good correlations observed between the values for total PEth versus PEth 16:0/18:1 ($r = 0.913$), versus 16:0/18:2 ($r = 0.793$), versus 16:0/20:4 ($r = 0.674$), and with the sum of PEth 16:0/18:1 and 16:0/18:2 ($r = 0.969$) ($P < 0.0001$ for all).

There were no significant differences in PEth values between male and female subjects. For example, the total PEth median values on Day 1 were 5.91 $\mu\text{mol/l}$ and 5.18 $\mu\text{mol/l}$ for men and women, respectively ($P = 0.520$, Mann–Whitney test), and 2.15 $\mu\text{mol/l}$ and 2.47 $\mu\text{mol/l}$, respectively, for the major PEth 16:0/18:1 form ($P = 0.626$).

The total PEth concentration on Day 1 showed a weak, positive correlation with the corresponding blood ethanol concentration on admission to hospital ($r = 0.371$, $P = 0.013$), but not with the

urinary EtG/creatinine value on Day 1 ($r = 0.289$, $P = 0.057$). The PEth 16:0/18:1 concentration on Day 1 also correlated positively with the blood ethanol concentration on admission to hospital ($r = 0.357$, $P = 0.018$), and with the urinary EtG/creatinine value on Day 1 ($r = 0.340$, $P = 0.024$).

Phosphatidylethanol elimination during alcohol detoxification

During the ~2 weeks of abstinence from alcohol, as confirmed by negative urinary EtG and EtS tests, a steady decline in the whole blood concentration of PEth forms was observed (Fig. 2). Two participants only provided one blood sample each (on the first day of the study), leaving 47 cases (range 3–9, median 8, blood samples/case) for the calculations of PEth half-lives. At the end of the study period, 3 patients showed indications of alcohol relapse despite inpatient treatment (it should be noted that the ward was not locked), based on positive urinary EtG values, and their PEth results after relapse were excluded from the half-life calculations.

The distribution of half-life values for total PEth (range 3.5–9.8 days), and for PEth 16:0/18:1 (range 3.7–10.4 days), 16:0/18:2 (range 2.7–8.5 days) and 16:0/20:4 (range 2.3–8.4 days), calculated from the regression equation for PEth logarithmic concentrations versus linear time values, are shown in Fig. 3. The time-course of decline of PEth 16:0/18:1, 16:0/18:2 and 16:0/20:4 concentrations for one representative subject are shown in Fig. 4.

There was a statistically significant, positive correlation between the half-lives for PEth 16:0/18:1 and 16:0/18:2 ($r = 0.537$, $P = 0.0001$). When the PEth values were normalized to the starting blood ethanol concentrations, the correlation was even better ($r = 0.783$, $P < 0.0001$). There was also a weak, negative correlation between the PEth 16:0/18:1 half-life value and the corresponding starting concentration on Day 1 ($r = -0.299$, $P = 0.046$).

When relapse drinking was indicated in three subjects, the declining trend for PEth values stopped and concentrations instead levelled out or slightly increased. The urinary EtG and whole blood

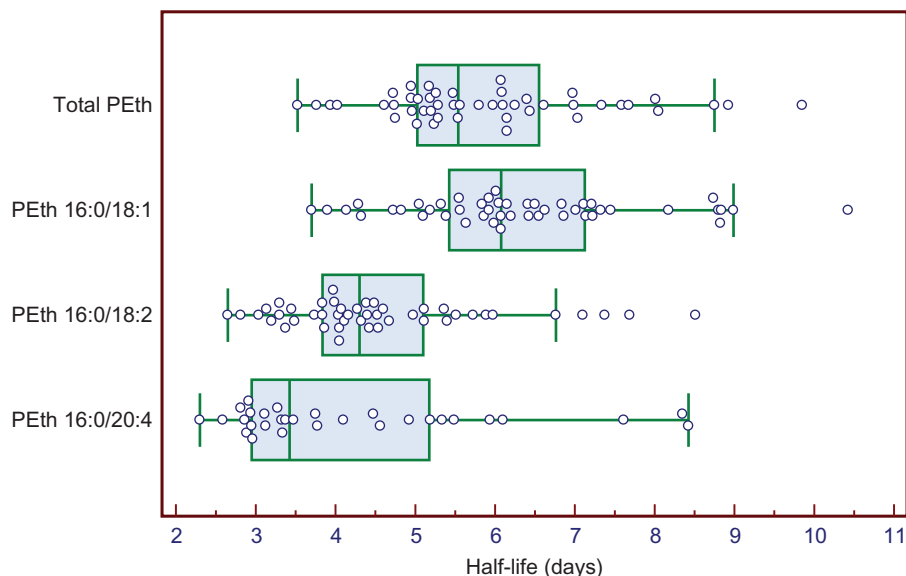


Fig. 3. Distribution of elimination half-lives of total PEth (i.e. sum of 9 PEth forms; mean 5.9 days, median 5.5 days), PEth 16:0/18:1 (mean 6.3 days, median 6.1 days), 16:0/18:2 (mean 4.6 days, median 4.3 days) and 16:0/20:4 (mean 4.2 days, median 3.4 days) in whole blood samples. There was no statistically significant difference in PEth 16:0/18:1 half-life between male (median 6.3 days; $n = 36$) and female (median 5.9 days; $n = 11$) subjects ($P = 0.305$, Mann–Whitney test).

PEth 16:0/18:1 and 16:0/18:2 concentrations over time for one relapsing patient are shown in Fig. 5.

DISCUSSION

The present study of heavy drinkers undergoing voluntary, inpatient alcohol detoxification demonstrated positive whole blood PEth tests in all subjects at the start. During alcohol abstinence, as confirmed by negative urinary EtG and EtS tests, a steady, exponential (first-order) elimination of PEth forms from the blood (i.e. erythrocyte membranes) (Varga *et al.*, 2000) was seen during the ~2-week study period. The elimination rates did not differ significantly between

men and women, but there were marked differences between individuals and between the PEth homologs.

When PEth was introduced as an alcohol biomarker for routine clinical use, studies of test characteristics reported half-life values for total PEth in blood of <5 days (Varga *et al.*, 2000; Wurst *et al.*, 2010). A half-life value of ~4 days, roughly half that of CDT (Jeppsson *et al.*, 1993), has since been the clinically commonly used figure (Isaksson *et al.*, 2011). However, the present study revealed a wide, ~3-fold, spread in half-life values for different individuals, ranging from ~3.5 to 10 days for total PEth. This large inter-individual spread in half-life values, and the slightly higher average value (mean ~6 days) than the one commonly cited, agrees with previous

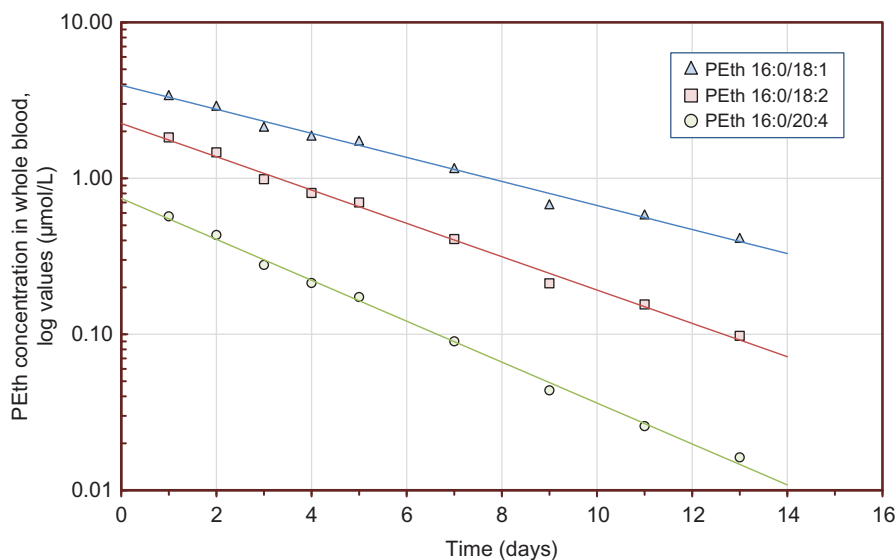


Fig. 4. Elimination half-lives of PEth 16:0/18:1, 16:0/18:2 and 16:0/20:4 in whole blood samples from one representative study participant. Data shown are the correlation curves for PEth logarithmic concentrations versus linear time values.

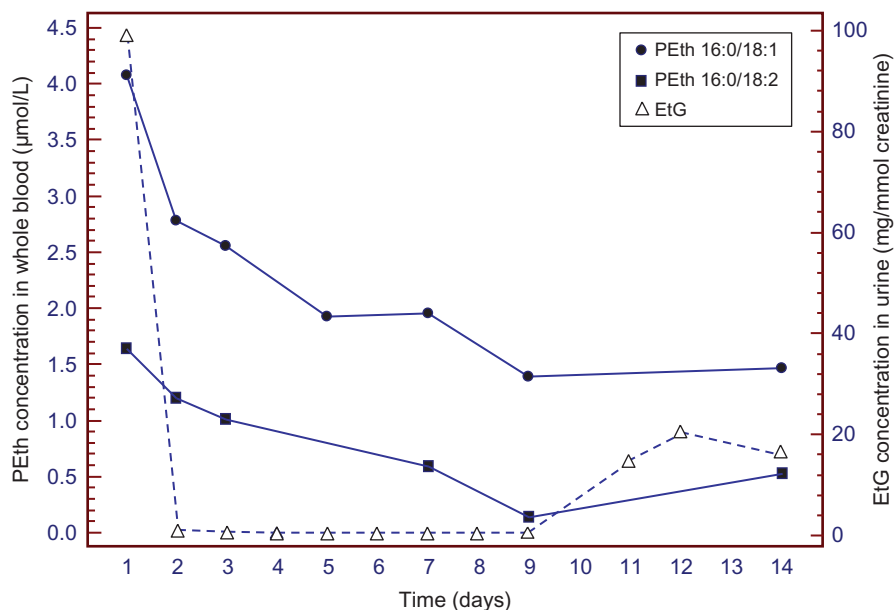


Fig. 5. Gradual declines and subsequent increases in whole blood PEth 16:0/18:1 and 16:0/18:2 concentrations in one subject with indications of alcohol relapse, according to three consecutive, positive urinary EtG tests, at the end of the study period (see Fig. 1). Blood samples were missing on Days 11 and 12.

observations both from drinking experiments with control subjects and in patients during treatment (Gnann *et al.*, 2012; Helander *et al.*, 2012; Javors *et al.*, 2016). This indicates that for some individuals it will take considerably longer for removal of PEth after abstinence from alcohol, in addition to the differences in detection window that are related to the starting concentration. For this reason, it would have been desirable to be able to collect samples for longer than 2 weeks.

When routine PEth measurement changed from using LC separation and evaporative light-scattering detection (Gunnarsson *et al.*, 1998) to LC-MS based methods (Zheng *et al.*, 2011), this meant that testing changed from non-selective measurement of a total PEth fraction to selective quantification of one or a few specific forms, usually the major and single most sensitive PEth 16:0/18:1 (Zheng *et al.*, 2011; Helander and Hansson, 2013). The results of the present study demonstrated marked differences in half-lives for different PEth forms, with 16:0/18:1 showing the longest and 16:0/20:4 the shortest mean and median times. This observation agrees with recent results (Hill-Kapturczak *et al.*, 2018; Lopez-Cruzan *et al.*, 2018). There is a continuous renewal of membrane phospholipids, and studies on erythrocyte membranes of rabbits demonstrated that the turnover of individual molecular species depends largely on the fatty acid composition with phosphatidylcholine 16:0/18:2 being eliminated faster than 16:0/18:1 (van den Boom *et al.*, 1994). In agreement with this observation, because PEth 16:0/18:2 was indicated to respond more rapidly to drinking than 16:0/18:1 (Helander *et al.*, 2012; Hill-Kapturczak *et al.*, 2018), it has been suggested that combined measurement of two or more PEth forms could make the biomarker more clinically accurate with respect to the time and dose of alcohol intake, but at the same time more analytically complex.

The introduction of LC-MS based methods meant that PEth testing became analytically, and hence also clinically, more sensitive. The original, non-selective PEth method could detect only markedly elevated levels occurring following prolonged excessive drinking, but was not sensitive enough to pick up minor elevations after a single, high alcohol intake (Varga *et al.*, 1998), similar to CDT. The currently used LC-MS methods allow detection of even a single drinking occasion and can thus be useful for abstinence monitoring (Helander *et al.*, 2012; Javors *et al.*, 2016; Schrock *et al.*, 2017). This was further indicated in the present study, where patients with indications of alcohol relapse showed increases in PEth 16:0/18:1 and 16:0/18:2 concentrations after the initial gradual decline.

CONCLUSIONS

The results of the present study involving heavy drinkers during alcohol detoxification demonstrated a very high sensitivity (100%) of PEth as alcohol biomarker, but also marked inter-individual differences in PEth half-lives and in the elimination rates for different PEth forms during abstinence. Accordingly, considering also the large inter-individual scatter in PEth values reported for a given alcohol intake (Aradottir *et al.*, 2006; Stewart *et al.*, 2009; Nalesso *et al.*, 2011), this indicates that it is possible to make only approximate estimates of the quantity and recency of drinking based on a single PEth value.

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CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

- Aradottir S, Asanovska G, Gjerss S, *et al.* (2006) Phosphatidylethanol (PEth) concentrations in blood are correlated to reported alcohol intake in alcohol-dependent patients. *Alcohol Alcohol* 41:431–7.
- Aradottir S, Seidl S, Wurst FM, *et al.* (2004) Phosphatidylethanol in human organs and blood: a study on autopsy material and influences by storage conditions. *Alcohol Clin Exp Res* 28:1718–23.
- Bergström JP, Helander A. (2008a) HPLC evaluation of clinical and pharmacological factors reported to cause false-positive carbohydrate-deficient transferrin (CDT) levels. *Clin Chim Acta* 389:164–6.
- Bergström JP, Helander A. (2008b) 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* 388:59–67.
- Bortolotti F, Sorio D, Bertaso A, *et al.* (2018) Analytical and diagnostic aspects of carbohydrate deficient transferrin (CDT): a critical review over years 2007–2017. *J Pharm Biomed Anal* 147:2–12.
- Dahl H, Stephanson N, Beck O, *et al.* (2002) Comparison of urinary excretion characteristics of ethanol and ethyl glucuronide. *J Anal Toxicol* 26: 201–4.
- GBD 2016 Alcohol Collaborators. (2018) Alcohol use and burden for 195 countries and territories, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet* 392:1015–35.
- Gnann H, Engelmann C, Skopp G, *et al.* (2010) Identification of 48 homologues of phosphatidylethanol in blood by LC-ESI-MS/MS. *Anal Bioanal Chem* 396:2415–23.
- 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* 36:1507–11.
- Gunnarsson T, Karlsson A, Hansson P, *et al.* (1998) 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* 705: 243–9.
- Gustavsson L. (1995) Phosphatidylethanol formation: specific effects of ethanol mediated via phospholipase D. *Alcohol Alcohol* 30:391–406.
- Helander A, Beck O. (2005) Ethyl sulfate: a metabolite of ethanol in humans and a potential biomarker of acute alcohol intake. *J Anal Toxicol* 29: 270–4.
- Helander A, Böttcher M, Fehr C, *et al.* (2009) Detection times for urinary ethyl glucuronide and ethyl sulfate in heavy drinkers during alcohol detoxification. *Alcohol Alcohol* 44:55–61.
- Helander A, Eriksson CJ. (2002) Laboratory tests for acute alcohol consumption: results of the WHO/ISBRA Study on State and Trait Markers of Alcohol Use and Dependence. *Alcohol Clin Exp Res* 26:1070–7.
- Helander A, Hansson T. (2013) [National harmonization of the alcohol biomarker PEth]. *Läkartidningen* 110:1747–8.
- Helander A, Peter O, Zheng Y. (2012) Monitoring of the alcohol biomarkers PEth, CDT and EtG/EtS in an outpatient treatment setting. *Alcohol Alcohol* 47:552–7.
- Helander A, Voltaire Carlsson A, Borg S. (1996) Longitudinal comparison of carbohydrate-deficient transferrin and gamma-glutamyl transferase: complementary markers of excessive alcohol consumption. *Alcohol Alcohol* 31:101–7.
- Helander A, von Wachenfeldt J, Hiltunen A, *et al.* (1999) Comparison of urinary 5-hydroxytryptophol, breath ethanol, and self-report for detection of recent alcohol use during outpatient treatment: a study on methadone patients. *Drug Alcohol Depend* 56:33–8.
- Helander A, Wielders J, Anton R, *et al.* (2016) Standardisation and use of the alcohol biomarker carbohydrate-deficient transferrin (CDT). *Clin Chim Acta* 459:19–24.

- Helander A, Zheng Y. (2009) Molecular species of the alcohol biomarker phosphatidylethanol in human blood measured by LC-MS. *Clin Chem* 55:1395–405.
- Hill-Kapturczak N, Dougherty DM, Roache JD, *et al.* (2018) Differences in the synthesis and elimination of phosphatidylethanol 16:0/18:1 and 16:0/18:2 after acute doses of alcohol. *Alcohol Clin Exp Res* 42:851–60.
- Isaksson A, Walther L, Hansson T, *et al.* (2011) Phosphatidylethanol in blood (B-PEth): a marker for alcohol use and abuse. *Drug Test Anal* 3:195–200.
- Javors MA, Hill-Kapturczak N, Roache JD, *et al.* (2016) Characterization of the pharmacokinetics of phosphatidylethanol 16:0/18:1 and 16:0/18:2 in human whole blood after alcohol consumption in a clinical laboratory study. *Alcohol Clin Exp Res* 40:1228–34.
- Jeppsson JO, Kristensson H, Fimiani C. (1993) Carbohydrate-deficient transferrin quantified by HPLC to determine heavy consumption of alcohol. *Clin Chem* 39:2115–20.
- Kummer N, Ingels AS, Wille SM, *et al.* (2016) Quantification of phosphatidylethanol 16:0/18:1, 18:1/18:1, and 16:0/16:0 in venous blood and venous and capillary dried blood spots from patients in alcohol withdrawal and control volunteers. *Anal Bioanal Chem* 408:825–38.
- Lopez-Cruzan M, Roache JD, Hill-Kapturczak N, *et al.* (2018) Pharmacokinetics of phosphatidylethanol 16:0/20:4 in human blood after alcohol intake. *Alcohol Clin Exp Res* 42:2094–99.
- Nalesso A, Viel G, Cecchetto G, *et al.* (2011) Quantitative profiling of phosphatidylethanol molecular species in human blood by liquid chromatography high resolution mass spectrometry. *J Chromatogr A* 1218:8423–31.
- Niemelä O. (2016) Biomarker-based approaches for assessing alcohol use disorders. *Int J Environ Res Public Health* 13:166.
- Schellenberg F, Wielders J, Anton R, *et al.* (2017) IFCC approved HPLC reference measurement procedure for the alcohol consumption biomarker carbohydrate-deficient transferrin (CDT): Its validation and use. *Clin Chim Acta* 465:91–100.
- Schrock A, Thierauf-Emberger A, Schurch S, *et al.* (2017) Phosphatidylethanol (PEth) detected in blood for 3 to 12 days after single consumption of alcohol—a drinking study with 16 volunteers. *Int J Legal Med* 131:153–60.
- Stewart SH, Reuben A, Brzezinski WA, *et al.* (2009) Preliminary evaluation of phosphatidylethanol and alcohol consumption in patients with liver disease and hypertension. *Alcohol Alcohol* 44:464–7.
- Stibler H. (1991) Carbohydrate-deficient transferrin in serum: a new marker of potentially harmful alcohol consumption reviewed. *Clin Chem* 37:2029–37.
- Ullwelling W, Smith K. (2018) The PEth blood test in the security environment: what it is; why it is important; and interpretative guidelines. *J Forensic Sci* 63:1634–40.
- van den Boom MA, Wassink MG, Westerman J, *et al.* (1994) *In vivo* turnover of phospholipids in rabbit erythrocytes. *Biochim Biophys Acta* 1215:314–20.
- Varga A, Hansson P, Johnson G, *et al.* (2000) Normalization rate and cellular localization of phosphatidylethanol in whole blood from chronic alcoholics. *Clin Chim Acta* 299:141–50.
- Varga A, Hansson P, Lundqvist C, *et al.* (1998) Phosphatidylethanol in blood as a marker of ethanol consumption in healthy volunteers: comparison with other markers. *Alcohol Clin Exp Res* 22:1832–7.
- Viel G, Boscolo-Berto R, Cecchetto G, *et al.* (2012) Phosphatidylethanol in blood as a marker of chronic alcohol use: a systematic review and meta-analysis. *Int J Molec Sci* 13:14788–812.
- Whitford JL, Widner SC, Mellick D, *et al.* (2009) Self-report of drinking compared to objective markers of alcohol consumption. *Am J Drug Alcohol Abuse* 35:55–8.
- Wurst FM, Thon N, Aradottir S, *et al.* (2010) Phosphatidylethanol: normalization during detoxification, gender aspects and correlation with other biomarkers and self-reports. *Addict Biol* 15:88–95.
- 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* 412:1428–35.