Alcohol and Alcoholism, 2019, 54(6) 567–573
doi: 10.1093/alcalc/agz064
Advance Access Publication Date: 14 September 2019
Article

OXFORD

Article

Dose–Response Characteristics of the Alcohol Biomarker Phosphatidylethanol (PEth)—A Study of Outpatients in Treatment for Reduced Drinking

Anders Helander[1,2,3,*], Ulric Hermansson[4], and Olof Beck[1,2]

[1]Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden, [2]Division of Clinical Pharmacology, Karolinska University Hospital, Stockholm, Sweden, [3]Division of Clinical Chemistry, Karolinska University Hospital, Stockholm, Sweden, and [4]Department of Clinical Neurosciences, Karolinska Institutet, Stockholm Centre for Dependence Disorders, Stockholm Health Care Services Riddargatan 1, Stockholm, Sweden

*Corresponding author: C1:74, Clinical Chemistry, Karolinska University Laboratory Huddinge, SE-141 86 Stockholm, Sweden. Tel: +46-8-58581293; E-mail: anders.helander@ki.se

Received 27 March 2019; Revised 13 June 2019; Accepted 02 July 2019

Abstract

Aim: Measurement of whole-blood phosphatidylethanol (PEth) offers high sensitivity and specificity as alcohol biomarker. A remaining issue of importance for the routine application is to better establish the relationship between PEth concentration and amount and duration of drinking.
Methods: The study included 36 subjects (32–83 years) voluntarily attending outpatient treatment for reduced drinking. At ~3- to 4-week intervals, they provided a diary on their daily alcohol intake and gave blood samples for measurement of PEth and carbohydrate-deficient transferrin (CDT). Whole-blood PEth 16:0/18:1 was measured by liquid chromatography-tandem mass spectrometry and serum CDT (%disialotransferrin) by high-performance liquid chromatography.
Results: At start, the self-reported past 2-week alcohol intake ranged 0–1260 (median 330) g ethanol, the PEth 16:0/18:1 concentration ranged 0.05–1.20 (median 0.23) μmol/L, and the CDT value ranged 0.7–13.0% (median 1.5%). At the final sampling after 5–20 (median 12) weeks, neither reported alcohol intake nor PEth and CDT levels differed significantly from the starting values. The PEth concentration showed best association with past 2-week drinking, followed by for intake in the next last week. The changes in PEth concentration vs past 2-week alcohol intake between two successive tests revealed that an increased ethanol intake by ~20 g/day elevated the PEth concentration by on average ~0.10 μmol/L, and *vice versa* for decreased drinking.
Conclusions: The PEth concentration correlated well with past weeks alcohol intake, albeit with a large inter-individual scatter. This indicates that it is possible to make only approximate estimates of drinking based on a single PEth value, implying risk for misclassification between moderate and heavy drinking.

INTRODUCTION

Many laboratory tests have been proposed and evaluated as objective measures to indicate acute or chronic heavy alcohol consumption and to monitor drinking behavior (e.g. confirmation of abstinence or detection of relapses) during treatment of persons with alcohol-related problems (Niemelä, 2016). Unfortunately, many of those tests have shown limited specificity for alcohol, implying risk of making incorrect conclusions, but despite this been widely used in clinical routine (e.g. liver function tests). Only few tests have gained acceptance as sensitive and specific enough alcohol biomarkers for medico-legal use.

The first alcohol-specific routine biomarker to indicate prolonged heavy drinking was carbohydrate-deficient transferrin (CDT), an alcohol-induced change in the glycosylation pattern of serum transferrin (Stibler, 1991; Bergström and Helander, 2008). During alcohol abstention, an elevated CDT level (Schellenberg *et al*., 2017) normalizes with a half-life of ∼10 days (Jeppsson *et al*., 1993), and the test reflects heavy drinking over the past weeks up to ∼1 month. Using the current analytical methods for CDT, there is very little risk for obtaining false-positive results (Bergström and Helander, 2008; Kenan *et al*., 2011; Helander *et al*., 2014). A limitation is that not all individuals respond to prolonged heavy alcohol consumption (i.e. at least 50–60 g ethanol/day on average) with an increased CDT value (Helander *et al*., 1996).

Phosphatidylethanol (PEth) was later introduced as a whole blood–based alcohol biomarker (Isaksson *et al*., 2011; Viel *et al*., 2012). PEth is the name for a large set of phospholipids formed in cell membranes from ethanol and the corresponding phosphatidylcholine by the enzyme phospholipase D (Helander and Zheng, 2009; Gnann *et al*., 2010), implying high specificity for use as alcohol biomarker. PEth formation and accumulation in erythrocyte membranes continue as long as ethanol is present in the body and, during alcohol abstention, it is eliminated with an average half-life of ∼6 days, albeit with considerable inter-individual variation (Helander *et al*., 2019).

When PEth measurement changed from using liquid chromatography (LC) combined with evaporative light-scattering detection (Gunnarsson *et al*., 1998) to mass-spectrometric detection (LC–MS) (Helander and Zheng, 2009; Nalesso *et al*., 2011), testing also changed from non-selective measurement of a total PEth fraction to quantification of one or a few main homologues. Current PEth testing focuses on the usually predominant and single most sensitive subform, PEth 16:0/18:1, containing one palmitic acid and one oleic acid and making up ∼40% of the total amount (Zheng *et al*., 2011; Helander and Hansson, 2013). The change to LC–MS-based analysis further meant that PEth testing became analytically, and hence also clinically, more sensitive. Today, the PEth test allows detection of not only prolonged heavy drinking (Varga *et al*., 1998) but even a single, large alcohol intake leads to a measurable concentration (Helander *et al*., 2012; Javors *et al*., 2016; Schrock *et al*., 2017).

A remaining issue with relevance for the routine application of PEth is, whether a test value can reliably indicate a specific amount and duration of drinking. Dose–response cut-offs to aid in the interpretation of a PEth test have been suggested (Helander and Hansson, 2013; Kummer *et al*., 2016; Ulwelling and Smith, 2018). However, besides the inter-individual variability in the elimination rate on abstinence (Helander *et al*., 2019), also has the individual PEth formation rate after alcohol intake been reported to vary considerably (Aradottir *et al*., 2006; Stewart *et al*., 2014; Walther *et al*., 2015; Wang *et al*., 2017).

This study was undertaken to provide additional information on the association between PEth concentration in whole blood and self-reported prior alcohol intake, based on repeated measurements in problem drinkers during voluntary outpatient treatment for reduced drinking. A comparison of parallel PEth and CDT levels was also performed.

METHODS

Patients and samples

The study was carried out at 'Riddargatan 1', an outpatient treatment unit at the Stockholm Centre for Dependence Disorders (Sweden) focusing on individuals with drinking problems (patients are diagnosed with harmful consumption or dependence of alcohol). The primary objective is to offer treatment before serious social and health-related consequences appear. All patients attend the unit on a voluntary basis.

At start, all patients are offered an alcohol assessment by self-report questionnaires and biological markers, focusing on the consequences of drinking, followed by a feedback session. The treatment is structured but individually calibrated, mainly psychologically or pharmacologically oriented, and typically last for ∼6 months including a follow-up. The psychological treatment focuses on behavioral self-control training (Walters, 2000) and motivational enhancement (Sellman *et al*., 2001). The pharmacological treatment aims at reinforcing control over bad alcohol habits and involves use of medications such as naltrexone or acamprosate (Maisel *et al*., 2013). It is also possible to combine the two (Anton *et al*., 2006).

For this study, consecutive patients attending treatment with the goal of learning how, or getting support, to control their drinking (i.e. not aimed at complete sobriety) received oral and written information about its purpose and content and were invited to participate. After giving written informed consent, they obtained an alcohol diary and training how to quantify their daily alcohol intake in number of standard drinks (12 g ethanol). It usually took several weeks from the first contact with the unit until they started the study. The time interval between sampling occasions was typically ∼3–4 weeks and, on each occasion, the patients should bring their alcohol diary and give venous blood samples for measurement of PEth (ethylenediaminetetraacetic acid whole blood) and CDT (serum). Feedback about previous biomarker results was also given.

The blood samples were forwarded daily to the Karolinska University Laboratory in Huddinge (Stockholm, Sweden) for measurement of PEth and CDT using standard routines.

The study was approved by the ethics committee in Stockholm (Nr 215/362–31).

Laboratory measurement of PEth and CDT

Measurement of PEth (16:0/18:1 homologue) in whole blood specimens was done essentially as previously described (Zheng *et al*., 2011; Ullah *et al*., 2017). In brief, 100 µL whole blood was mixed with 50 µL internal standard solution (PEth-d_{31}; Avanti Polar Lipids, Alabaster, AL, USA), 75 µL acetonitrile, and 150 µL acetone. The mixture was gently shaken (40 rpm) for 20 min at room temperature, centrifuged at 4000*g* for 20 min, and the supernatant transferred to a new vial and centrifuged for another 10 min. LC–MS/MS quantification of PEth 16:0/18:1 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

(Avanti Polar Lipids). The detection limit and lower quantification limit were 0.01 and 0.03 μmol/L, respectively. A PEth 16:0/18:1 value ≥0.30 μmol/L was used as cut-off to indicate excessive alcohol consumption (Helander and Hansson, 2013).

Measurement of CDT (the relative amount of disialotransferrin to total transferrin expressed as percentage peak area) in serum specimens was done by an International Federation of Clinical Chemistry and Laboratory Medicine high-performance liquid chromatography reference method (Helander *et al.*, 2003; Schellenberg *et al.*, 2017). A CDT value ≥2.0% was used as cut-off to indicate prolonged heavy alcohol consumption (Helander *et al.*, 2016; Schellenberg *et al.*, 2017).

Statistics

Statistical calculations were carried out using non-parametric tests, the Mann–Whitney test to test the significance of differences between groups, Wilcoxon paired test in case of pairwise comparison, and Spearman rank correlation to analyze the degree of association between two variables (MedCalc software). Results are presented for all observations and, to control for subject effects, for single observations (the first) of each subject.

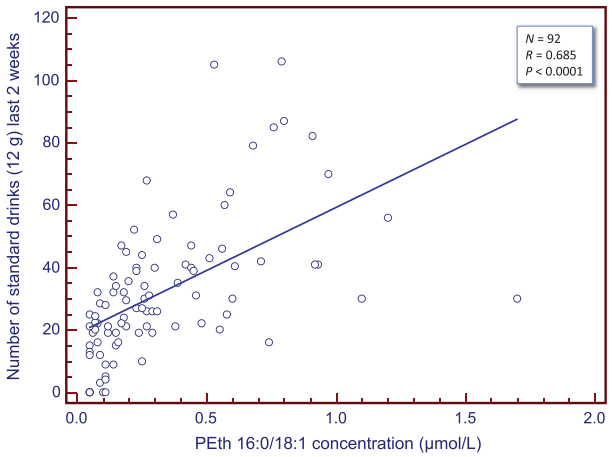

Fig. 1. Correlation between number of standard alcohol drinks (12 g ethanol) consumed in the previous 2 weeks, according to self-report using a daily diary, and the corresponding PEth 16:0/18:1 concentration in whole blood (Spearman rank correlation). Corresponding calculations based on only one (the first) observation from each subject gave similar results ($N = 36$, $R = 0.735$, $P < 0.0001$).

RESULTS

Study population

The study population comprised 36 patients aged 32–83 (mean 53, median 52) years, 25 of which were men aged 35–83 (mean 52, median 50) years, and 11 were women aged 32–66 (mean 55, median 56) years. There was no statistically significant difference in age between men and women ($P = 0.272$, Mann–Whitney test).

Alcohol consumption and biomarker levels

At the first session for collection of study data, which usually took place several weeks after the first contact with the unit, the total alcohol consumption recorded in the previous 2 weeks according to the diary ranged 0–105 (mean 32.4, median 27.5) standard drinks or 0–1260 (mean 389, median 330) g ethanol. PEth 16:0/18:1 was measured in all patients, the concentration ranging 0.05–1.2 (mean 0.32, median 0.23) μmol/L, and with 16 of the 36 (44%) patients showing a value ≥0.30 μmol/L (range 0.30–1.2 (mean 0.55, median 0.51) μmol/L), the cut-off used to indicate excessive alcohol consumption. The CDT values at the first collection ranged 0.7–13.0% (mean 1.9%, median 1.5%), with 8 of 36 (22%) patients showing a value ≥2.0% (range 2.0–13.0 (mean 3.9, median 2.4)%) indicating sustained heavy alcohol consumption.

The number of individual sessions when study data were collected, including providing 2-week retrospective self-reports of alcohol consumption and giving blood samples for PEth and CDT measurement, ranged from 1 to 4 (mean 2.8, median 3.0) times. The time span between the first and last session ranged 5–20 (median 12) weeks. In total, 100 pairs of biomarker results vs daily alcohol intake were obtained, but 8 diaries did not cover the entire 2-week period prior to blood sampling.

At the last collection of study data, the reported total alcohol consumption in the previous 2 weeks ranged 0–79 (mean 30.1, median 27.0) standard drinks, the PEth values ranged 0.05–1.70 (mean 0.34, median 0.24) μmol/L with 12 of 34 (35%) exceeding the ≥0.30 μmol/L cut-off (range 0.31–1.7 (mean 0.67, median 0.58) μmol/L), and the CDT values ranged 0.8–16.1% (mean 2.0%, median 1.4%) with 5 of 34 (15%) exceeding the ≥2.0% cut-off (range 2.0–16.1 (mean 6.5, median 3.5)%). Overall, there were no statistically significant differences compared to the first collection ($P = 0.808$ for reported drinking, $P = 0.915$ for PEth values, and $P = 0.734$ for CDT values; Wilcoxon paired test).

Dose–response between PEth levels and self-reported alcohol consumption

The PEth 16:0/18:1 concentration in whole blood correlated significantly with reported alcohol intake in the past 2-week period (Fig. 1). To examine this further, the PEth results were separated into different concentration subgroups and compared with the corresponding alcohol consumption over different time intervals prior to blood sampling. The subgroup with a PEth concentration <0.05 μmol/L, indicating no, very low, or only occasional alcohol intake (Helander and Hansson, 2013), had reported a total intake of 0–25 (mean 9.6, median 12.0) standard drinks (0–300, mean 115, median 144 g ethanol) in the last 2 weeks (Fig. 2). The subgroups with PEth concentrations ≥0.30 μmol/L, indicating excessive alcohol consumption, had reported intake of 16–106 (mean 48.7, median 41.0) standard drinks (192–1270, mean 584, median 492 g ethanol) in the last 2 weeks. Although data revealed considerable overlaps between all subgroups (Fig. 2), the best dose–response association overall with past alcohol intake was obtained for previous 2-week total drinking, followed by for intake in the next last week.

Comparison of individual changes in PEth concentration vs past 2-week alcohol consumption between two successive study visits revealed that, based on the slope of the regression line, an average increase in alcohol intake by ~1.5 standard drinks/day (~20 g ethanol/day) would raise the PEth 16:0/18:1 concentration by ~0.10 μmol/L, and *vice versa* if the alcohol consumption had decreased (Fig. 3). However, the results demonstrated considerable inter-individual differences in test response.

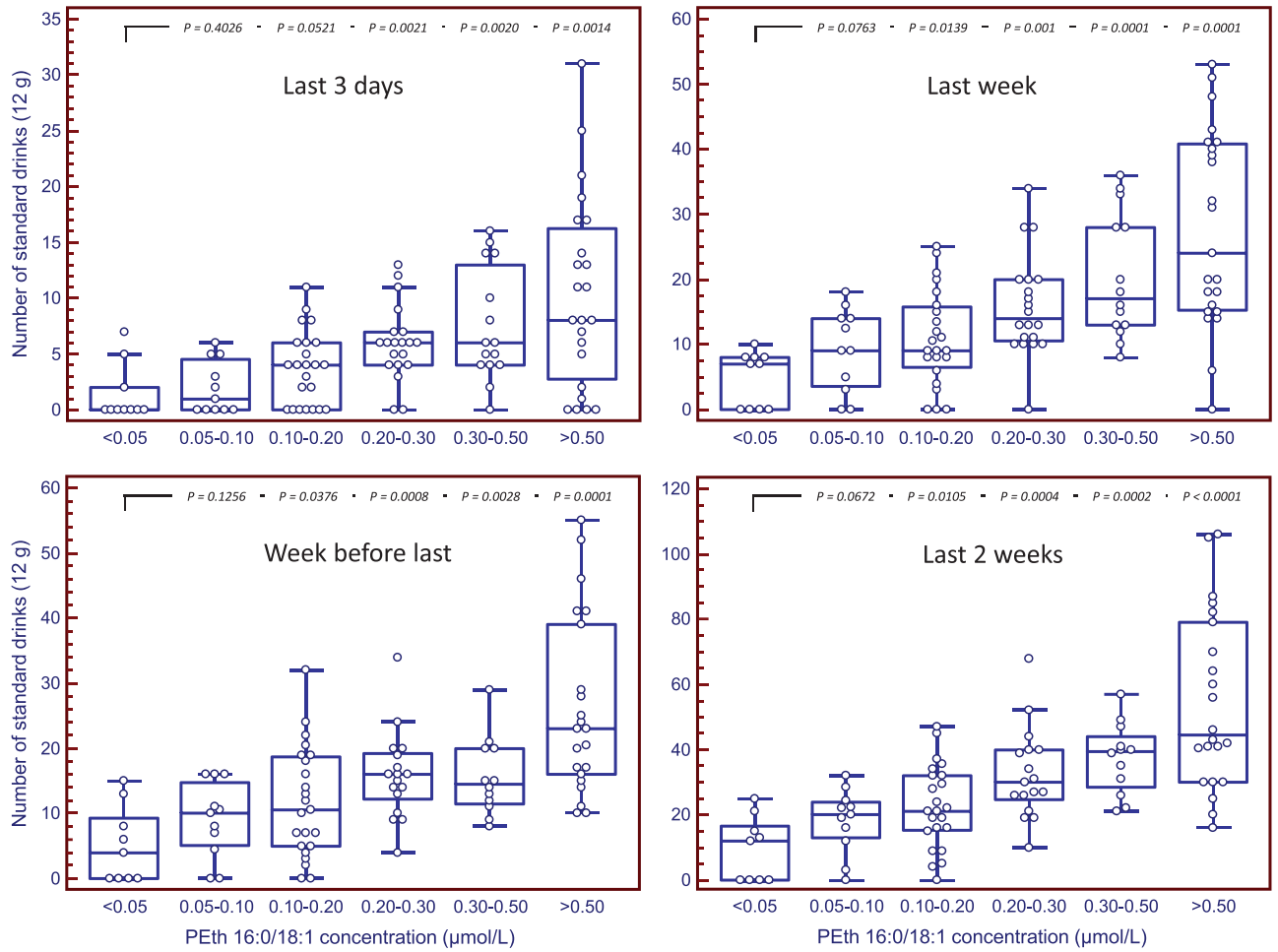

Fig. 2. Distribution of number of standard drinks (12 g ethanol) consumed during different times over the previous 2 weeks, according to self-report using a daily alcohol diary, for different PEth 16:0/18:1 concentration subgroups. The *P* values represent statistical comparison with the <0.05 µmol/L PEth subgroup (Mann–Whitney test).

Correlation between PEth and CDT levels

There was a significant correlation between the whole-blood PEth concentration and the corresponding serum CDT level (Fig. 4). It should be noted that most patients had recorded alcohol consumption levels below the threshold suggested necessary to cause an elevated CDT value (i.e. at least ~60 g ethanol/day on average). However, in 7 cases where the reported past 2-week alcohol consumption was 840–1272 g ethanol (i.e. 60–91 g/day on average), the CDT level was always higher than the ≥2.0% cut-off ranging 2.5–15.2% (mean 6.3%, median 3.5%). In these 7 cases, the PEth concentrations ranged 0.53–0.97 (mean 0.78, median 0.79) µmol/L, which was also well above the corresponding cut-off for PEth 16:0/18:1. For a lower drinking threshold for CDT at ≥700 g/2 weeks (i.e. at least 50 g ethanol/day on average), still 9 of 10 CDT values exceeded the ≥2.0% cut-off (range 2.0–16.1%).

DISCUSSION

The present results demonstrated a significant, dose-dependent positive association between reported total amount of alcohol consumed in the past weeks and the concentration of the alcohol biomarker PEth 16:0/18:1 in whole blood, based on repeated measurements in subjects undergoing voluntary outpatient treatment aiming for reduced drinking. These results agree with observations from previous studies, both on subjects with alcohol-related problems (Aradottir *et al.*, 2006; Stewart *et al.*, 2014; Walther *et al.*, 2015) and from experimental studies where control subjects were administered a standardized ethanol dose (Javors *et al.*, 2016; Schrock *et al.*, 2017).

The overall best statistical correlation was seen with past 2-week alcohol intake, whereas past 3 days drinking had less influence. This is somewhat in disagreement with the results of a simulation statistics study, suggesting that the PEth concentration should be most strongly correlated with alcohol intake over the previous 5 days and with less influence from day 6 to 12 (Helian *et al.*, 2017). However, a clinical study reported a similar test performance for PEth, whether compared with past 1-week, 2-week, or 3-week self-reported alcohol intake (Hahn *et al.*, 2011). Nevertheless, considering the sometimes long half-life of PEth in blood up to about 10 days (Helander *et al.*, 2019), it is evident that drinking dating back more than 2 weeks, and also recent, occasional heavy drinking (Helander *et al.*, 2012), should be taken into account.

There was a considerable inter-individual variability in the association between PEth level and alcohol intake with overlaps between the PEth concentration subgroups. There are several possible reasons for this, such as individual differences in ethanol metabolism,

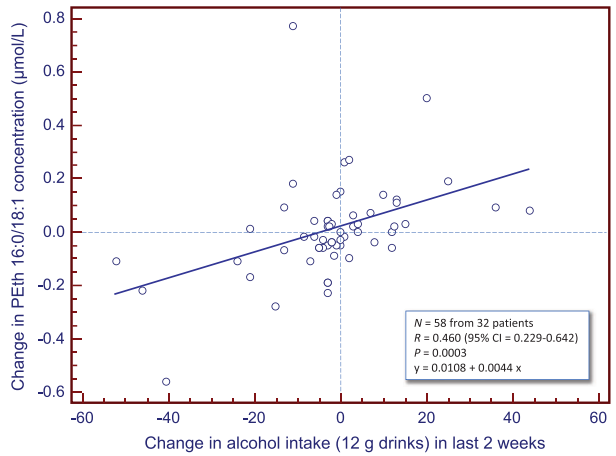

Fig. 3. Comparison of changes in PEth 16:0/18:1 concentration vs number of standard drinks (12 g) between two successive sampling occasions (Spearman rank correlation). The correlation results indicated that, on average, an increased alcohol intake by ~1.5 drinks/day (~20 g ethanol/day) would increase the PEth 16:0/18:1 concentration by ~0.10 µmol/L, and *vice versa* for decreased drinking, albeit with a considerable inter-individual scatter. Corresponding calculations based on only one (the first) observation from each subject gave similar results ($N = 28$, $R = 0.558$, $P = 0.0020$), but the average drinking level needed to change the PEth concentration by ~0.10 µmol/L was somewhat higher (~30 g/day).

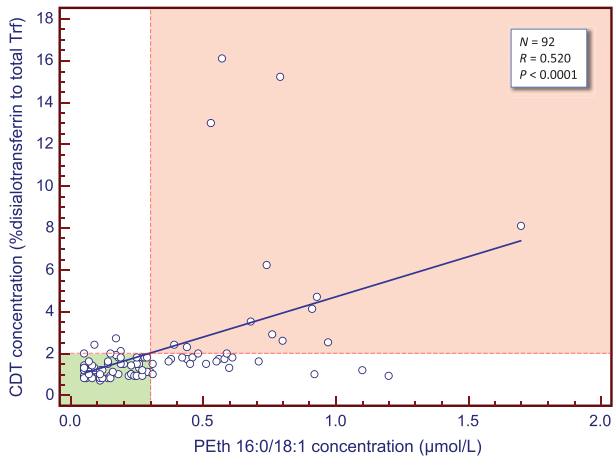

Fig. 4. Correlation between PEth 16:0/18:1 concentration in whole blood samples and the relative CDT level (%disialotransferrin) in serum samples (Spearman rank correlation). The routinely applied cut-offs for PEth 16:0/18:1 and %CDT to indicate excessive alcohol consumption (>0.30 µmol/L and ≥2.0%, respectively) are indicated by broken lines. Corresponding calculations based on only one (the first) observation from each subject gave similar results ($N = 35$, $R = 0.543$, $P = 0.0008$).

phospholipase D activity, and PEth elimination rate. Another reason could be that the self-reported drinking level suffered from uncertainties, as underreporting and denial of alcohol intake is a well-known problem (Helander *et al.*, 1999; Helander and Eriksson, 2002; Del Boca and Darkes, 2003; Whitford *et al.*, 2009). Although the use of self-report to estimate alcohol intake is a limitation of the present study, all participants were voluntarily seeking help for drinking problems, suggesting they had little reason not to give a reliable report, and there were no negative consequences associated with their drinking level. To maximize reporting accuracy of daily alcohol intake, the patients were instructed how to best estimate their alcohol intake, using example drink types and sizes. Using a daily diary instead of retrospective calculation also seems more useful, to gather information about drinking (Leigh, 2000). The small changes observed in reported drinking levels as well as in PEth and CDT values between the first and last collection of study data further supported that the reported amounts were reliable. Nevertheless, caution is needed if using self-reports as a sole outcome measure for alcohol intake, in evaluation of treatments and clinical trials (Wang *et al.*, 2018).

When comparing the individual changes in PEth concentration vs past 2-week alcohol consumption between two successive sessions, it was concluded that an average increase in alcohol intake by ~20 g ethanol/day would raise the PEth 16:0/18:1 concentration by ~0.10 µmol/L, and *vice versa*, albeit with a large inter-individual scatter. Accordingly, the PEth threshold currently used in Sweden to indicate excessive drinking (≥0.30 µmol/L) (Helander and Hansson, 2013) would correspond to an average daily intake of about 60 g ethanol, which is in the range for harmful drinking suggested by many authorities (WHO, 2000). This dose–response effect is considerably different from that in a study of 44 healthy volunteers who were instructed to drink 16 g (women) or 32 g (men) ethanol/day over 3 months (Kechagias *et al.*, 2015). The mean whole-blood PEth 16:0/18:1 concentration reported after this alcohol consumption level was only 0.02 µmol/L, which is much lower than would be expected from the present results, but it should be considered that there was no compliance control of alcohol intake in that study (Kechagias *et al.*, 2015).

Compared with CDT, the present results demonstrated that PEth 16:0/18:1, measured by LC–MS and using the nationally harmonized ≥0.30 µmol/L cut-off indicating excessive drinking, was the more sensitive blood-based alcohol biomarker, which agrees with previous observations (Helander *et al.*, 2012; Andresen-Streichert *et al.*, 2017). It should be noted that most participants reported an average alcohol intake that is considered insufficient to trigger an elevation of CDT (Helander *et al.*, 2016; Schellenberg *et al.*, 2017), but among those who reported intake of at least ~50 g or 60 g ethanol/day on average, the test positive rate was 90 and 100%, respectively.

CONCLUSIONS

The present results from repeated alcohol biomarker measures in subjects undergoing voluntary outpatient treatment for reduced drinking demonstrated that the PEth concentration in whole blood correlated well with self-reported alcohol intake in the last weeks. However, a large inter-individual scatter in PEth concentration vs alcohol intake indicated that it is possible to make only approximate estimates of the quantity of drinking based on a single PEth value, implying risk for misclassification. Because PEth is an ethanol metabolite, its presence in blood is useful as a specific biomarker to distinguish between alcohol abstinence and any drinking over the past weeks to about 1 month. CDT, on the other hand, was useful to identify subjects with the highest alcohol consumption level. Accordingly, for diagnostic purposes and treatment follow-up, these biomarkers with somewhat different features may be used together and also combined with a sensitive test for recent drinking such as ethyl glucuronide and ethyl sulfate (Helander *et al.*, 2009).

ACKNOWLEDGEMENTS

This work was supported by a grant provided by the Stockholm County Council to AH (ALF project 20160517).

CONFLICT OF INTEREST STATEMENT

None.

REFERENCES

Andresen-Streichert H, Beres Y, Weinmann W, *et al.* (2017) Improved detection of alcohol consumption using the novel marker phosphatidylethanol in the transplant setting: results of a prospective study. *Transpl Int* **30**:611–20.

Anton RF, O'Malley SS, Ciraulo DA, *et al.* (2006) Combined pharmacotherapies and behavioral interventions for alcohol dependence: the COMBINE study: A randomized controlled trial. *JAMA* **295**:2003–17.

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.

Bergström JP, Helander A. (2008) HPLC evaluation of clinical and pharmacological factors reported to cause false-positive carbohydrate-deficient transferrin (CDT) levels. *Clin Chim Acta* **389**:164–6.

Del Boca FK, Darkes J. (2003) The validity of self-reports of alcohol consumption: State of the science and challenges for research. *Addiction* **98**:1–12.

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.

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.

Hahn JA, Dobkin LM, Mayanja B, *et al.* (2011) Phosphatidylethanol (PEth) as a biomarker of alcohol consumption in HIV-positive patients in sub-Saharan Africa. *Alcohol Clin Exp Res* **36**:854–62.

Helander A, Böttcher M, Dahmen N, *et al.* (2019) Elimination characteristics of the alcohol biomarker phosphatidylethanol (PEth) in blood during alcohol detoxification. *Alcohol Alcohol* **54**:251–7.

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, Husa A, Jeppsson J-O. (2003) Improved HPLC method for carbohydrate-deficient transferrin in serum. *Clin Chem* **49**:1881–90.

Helander A, Jaeken J, Matthijs G, *et al.* (2014) Asymptomatic phosphomannose isomerase deficiency (MPI-CDG) initially mistaken for excessive alcohol consumption. *Clin Chim Acta* **431**:15–8.

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.

Helian S, Brumback BA, Cook RL. (2017) Sparse canonical correlation analysis between an alcohol biomarker and self-reported alcohol consumption. *Commun Stat Simul Comput* **46**:7924–41.

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 J-O, Kristensson H, Fimiani C. (1993) Carbohydrate-deficient transferrin quantified by HPLC to determine heavy consumption of alcohol. *Clin Chem* **39**:2115–20.

Kechagias S, Dernroth DN, Blomgren A, *et al.* (2015) Phosphatidylethanol compared with other blood tests as a biomarker of moderate alcohol consumption in healthy volunteers: a prospective randomized study. *Alcohol Alcohol* **50**:399–406.

Kenan N, Larsson A, Axelsson O, *et al.* (2011) Changes in transferrin glycosylation during pregnancy may lead to false-positive carbohydrate-deficient transferrin (CDT) results in testing for riskful alcohol consumption. *Clin Chim Acta* **412**:129–33.

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.

Leigh BC. (2000) Using daily reports to measure drinking and drinking patterns. *J Subst Abuse* **12**:51–65.

Maisel NC, Blodgett JC, Wilbourne PL, *et al.* (2013) Meta-analysis of naltrexone and acamprosate for treating alcohol use disorders: When are these medications most helpful? *Addiction* **108**:275–93.

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.

Sellman JD, Sullivan PF, Dore GM, *et al.* (2001) A randomized controlled trial of motivational enhancement therapy (MET) for mild to moderate alcohol dependence. *J Stud Alcohol* **62**:389–96.

Stewart SH, Koch DG, Willner IR, *et al.* (2014) Validation of blood phosphatidylethanol as an alcohol consumption biomarker in patients with chronic liver disease. *Alcohol Clin Exp Res* **38**:1706–11.

Stibler H. (1991) Carbohydrate-deficient transferrin in serum: a new marker of potentially harmful alcohol consumption reviewed. *Clin Chem* **37**:2029–37.

Ullah S, Helander A, Beck O. (2017) Identification and quantitation of phosphatidylethanols in oral fluid by liquid chromatography-tandem mass spectrometry. *Clin Chem Lab Med* **55**:1332–9.

Ulwelling 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.

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 Mol Sci* **13**:14788–812.

Walters GD. (2000) Behavioral self-control training for problem drinkers: a meta-analysis of randomized control studies. *Behavior Therapy* **31**: 135–49.

Walther L, de Bejczy A, Lof E, *et al.* (2015) Phosphatidylethanol is superior to carbohydrate-deficient transferrin and gamma-glutamyltransferase as an alcohol marker and is a reliable estimate of alcohol consumption level. *Alcohol Clin Exp Res* **39**:2200–8.

Wang S, Yang R, Ji F, *et al.* (2017) Sensitive and precise monitoring of phosphatidylethanol in human blood as a biomarker for alcohol intake by ultrasound-assisted dispersive liquid-liquid microextraction combined with liquid chromatography tandem mass spectrometry. *Talanta* **166**:315–20.

Wang Y, Chen X, Hahn JA, *et al.* (2018) Phosphatidylethanol in comparison to self-reported alcohol consumption among HIV-infected women in a randomized controlled trial of naltrexone for reducing hazardous drinking. *Alcohol Clin Exp Res* **42**: 128–34.

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.

WHO. (2000) *International Guide for Monitoring Alcohol Consumption and Related Harm*. https://apps.who.int/iris/bitstream/handle/10665/66529/WHO_MSD_MSB_00.4.pdf;jsessionid=843508D6B7B9829B118D02CE62156CF1?sequence=1

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.