Commentary on the Paper of Thompson P. et al.:

Phosphatidylethanol in Postmortem Brain and Serum Ethanol at Time of Death

Wolfgang Weinmann[1], Alexandra Schröck[1,2], Friedrich Martin Wurst[3,4]

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

[2] Institute of Forensic Medicine, Kantonsspital Aarau, Tellstrasse, 5001 Aarau, Switzerland

[3] Paracelsus Medical University, Strubergasse 21, A-5020 Salzburg, Austria

[4] Center for Interdisciplinary Addiction Research, University of Hamburg, Germany

Wolfgang.weinmann@irm.unibe.ch

Alexandra.Schroeck@ksa.ch

Corresponding author:

Friedrich M. Wurst

Paracelsus Medical University,

Strubergasse 21

5020 Salzburg

Email: frieder.wurst@gmx.de

The paper of Thompson et al. contributes from both a scientific and a clinical point of view to a timely and relevant issue, namely the potential role of the ethanol metabolite phosphatidylethanol (PEth) as a biomarker for assessing ethanol intake in autopsy cases.

To facilitate early diagnosis and therapy of alcohol-related disorders and thus prevent secondary complications, questionnaires like the Alcohol Use Disorders Identification Test (AUDIT) (Saunders et al., 1993) together with reliable and valid biomarkers are useful. Clearly, biomarkers have the advantage to indicate and reflect alcohol intake, independent of recall bias of the interviewed subjects. Among biomarkers of ethanol intake, ethanol metabolites such as ethyl glucuronide (EtG) and, more recently, PEth have been investigated. PEth is now recommended by the German evidence- and consensus-based Guideline on alcohol use disorders for screening chronic alcohol intake at the highest level of evidence (level 1) in all settings for assessing drinking habits (Weinmann et al., 2016). PEth has been employed in settings such as the assessment of driving ability, in forensic psychiatry, in monitoring programs, and for identifying alcohol intake in specific risk groups, e.g., for neonatal screening of prenatal alcohol exposure (for review see Wurst et al., 2015).

It should be noted that PEth is not a single molecule but a family of phospholipids with different fatty acids at the sn-1 and sn-2 positions (Gnann et al., 2010). Nutrition can influence the lipids available to be transformed into PEth homologues, and thus mediate the substitution of different fatty acids.

LC-MS/MS allows PEth to be detected in blood after a single drinking episode, while multiple drinking on five subsequent days leads to the accumulation of PEth in blood (Gnann et al., 2012). In volunteers a single drinking episode to a blood alcohol concentration (BAC) of 1.0 g/kg (approx. 0.1 g/dL) resulted in a detection window of 3 to 12 days (Schröck et al., 2016b). During abstinence the ratios of different PEth homologues change due to their differing elimination times (Gnann et al., 2014). As PEth seems to be formed as long as alcohol is present in the blood, and higher BAC levels lead to a higher formation rate, the manner of drinking (speed, frequency, single-drinking amounts) and the BAC reached per drinking event may play roles in PEth production. However, this has not yet been investigated in detail.

No paper has so far looked at PEth degradation in vivo, nor its mechanism of degradation and which products are formed. Apart from phospholipase D (PL D) activity, PL A1, PL A2, PL B and PL C can also lead to different forms of “lyso”-PEth,

which are currently not detected by LC-MS/MS methods. A variety of possibilities might explain inter-individual differences in the formation of certain PEth homologues.
The investigated samples were autopsy cases from a brain bank with death by suicide or from natural causes. To establish a diagnosis, the Mini-International Neuropsychiatric Interview (M.I.N.I.) with DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, American Psychiatric Association) criteria was employed. Of note, a differentiation between a lifetime and a current diagnosis of AUD was not possible with the given information. This would have been of interest for data interpretation. In addition to this excellent diagnostic tool, it would be beneficial in future studies to have information about ethanol intake over the days, weeks and months prior to death. Such information could have been provided by the AUDIT or by time-line follow back (TLFB), as used for many other studies with living persons.
The study is based on PEth concentrations, serum blood levels, and coroner's reports, reports from next of a kin, on the manner of death and circumstances with the differentiation of suicide and death from natural causes, with a relatively small number of autopsy samples, and thus should be regarded as a pilot study.
The samples used were classified into three groups: AUD-W (with positive serum ethanol levels present at the time of autopsy), AUD-WO (without positive serum ethanol levels), and controls. Since the control group was positive for PEth, it should be assumed that these persons were no teetotalers, but showed at least occasional alcohol consumption. It is not clear if the diagnosis was lifetime or current for AUD-W or AUD-WO.
Compared with other forensic autopsy case reports, no morphological characteristic signs for alcohol abuse, such as structural liver damage, are mentioned in the study. Furthermore, no analysis of other autopsy material, such as urine or hair for EtG, was performed to assess consumption habits prior to death. Correlations between hair EtG concentrations and blood PEth concentrations have recently been shown by Schröck et al. (2016a).
Alcohol concentration has been determined in serum, and the complementary determination of EtG in serum and γ-glutamyl-transferase in serum (GGT) would have added aspects from different time frames of alcohol intake.
In contrast to earlier papers on PEth in tissue samples from rats and human subjects (Aradottir et al., 2002, 2004), where HPLC with evaporative light scattering detection

was used to quantify the sum of all PEth homologues, in the present paper individual PEth homologues were quantified by LC-MS/MS.

Interestingly, brain tissue seems to show some differences from erythrocytes or blood samples in the profile of PEth homologues after ethanol consumption. This might be due to turnover rate, which is dependent on enzymatic activity and the presence of educts for PEth homologue biosynthesis across brain regions. Also, steric issues with the multiple unsaturated lipid moieties, or oxidative processes, might play roles. There are few data on the presence of PL D (Exton, 2002), which catalyzes the reaction of phosphatidylcholine with ethanol to form PEth (Kobayashi and Kanfer, 1987), and no mention of the presence of other phospholipases that could influence PEth concentrations. The degree of unsaturation of the fatty acid moieties influences their stability. Post-mortem redistribution of metabolites might play a role, including in brain.

It should also be mentioned that blood is still present in the brain after death, and as a result there might be diffusion between erythrocytes and brain cells. Furthermore, the possibility of continued formation of PEth in tissue in the presence of alcohol after death, which has been observed in blood if not stored at -80°C, might lead to somewhat falsely elevated PEth concentrations.

From a technical point of view, other factors could influence the ratio of PEth homologues: the liquid-liquid extraction technique used with brain tissue is very similar to techniques used for whole blood. The paper does not mention how recovery and extraction efficiency were determined for brain tissue. Control samples containing PEth are not commercially available, and are usually only generated by spiking blank material with the analytes of interest. Systematic variations in extraction need to be compensated for. As no deuterated standard was used to determine PEth 16:0/18:2, only 16:0/18:1, it is unclear if PEth 16:0/18:2 recovery was tested. Some chemical suppliers are now producing control material for the quantification of PEth homologues (in dried blood spots), since stability issues are a major problem. Inter-laboratory comparability of results remains an unsolved problem, especially for tissue samples.

Finally, the authors discuss possible limitations of their study, and state that “our results are not conclusive”. The results need to be verified by further investigations that include a range of markers and signs of prolonged alcohol abuse – and also by the evaluation of storage conditions, pre-analytical stability, and the diffusion rates of

these highly lipophilic compounds. Studies on lipase activity in brain tissue are also important.

In summary, the study adds to the increasing body of evidence for the advantages of PEth as biomarker of ethanol intake. Of note, a plethora of variables apart from daily amount of alcohol consumed that influence PEth concentrations, such as gender (which influences the area-under-the-curve for BAC), nutrition, and inter-individual differences in the activities of enzymes that form and degrade PEth homologues, among others, might contribute to the variation found. Additional information on current drinking status should be considered in future work.

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