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Phosphatidylethanol in blood (B-PEth): A marker for alcohol use and abuse

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Phosphatidylethanol (PEth) represents a group of glycerophospholipid homologues where ethanol by phospholipase D has been bound at the position that normally contains an amino-alcohol. Since the formation of PEth is specifically dependent on ethanol, the diagnostic specificity of PEth as an alcohol biomarker is theoretically 100%. The half-life of PEth in blood is approximately 4 days. The amount of alcohol consumed correlates to blood concentration of PEth and PEth has been shown to be a more sensitive indicator of alcohol consumption than traditional alcohol markers, such as CDT (carbohydrate-deficient transferrin), GGT (γ-glutamyl transferase), and MCV (mean corpuscular volume) or a combination of these. Almost all clinical data so far available are based on a high performance liquid chromatography (HPLC) method with limited analytical sensitivity. With the advent of methods with considerably higher analytical sensitivity (e.g. mass spectrometric methods), clinical sensitivity will increase correspondingly. The possibility of determining very low concentrations of PEth by new sensitive analytical techniques may, however, have both ethical and legal consequences that have to be considered.

Keywords: phosphatidylethanol; PEth; blood; alcohol; ethanol

Introduction

Alcohol is a common cause of disability, disease, and premature death. Guidance on the investigation of suspected alcohol dependence or abuse includes symptoms, medical history, self-report forms, special questionnaires (e.g. AUDIT), and alcohol biomarkers. Aside from ethanol in blood, urine, or exhaled air, there are several direct markers of alcohol consumption that can be measured in blood or urine, for example, some minor ethanol metabolites such as ethyl glucuronide (EtG), ethyl sulfate (EtS), and fatty acid ethyl esters (FAEE). Common to EtG and EtS is that specificity is high, that single intake will give a positive result, and that they are detected in urine one or a few days after alcohol intake and a somewhat shorter time period when measured in serum.[1–5] It is, however, described that EtG, but not EtS, can be produced post-sampling if urine specimens contain E. coli[6] and that EtG but not EtS is sensitive to bacterial hydrolysis if samples are stored improperly.[7,8] To increase clinical specificity, a combination of EtG and EtS has been recommended. FAEE in serum has a similar window of detection as EtG and EtS.[9] Longer time windows of several months are seen when these markers, i.e. FAEE or EtG, are determined in hair,[10–13] but the use of hair as sample material is somewhat hampered for practical reasons. Slowly eliminated indirect markers of alcohol consumption such as GGT (γ-glutamyltransferase) and MCV (mean corpuscular volume) are therefore still used for detection and follow up of long-term risk consumption or heavy drinking. Common to these are, however, their low clinical sensitivity and specificity, which means that a large proportion of high consumers may have normal values and that an elevated value in a significant proportion of cases has a reason other than alcohol. CDT (carbohydrate-deficient transferrin), another indirect marker of alcohol consumption with a wide window of detection, shows a considerably higher clinical specificity but only a minor improvement in sensitivity. Slowly eliminated direct biomarkers of ethanol with inherent improved specificity and sensitivity would therefore seem to be highly desirable. One such biomarker that has gained increasing interest over the last few years is phosphatidylethanol in blood (B-PEth).

Phosphatidylethanol (PEth)

PEth represents a group of glycerophospholipid homologues (Figure 1) with phosphoethanol as the head group, each with a unique set of long chain carboxylic acid residues (two per molecule), typically containing 14 to 22 carbon atoms with 0 to 6 double bonds, as substituents.[14] These homologues are commonly named in the form 'PEth A:B/C:D'; wherein A indicates the number of carbons in the carboxylic acid substituent at the first position of the glycerol backbone and B indicates the number of double bonds encompassed by that carbon chain; C indicates the number of carbons in the carboxylic acid substituent at the second position of the glycerol backbone and D indicates the number of double bonds encompassed by that carbon chain. This nomenclature, however, does not indicate the position, or the stereochemistry, of any existing double bonds. For example, the regio- and stereo-isomers 1-Hexadecanoic acid-2-[(Z)-Octadec-9-enoic acid]-sn-Glycero-Phosphatidylethanol and 1-Hexadecanoic acid-2-[(E)-Octadec-11-enoic acid]-sn-Glycero-Phosphatidylethanol are both named PEth 16:0/18:1. Theoretically, there are a large number of different PEth homologues and so far 48 homologues of

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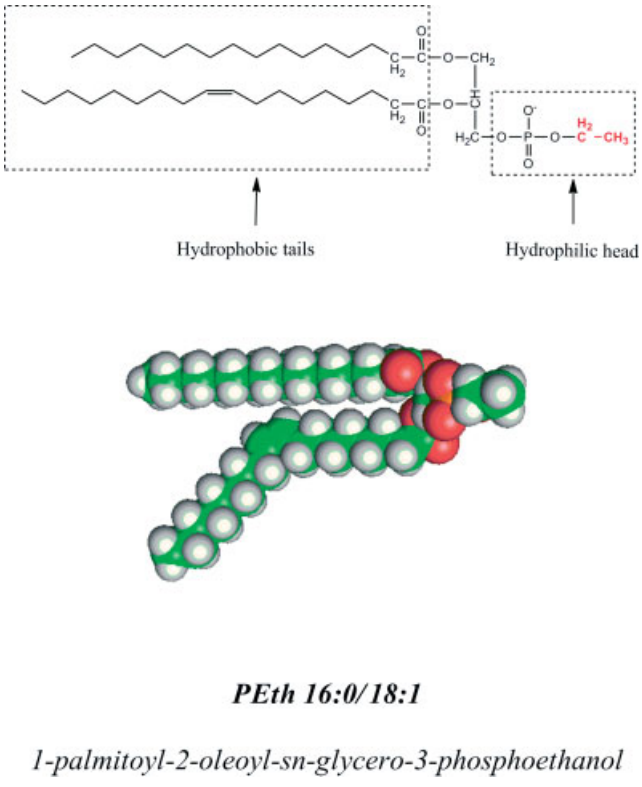

Figure 1. The structure of the most common PEth homologue, PEth 16:0/18:1.

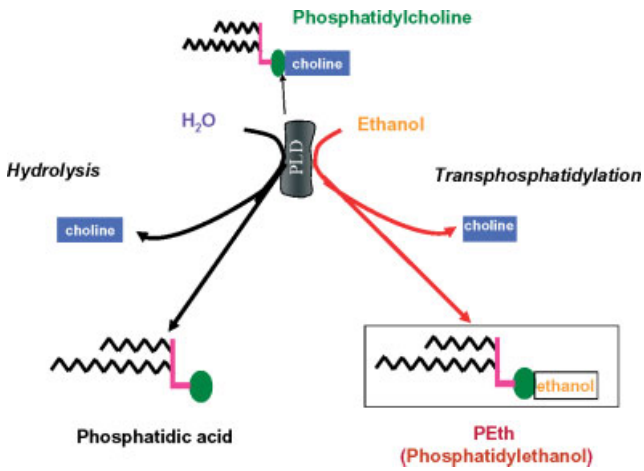

Figure 2. Schematic representation of the formation of phosphatidylethanol (PEth) from phosphatidylcholine. Normally, in the presence of water, phospholipase D (PLD) hydrolyzes phosphatidylcholine to phosphatidic acid and choline. In the presence of alcohol (ethanol), however, PLD synthesizes phosphatidylethanol (PEth) from phosphatidylcholine.

PEth have been identified in blood.[14] PEth is an abnormal phospholipid formed by transphosphatidylation of precursor phospholipids with a glycerol backbone under catalytic influence of phospholipase D in the presence of ethanol.[15–17] In the absence of ethanol, phosphatidic acid is formed (Figure 2).

After exposition to ethanol, PEth can be detected in different organs of both humans and rats with the highest concentrations in the gastrointestinal tract (especially when alcohol is given orally), kidney, lung, and spleen, but also in the CNS.[18–19] In contrast to the rat, PEth in humans is also demonstrated in the blood.[20] *In vitro* incubation of human blood with ethanol results in formation of PEth, unlike blood from rats and several other investigated species,[21] suggesting that humans may be different with regard to the formation and accumulation of PEth in blood. Formation of PEth in human blood takes place both at room temperature and at $-20\,^{\circ}C$ but no significant formation is seen when blood is incubated at $+4\,^{\circ}C$ in the presence of ethanol for up to 3 weeks.[22]

In blood from humans, PEth can be detected in leukocytes and erythrocytes. Since erythrocyte counts exceed leukocyte counts by a factor of about 1000, almost all PEth in blood is, however, found within the erythrocyte fraction.[23] Like other phospholipids, PEth is probably concentrated in the membrane fraction of cells.

Evaluation of PEth as an Alcohol Biomarker

Data on the formation and degradation of PEth *in vivo* were needed to establish PEth as an alcohol marker. It has been shown that more than a week of regular consumption of more than moderate amounts of alcohol is required before PEth is detectable in blood and that a single dose of ethanol (50 g) does not give measurable PEth-concentrations ($>0.8\,\mu mol/L$).[24] In a study by Aradottir *et al.*[25] involving 144 actively drinking patients (123 men, 21 women) consisting of both outpatients and inpatients, PEth was detectable ($\geq 0.22\,\mu mol/L$) in all patients except one. Other measured alcohol markers, including CDT and GGT, were increased in a considerably lower proportion of the patients. In this study it was also observed that PEth was positive at a lower consumption level than was required for the other alcohol markers (Figure 3). Various studies have reported a sensitivity of PEth as an alcohol marker between 94 and 100% depending on study group and decision-limit[24–26] and that there is a clear correlation between reported alcohol consumption and the concentration of PEth in blood.[25] One of these studies[26] comprised 35 forensic psychiatric patients, who had not been drinking alcohol for at least 4 weeks prior to sampling and 56 alcohol-dependent patients with a self-reported intake of 280–5320 g of alcohol during the 7 days immediately prior to admission to hospital for detoxification. ROC (receiver operating characteristics) analysis in this study showed 94.5% sensitivity and 100% specificity at a decision-limit for PEth of $0.36\,\mu mol/L$. The corresponding values for both sensitivity and specificity of GGT, MCV, and CDT were significantly lower. Studies of different patient groups including both active alcoholics and non-drinking subjects with previous alcohol abuse demonstrated no false positive results for PEth.[12,26,27] The half-life of PEth in the blood is about 4 days, which means that PEth, depending on the value at baseline, can be detected up to 4 weeks after alcohol has been eliminated from the body.[23,27–29] No effects of gender were seen when correlating reported alcohol consumption to PEth concentration at admission to hospital or at any time point during abstinence.[29]

PEth as a Clinical Alcohol Biomarker

Since May 2006 our laboratory has made PEth accessible as a clinical alcohol marker to health care organisations and other clients. Determination of PEth has been performed with a high performance liquid chromatography (HPLC) method. The

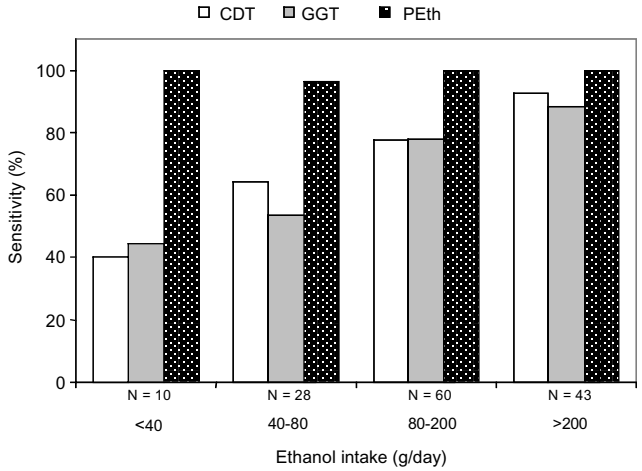

Figure 3. Sensitivity of CDT, GGT and PEth is shown in relation to alcohol intake (g ethanol/day) at four different levels of average alcohol intake (from low, moderate to very high consumption) during the last two weeks prior to sampling. Decision-limits are 0.22 μmol/L, 1.7% and 50 U/L (0.83 μkat/L) for PEth, CDT (HPLC method) and GGT, respectively. The total number of individuals (N) is shown for each level of consumption.

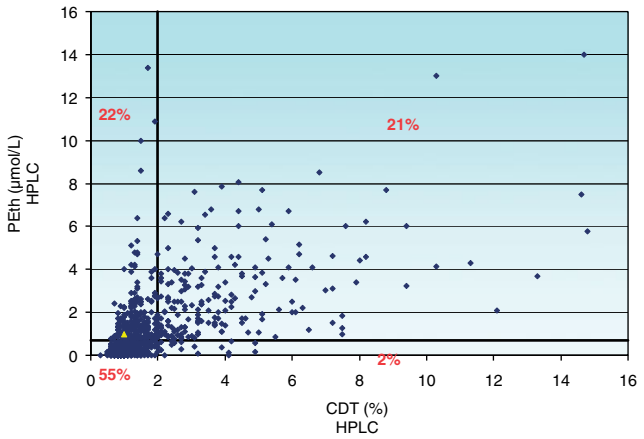

Figure 4. Scatter plot of CDT and PEth for all patient samples analyzed in clinical routine where both alcohol markers were requested (N = 969). The lines in the graph represent the decision-limits for CDT (HPLC method) and PEth (2.0% and 0.7 μmol/L, respectively).

consistent lower limit of quantification (LOQ) of this method is ≤0.7 μmol/L. The number of requests has continuously increased and now exceeds that of CDT (9205 compared to 7381 during January–October 2010), heretofore the most requested alcohol marker. In a large number of consecutive patient samples, 67% showed values <0.7 μmol/L, which is currently considered to correspond to moderate or low alcohol consumption during the 2 weeks immediately prior to sampling. Nineteen percent had values ranging from 0.7 to 2.0 μmol/L, 12% had values ranging from 2.0 to 5.5 μmol/L, and 2.6% had values >5.5 μmol/L, levels corresponding to an increasingly pronounced consumption of alcohol. In many cases, both PEth and CDT were ordered simultaneously and values of the two alcohol markers have been compared in 969 patient samples (Figure 4). Fifty-five percent of the samples were below the decision-limit for both markers (<0.7 μmol/L for PEth and <2.0% for CDT); 22% were above the decision-limit only for PEth; while 2% of patients had CDT >2.0% in combination with PEth <0.7 μmol/L. Overall, this is consistent with results from published studies, which have demonstrated that PEth is a much more sensitive alcohol marker than CDT. The reasons for the combination of elevated CDT but normal PEth may be several. One important reason is that the decision-limit for PEth has been dictated by the LOQ of the HPLC method. On the other hand, this decision-limit also reduces the risk for normal consumption in social drinkers to be detected. A further reason for the rare cases of relatively low PEth values relative to CDT may also be differences in half-lives in blood, where CDT on average has a half-life of about 10 days compared with a half-life of PEth of about 4 days. Another and probably more important reason is differences in clinical specificity (100% for PEth compared with about 90–95% for CDT).

Case Reports

These cases illustrate the difference in clinical specificity (Case 1) and sensitivity between CDT and PEth as well as the time pattern of these (Cases 2–4, Figure 5) and results from a patient with alcoholic hepatitis (Case 5).

Case 1

In a driver's license issue, a man was refused a new license because of repeatedly elevated CDT values (2.4 to 2.8%). He denied drinking. Further samples on several later occasions showed CDT values at constant elevated level despite persistently undetectable PEth concentrations even using an alternative method (mass spectrometry) with increased analytical sensitivity. He subsequently received a new license.

Case 2

A 54-year-old man with obesity, hypertension and known alcohol abuse for at least 10 years with a reported alcohol consumption of one bottle of vodka per weekend, and 0–4 cans of beer daily. PEth was elevated at all sampling occasions, but CDT was constantly below decision-limit.

Case 3

A 51-year-old man with known long-term alcohol abuse had elevated PEth values on seven of the eight sampling occasions, but only at one of four sampling occasions for CDT. The lower values of PEth and CDT in May/June coincides with treatment on an outpatient basis.

Case 4

A 52-year-old woman with longstanding alcohol abuse was hospitalized in 2008 after an epileptic seizure. She had elevated PEth on 13 out of 14 sampling occasions and on 19 of 19 occasions for CDT with similar temporal patterns for the two markers.

Case 5

A 56-year-old man with well known alcohol problems was admitted to hospital with a diagnosis of alcoholic hepatitis. At admission, liver tests and alcohol markers were significantly increased (PEth = 32 μmol/L, CDT = 11%, GGT = 84 μkat/L, AST (aspartate aminotransferase) = 12 μkat/L, ALT (alanine aminotransferase) = 3.4 μkat/L and bilirubin = 113 μmol/L). The value of PEth is the highest so far recorded at our laboratory.

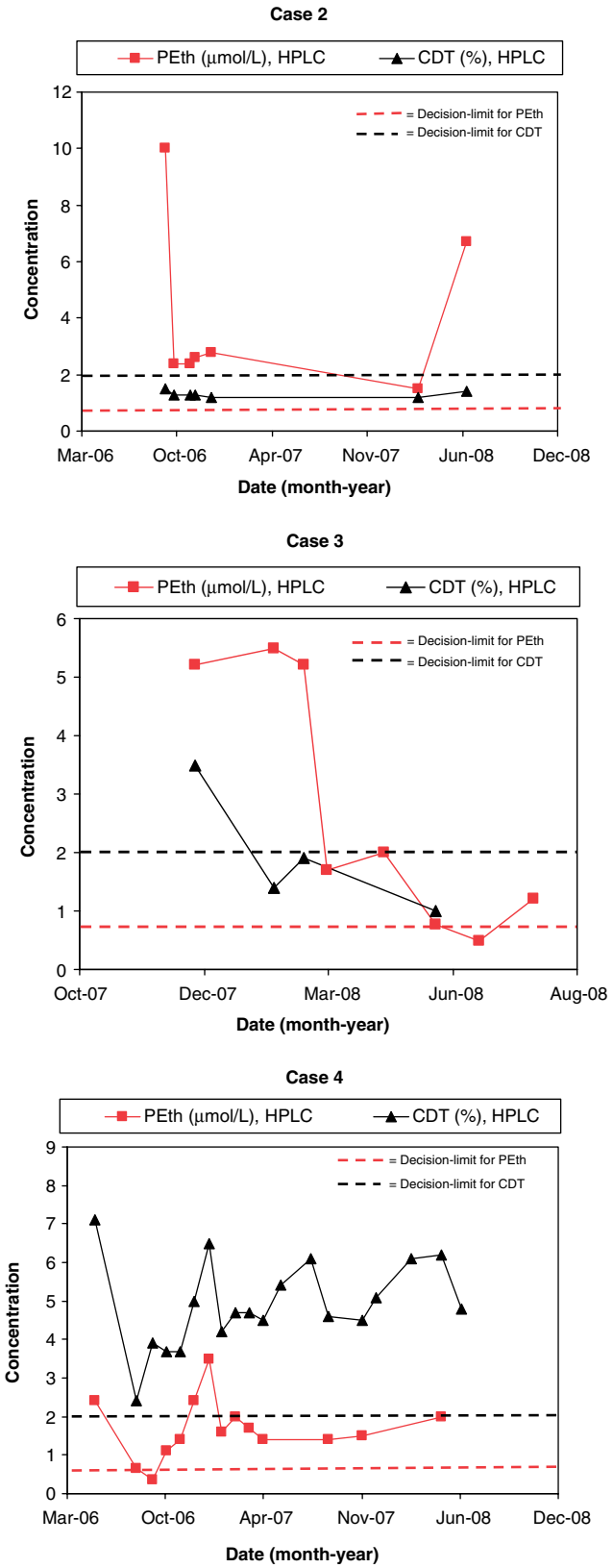

Figure 5. The concentration of PEth and CDT (HPLC method) at different sampling occasions. Dashed lines represent decision-limits for PEth (0.7 µmol/L, red line) and CDT (2.0%, black line).

Analytical Methods

For measurement of B-PEth, venous blood should be obtained in a tube containing ethylenediaminetetraacetic acid (EDTA) that should not be centrifuged. The sample is stable for 24 h at room temperature and for 3 weeks at +4 °C. For longer periods of storage, whole blood is frozen in a plastic tube and kept at −80 °C. The sample should not be stored at −20 °C.

Originally, PEth was discovered using thin layer chromatography.[15] This is a manual and semi-quantitative method with limited sensitivity and throughput and was found unsuitable for further experimental or clinical studies. For these purposes an HPLC method was developed and has also been used for determination of PEth in clinical samples. In this method, whole blood together with the internal standard (Phosphatidylbutanol 18 : 1/18 : 1) are extracted with 2-propanol and hexane, followed by quantification of PEth in the extract on an HPLC system equipped with an evaporative light scattering detector (ELSD) and with PEth 18 : 1/18 : 1 as calibrator.[22,28] The HPLC method determines the sum of all PEth homologues, which are numerous due to variations in fatty acid substituents. As PEth in blood is a mixture of different PEth homologues, the peak representing PEth in a patient sample is considerably broader than the peak from the calibrator sample (Figure 6). In the beginning of April 2010 we introduced a liquid chromatography tandem mass spectrometric (LC-MS/MS) method for the determination of PEth in clinical samples. The LC-MS/MS method has a considerably higher analytical sensitivity (more than two orders of magnitude), shorter turnaround time, and shows excellent correlation to the HPLC method [manuscript in preparation].

During the last one or two years, there have been several publications on LC-MS and LC-MS/MS methods for PEth from a methodological point of view[14,28,30–31] as well as reports of results from different study populations such as women of reproductive age[32] and patients with liver disease and hypertension.[33] Due to the high analytical sensitivity of LC-MS/MS, PEth was considered a promising marker for differentiating abstinence or light drinking from moderate or heavy consumption as well as a marker of alcohol abuse. Methods based on gas chromatography mass spectrometry (GC-MS)[34] and non-aqueous capillary electrophoresis using UV[35] or mass spectrometric detection[36] have also been described with similar LOQ as the HPLC method with ELSD. In addition, monoclonal antibodies against PEth have been produced[37] possibly enabling the development of an immunoassay.

In mass spectrometric methods for PEth, the MS-detector is typically tuned to detect one or a few of the more commonly occurring PEth-homologues.[30] Since phosphatidylcholine is a precursor of PEth, the concentrations of PEth-homologues containing, for example, oleic acid (18 : 1) and linoleic acid (18 : 2), such as the more commonly occurring PEth 16 : 0/18 : 1 and PEth 16 : 0/18 : 2 are likely to be dependent on a person's diet as has been shown for phosphatidylcholine.[38] Other factors that might influence the fatty acid pattern in phosphatidylcholine and PEth are diseases and alcohol habits. Hence, the level of previous ethanol intake may be over- or underestimated when only one or a few PEth homologues are determined. This might be compensated for if the sum of the most prevalent PEth homologues is reported. To simplify and standardize future analysis of PEth with mass spectrometry, it should, however, be advantageous to focus on distinct molecular species but this requires further evaluation, access to commercial reference substances for other

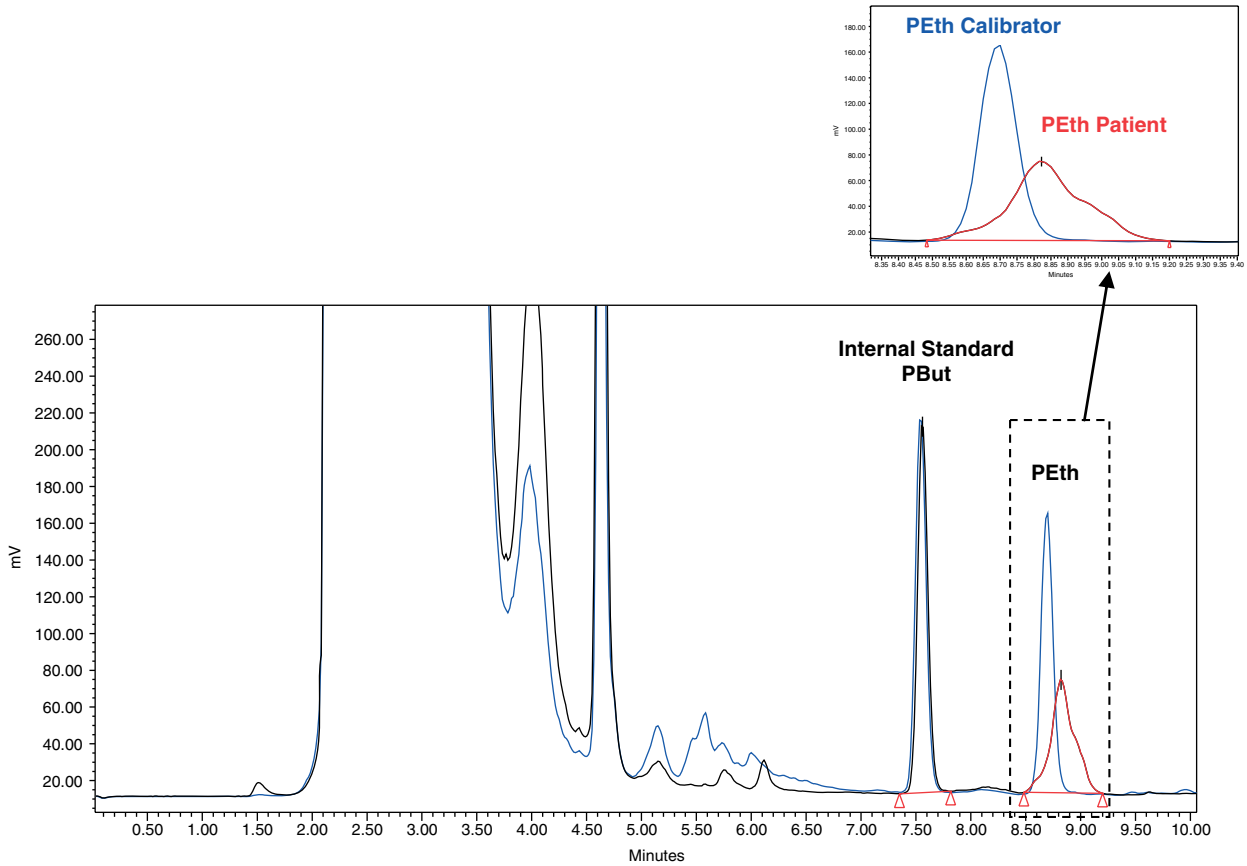

Figure 6. HPLC-chromatograms from an extract of a patient sample containing 4.9 µmol/L of PEth (red line) and a calibrator sample with 6.0 µmol/L of PEth 18 : 1/18 : 1 (blue line). Phosphatidylbutanol 18 : 1/18 : 1 (PBut) is used as the internal standard and elutes at 7.55 min. PEth in the calibrator sample elutes as a narrow peak at 8.7 min, whereas the different PEth homologues in the patient sample elutes as a broader peak at 8.9 min.

PEth homologues than presently available (PEth 16 : 0/16 : 0; PEth 16 : 0/18 : 1 and PEth 18 : 1/18 : 1) and comparison with a method measuring 'total PEth' (HPLC) [manuscript in preparation].

Clinical Applicability of PEth

The primary use of PEth is to detect and monitor patients with alcohol dependence or abuse and in the early identification of people with risk behaviour or harmful alcohol consumption. PEth can also be an aid in the investigation of several medical conditions that may be alcohol related or pathological conditions where alcohol may have an aggravating effect. Determination of PEth has begun to be used in preoperative evaluation before liver transplantation. Other uses are in emergency room setting,[39] occupational health service, in driving license issues,[40] workplace testing, and possibly in forensic medicine.[13,41] In the latter case, potential post-mortem formation of PEth may, however, lead to an interpretation problem when ethanol is present in blood. Another use of PEth is in the investigation of abnormal values for AST, ALT, GGT, or MCV. PEth is also reported to be of considerable value in the evaluation of patients with gastroenterological symptoms or diseases. With the combination of relatively slowly (i.e. PEth) and rapidly eliminated markers (i.e. EtG and EtS) of alcohol, it might be possible to determine both when and how much alcohol has been consumed.

The significant increase in analytical sensitivity using mass spectrometric methods for PEth results in a broadening of its clinical applicability. In a study of patients with liver disease, it was found that there was a correlation between reported alcohol consumption and PEth, that PEth could discriminate between abstinence or light drinking from moderate or heavy consumption, and that PEth concentration is not influenced by liver function or the presence of liver disease.[33] In a study of women of reproductive age, it was observed that PEth was detectable in 93% of women reporting a consumption of alcohol corresponding to 2 or more drinks per day.[32] PEth was therefore suggested as a complement to the use of self-report alcohol screens. Detection of regular alcohol use in pregnant women is important for reducing the risk of FASD (foetal alcohol spectrum disorders) and in particular its severe form, FAS (foetal alcohol syndrome), which is related to chronic heavy drinking during pregnancy. Through the highly sensitive mass spectrometric methods for PEth, it may be possible to discriminate between no or minute and low to moderate alcohol consumption. This might be of relevance when monitoring compliance in situations where total abstinence is required, e.g. for reissuing driver's licenses.[42] The possibility of determining very low concentrations of PEth by new sensitive analytical techniques may, however, have both ethical and legal consequences. This must be considered before reporting PEth concentrations at levels corresponding to low alcohol consumption. Caution in this regard is also motivated by the fact that so far there are only limited data on the relationship between PEth concentration and consumption level,[26] particularly at low levels.

Conclusions and Perspectives

Phosphatidylethanol (PEth) is an abnormal phospholipid that is formed only in the presence of alcohol (ethanol). Hence, the diagnostic specificity of PEth as an alcohol marker is theoretically 100%. No false positive results have been recorded using the HPLC method with an LOQ of 0.7 µmol/L. When more than moderate amounts of alcohol are regularly consumed, blood cells become enriched with PEth. Regular consumption for at least one week appears to be needed to give concentrations of PEth exceeding the LOQ of the HPLC method. There is a positive correlation between the amount of alcohol consumed and blood concentration of PEth. PEth has been shown to be a more sensitive indicator of alcohol consumption than CDT, GGT, and MCV or a combination of these. The half-life of PEth in circulation is about 4 days, which means that PEth can be detected up to 4 weeks after ethanol has been cleared from the body. All these conclusions are based on results obtained from the HPLC method. The development of mass spectrometric methods for PEth with a considerably higher analytical sensitivity results in a corresponding increase in clinical sensitivity. The sensitive mass spectrometric methods can be expected to be able to differentiate no or minute alcohol consumption from low consumption such as monitoring compliance during periods of required abstinence. Other situations where sensitive PEth methods might be of value are during pregnancy and in the pre-operative evaluation before liver transplantation. Standardization, quality assurance, and further clinical evaluation of these methods are needed, however, to establish their clinical applicability. Another issue is to investigate whether it is possible to link PEth concentration to a precise drinking level throughout the whole spectrum from no or minute to heavy consumption.

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References

[1] J. C. Kissack, J. Bishop, A. L. Roper, *Pharmacotherapy* **2008**, *28*, 769.
[2] T. Neumann, A. Helander, H. Dahl, T. Holzmann, B. Neuner, E. Weiss-Gerlach, C. Muller, C. Spies, *Alcohol Alcoholism* **2008**, *43*, 431.
[3] C. C. Halter, S. Dresen, V. Auwaerter, F. M. Wurst, W. Weinmann, *Int. J. Legal Med.* **2008**, *122*, 123.
[4] A. Helander, O. Beck, *J. Anal. Toxicol.* **2005**, *29*, 270.
[5] A. Helander, M. Bottcher, C. Fehr, N. Dahmen, O. Beck, *Alcohol Alcoholism* **2009**, *44*, 55.
[6] A. Helander, I. Olsson, H. Dahl, *Clin. Chem.* **2007**, *53*, 1855.
[7] S. Baranowski, A. Serr, A. Thierauf, W. Weinmann, M. Grosse Perdekamp, F. M. Wurst, C. C. Halter, *Int. J. Legal. Med.* **2008**, *122*, 389.
[8] A. Helander, H. Dahl, *Clin. Chem.* **2005**, *51*, 1728.
[9] K. Borucki, J. Dierkes, J. Wartberg, S. Westphal, A. Genz, C. Luley, *Alcohol. Clin. Exp. Res.* **2007**, *31*, 423.
[10] F. Pragst, V. Auwaerter, F. Sporkert, K. Spiegel, *Forensic Sci. Int.* **2001**, *121*, 76.
[11] F. Pragst, M. Yegles, *Ther. Drug Monit.* **2008**, *30*, 255.
[12] F. M. Wurst, S. Alexson, M. Wolfersdorf, G. Bechtel, S. Forster, C. Alling, S. Aradottir, K. Jachau, P. Huber, J. P. Allen, V. Auwarter, F. Pragst, *Alcohol Alcoholism* **2004**, *39*, 33.
[13] P. Bendroth, R. Kronstrand, A. Helander, J. Greby, N. Stephanson, P. Krantz, *Forensic Sci. Int.* **2008**, *176*, 76.
[14] H. Gnann, C. Engelmann, G. Skopp, M. Winkler, V. Auwarter, S. Dresen, N. Ferreiros, F. M. Wurst, W. Weinmann, *Anal. Bioanal. Chem.* **2010**, *396*, 2415.
[15] C. Alling, L. Gustavsson, E. Anggard, *FEBS Lett.* **1983**, *152*, 24.
[16] C. Alling, W. Becker, A. W. Jones, E. Anggard, *Alcohol Clin. Exp. Res.* **1984**, *8*, 238.
[17] L. Gustavsson, C. Alling, *Biochem. Bioph. Res. Co.* **1987**, *142*, 958.
[18] S. Aradottir, C. Lundqvist, C. Alling, *Alcohol Clin. Exp. Res.* **2002**, *26*, 514.
[19] S. Aradottir, S. Seidl, F. M. Wurst, B. A. Jonsson, C. Alling, *Alcohol Clin. Exp. Res.* **2004**, *28*, 1718.
[20] P. Hansson, M. Caron, G. Johnson, L. Gustavsson, C. Alling, *Alcohol Clin. Exp. Res.* **1997**, *21*, 108.
[21] S. Aradottir, K. Moller, C. Alling, *Alcohol Alcoholism* **2004**, *39*, 8.
[22] S. Aradottir, B. L. Olsson, *BMC Biochem.* **2005**, *6*, 18.
[23] A. Varga, P. Hansson, G. Johnson, C. Alling, *Clin. Chim Acta* **2000**, *299*, 141.
[24] A. Varga, P. Hansson, C. Lundqvist, C. Alling, *Alcohol Clin. Exp. Res.* **1998**, *22*, 1832.
[25] S. Aradottir, G. Asanovska, S. Gjerss, P. Hansson, C. Alling, *Alcohol Alcoholism* **2006**, *41*, 431.
[26] S. Hartmann, S. Aradottir, M. Graf, G. Wiesbeck, O. Lesch, K. Ramskogler, M. Wolfersdorf, C. Alling, F. M. Wurst, *Addict. Biol.* **2007**, *12*, 81.
[27] F. M. Wurst, R. Vogel, K. Jachau, A. Varga, C. Alling, A. Alt, G. E. Skipper, *Alcohol Clin. Exp. Res.* **2003**, *27*, 471.
[28] T. Gunnarsson, A. Karlsson, P. Hansson, G. Johnson, C. Alling, G. Odham, *J. Chromatogr. B Biomed. Sci. Appl.* **1998**, *705*, 243.
[29] F. M. Wurst, N. Thon, S. Aradottir, S. Hartmann, G. A. Wiesbeck, O. Lesch, K. Skala, M. Wolfersdorf, W. Weinmann, C. Alling, *Addict. Biol.* **2010**, *15*, 88.
[30] A. Helander, Y. Zheng, *Clin. Chem.* **2009**, *55*, 1395.
[31] H. Gnann, W. Weinmann, C. Engelmann, F. M. Wurst, G. Skopp, M. Winkler, A. Thierauf, V. Auwarter, S. Dresen, N. Ferreiros Bouzas, *J. Mass Spectrom.* **2009**, *44*, 1293.
[32] S. H. Stewart, T. L. Law, P. K. Randall, R. Newman, *Alcohol Clin. Exp. Res.* **2010**, *34*, 488.
[33] S. H. Stewart, A. Reuben, W. A. Brzezinski, D. G. Koch, J. Basile, P. K. Randall, P. M. Miller, *Alcohol Alcoholism* **2009**, *44*, 464.
[34] C. Yon, J. S. Han, *Exp. Mol. Med.* **2000**, *32*, 243.
[35] A. Varga, S. Nilsson, *Electrophoresis* **2008**, *29*, 1667.
[36] A. Nalesso, G. Viel, G. Cecchetto, G. Frison, S. D. Ferrara, *Electrophoresis* **2010**, *31*, 1227.
[37] A. E. Nissinen, S. M. Makela, J. T. Vuoristo, M. K. Liisanantti, M. L. Hannuksela, S. Horkko, M. J. Savolainen, *Alcohol Clin. Exp. Res.* **2008**, *32*, 921.
[38] L. Hodson, C. M. Skeaff, B. A. Fielding, *Prog. Lipid Res.* **2008**, *47*, 348.
[39] M. J. Kip, C. D. Spies, T. Neumann, Y. Nachbar, C. Alling, S. Aradottir, W. Weinmann, F. M. Wurst, *Alcohol Clin. Exp. Res.* **2008**, *32*, 1284.
[40] P. Marques, S. Tippetts, J. Allen, M. Javors, C. Alling, M. Yegles, F. Pragst, F. Wurst, *Addiction* **2010**, *105*, 226.
[41] P. Hansson, A. Varga, P. Krantz, C. Alling, *Int. J. Legal Med.* **2001**, *115*, 158.
[42] P. Marques, T. Hansson, A. Isaksson, L. Walther, J. Jones, D. Lewis, M. Jones, *Traffic Inj. Prev.* **2010**, (In press).