Title

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Journal

Alcoholism Clinical and Experimental Research, 40(11)

ISSN

0145-6008

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Publication Date

2016-11-01

DOI

10.1111/acer.13213

Peer reviewed

The Formation, Elimination, Interpretation and Future Research Needs of Phosphatidylethanol (PEth) for Research Studies and Clinical Practice

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Funding: NIAAA K24AA022586 (Hahn), NIAAA U01AA020776 (Hahn), NIAAA K05AA017435 (Anton), R01AA022361 (Javors)

In this journal, Papas and colleagues report on comparing phosphatidylethanol (PEth) to self-reported alcohol use in a behavioral alcohol intervention trial of 127 HIV infected adults in Kenya (Papas et al., 2016). Study eligibility included any self-reported prior 30-day drinking and scoring ≥3 on the Alcohol Use Disorders Identification Test – Consumption (AUDIT-C) or drinking ≥6 drinks per occasion at least monthly in the past year. Self-reported alcohol consumption was in the National Institute of Alcohol and Alcoholism (NIAAA) risky range(2016), with drinking reported on a median of 50% of the prior 30 days, and a median of 4.5 drinks per drinking day. No differences in self-reported consumption were observed by sex. At baseline, the proportion with positive PEth tests (PEth homologue 16:0/18:1 ≥8 ng/ml) was 54% in women (n=67) and 92% in men (n=60). At the 3-month follow-up, after engaging in a six-session alcohol intervention or control condition, of those reporting any 30-day alcohol consumption, the proportions testing PEth positive among the women and men, respectively, were 93% (n=27) and 97% (n=31). Of those who reported abstaining for ≥30 days, the proportions PEth positive were 30% (n=40) and 65% (n=29) among the women and men, respectively.

These data highlight discrepancies between PEth results and self-reported alcohol use that can occur in real world settings, especially in those that involve persons drinking alcohol at levels considered risky (i.e. more than 7 drinks per week and 3 drinks per occasion among women and more than 14 drinks per week and 4 drinks per occasion among men) but below the levels considered heavy (i.e. 5 or more drinks per occasion on 5 or more days per month) (2016). Such lower levels of alcohol use may have important biological or behavioral impact on HIV outcomes, and the level of drinking needed to cause harm may be lower than the levels needed to cause harm in HIV uninfected persons (Justice et al., 2016). In addition, trials of interventions to reduce alcohol use in persons with and without HIV require valid measurement of alcohol consumption; thus utilizing PEth to corroborate self-reported alcohol use in the trial conducted by Papas et al is an important step forward. But like most alcohol biomarkers, PEth has some limitations. In this commentary, we (1) summarize the current knowledge of the formation and elimination of PEth in whole blood and the factors that may influence them, (2) make recommendations for the use and interpretation of PEth in research and clinical settings, and (3) suggest directions for research and practice.

PEth formation and elimination.

PEth is a lipid metabolite of ethanol formed from phosphatidylcholine by a reaction catalyzed by phospholipase D (PLD) in red blood cells (RBCs). PEth has been detectable at high levels in whole blood in persons entering alcohol treatment/detoxification (Aradottir et al., 2006, Wurst et al., 2010, Winkler et al., 2013) and absent from persons in closed-ward facilities without access to alcohol (Hartmann et al., 2007). Less is known about PEth formation after lower levels of consumption. Laboratory studies in which volunteers were given standard doses of alcohol showed that PEth forms soon after alcohol consumption and begins to degrade after drinking has ceased (Javors et al., 2016, Gnann et al., 2012). In one experiment, in which volunteers drank alcohol to reach a blood alcohol concentration of approximately 1% (0.10 gm/dl) daily over 5 days and were followed for 16 more days, the range of maximum PEth levels reached was 74 to 237 ng/ml; the maximum level was observed between three to six six days after the drinking began (Gnann et al., 2012). The PEth elimination rate, i.e. the time after last drink to PEth levels below the limit of quantification (LOQ), varied between persons, ranged from 4 to 12 days. Such variability in PEth formation and degradation may have a variety of explanations, as follows.

The role of alcohol absorption. The synthesis of PEth is directly proportional to the concentration of ethanol located at the site of PLD (Weinmann et al., 2016). The rate of absorption of ethanol from the intestine impacts PEth formation and may vary widely between persons (Javors et al., 2016). Ethanol absorption is affected by sex, percent body fat, genetically determined alcohol and acetaldehyde dehydrogenases, stomach contents, and the rate of drinking, while ethanol elimination usually occurs at a fairly constant rate.

The role of phospholipase D (PLD) and phosphatidylcholine. In addition to ethanol, PEth synthesis requires the presence of phosphatidylcholine and PLD. The rate of PEth formation is a measure of PLD activity and represents PLD concentration; PLD activity can have large inter-individual variation (Aradottir et al., 2004). A better understanding of this variability may explain PEth variability and might be used to normalize PEth measurements (Weinmann et al., 2016) in the future.

Elimination of PEth. The average time in which PEth concentration is halved in the absence of new PEth formation (the PEth half-life) is approximately 4 days, with half-lives reported up to 12 days in recent publications (Gnann et al., 2012, Javors et al., 2016). The causes of variability in PEth elimination are unknown, but PEth concentration at the beginning of

abstinence affects the time to reach undetectable levels. Thus, after 30 days of abstinence from alcohol, a person with a starting PEth of <250 ng/ml and a PEth half-life of 4 to 6 days will have PEth below the LOQ at 30 days. On the other hand, someone with a high starting PEth level (e.g. PEth >2000 ng/ml) with a 4-day half-life, or someone with a lower PEth level (e.g. 250 ng/mL) and a long PEth half-life (e.g. 10 days) may still test positive for PEth after 30 days of abstinence. These examples show that one cannot be certain regarding timing of last drink from PEth levels alone.

Laboratory determination of PEth and PEth homologues. The primary method of measuring PEth concentration was originally high performance liquid chromatography with evaporative light scattering detection, which detects total PEth, i.e. all PEth homologues, with a LOQ of 0.22 µmol/L (approximately 154 ng/ml) (Wurst et al., 2015). More recently, individual PEth homologues have been detected using liquid chromatography with tandem mass spectrometry (MS). The most prevalent homologue is PEth 16:0/18:1, comprising about 40% of total PEth (Helander and Zheng, 2009), with a limit of detection (LOD) of 2 to 5 ng/ml and a LOQ of 8 to10 ng/ml. While this LOQ and further reductions in the LOQ could allow the detection of low or at-risk drinking over a longer time window, this could be at risk of increasing the rate of false positives, an important concern in legal and forensic work. The pattern of PEth homologue formation and elimination may differentiate between levels of drinking (Gnann et al., 2014, Nalesso et al., 2011, Javors et al., 2016). However, after an experimentally administered low single alcohol dose, the detection of the second most common PEth homologue, 16:0/18:2, yielded only a 1% improvement in PEth detection over the PEth 16:0/18:1 (Javors et al., 2016).

PEth interpretation and recommendations for research and clinical settings.

Given the high degree of variability in PEth formation and elimination described above, what can PEth levels validly tell us? Because PEth forms only in the presence of alcohol, positive PEth results clearly represent some amount of past drinking. However, we would suggest that the data reporting positive PEth tests in 30% of women and 65% of men who reported 30 days of abstinence should be interpreted cautiously. There are several reasons why results might differ from those reported. One possibility is under-report of alcohol use, which may occur in alcohol reduction trials, due to social desirability. It is also possible that some starting levels of PEth were high enough to be detectable even after 30 days of abstinence, or

that some people had slow PEth elimination rates, or some combination of both as previously described.

Interpretative validity of high PEth levels is more straightforward. Treatment-seeking persons have exhibited high mean PEth levels at the start of alcohol treatment (Aradottir et al., 2006, Hartmann et al., 2007), and studies in which self-reported alcohol use has been corroborated have shown reasonably strong correlations of PEth level with the volume of alcohol consumed (Spearman correlations of 0.57 to 0.80) (Aradottir et al., 2006, Hartmann et al., 2007, Hahn et al., 2012). Accordingly, Swedish laboratories have set PEth >0.3 □mol/L (approximately 210 ng/ml) as the cutoff for “excessive” alcohol use (Helander and Hansson, 2013).

Positive PEth levels below this cutoff indicate drinking but the level of drinking is not clear. Lower PEth levels could represent (1) low level recent alcohol consumption, in which PEth has formed but has not yet completely degraded; (2) remote heavy alcohol consumption, as high PEth levels take longer to degrade; or (3) very heavy drinking by someone for whom, for the reasons noted previously, PEth forms at a lower rate or is eliminated faster. Investigations into several cutoffs from 10 to 80 ng/ml for various drinking levels and time periods have yielded inconsistent results (Stewart et al., 2014, Hahn et al., 2012). Clearly cut-off levels need to be further investigated using valid measures of drinking, days since last drink, and other factors influencing formation and elimination. Optimally, drinking would be in a controlled experimental setting or measured using frequent biological sampling such as frequent breathalyzer tests or wearable biosensors. As with all diagnostic tests using a continuous metric (such as PEth concentration) a high cutoff value will yield high specificity, while a lower cutoff will reduce specificity in favor of sensitivity. Thus, in the absence of clear thresholds for drinking levels, the context of the testing is important in deciding appropriate cutoff levels for various levels of drinking.

Negative PEth tests generally imply abstinence or low level drinking in the recent past, and thus could be useful in settings where abstinence monitoring and/or heavy drinking reduction is required. Studies found that the sensitivity of PEth above its LOQ for detecting any recent drinking was 78% to88% (Stewart et al., 2014, Hahn et al., 2012), consistent with the variability noted in PEth formation and elimination. Thus, it is not surprising that several of the women in the study by Papas et al, some who reported occasional or low-risk drinking on

subsequent qualitative interviews concerning the pre-baseline period, did not test PEth positive at baseline, although the proportion PEth positive among the women is lower than expected.

In circumstances in which self-reported alcohol use may not be accurate, such as research studies providing incentives for participation based on self-reported behaviors (Devine et al., 2013), PEth levels could be used to confirm study eligibility. Even when self-reported alcohol use appears to be accurate (e.g. for those entering an alcohol treatment program) baseline PEth provides a biological estimate of drinking from which to measure change during treatment. Changes in PEth levels should reflect changes in drinking within a given individual, because the biological factors that affect PEth formation and elimination discussed above are, for the most part, invariant within persons. This would imply that a change from baseline PEth level for each individual would be the best metric, although PEth will be influenced by recent drinking as well as overall drinking in the prior month.

Combinations of alcohol measures can be useful. In settings in which over-reporting of alcohol use is unlikely, self-report is a specific measure of alcohol use (few false positives), thus a compound measure can use both self-report and PEth. Using this approach, if either PEth or self-report is positive, then the combined test is positive, which improves sensitivity over either measure alone, with no loss in specificity. One example is the increase in detection of unhealthy alcohol use when PETH was 50 ng/ml, compared to the use of AUDIT-C alone (Hahn et al., 2016). Other highly specific alcohol biomarkers may also be used in combination with PEth in various ways. Urinary ethyl glucuronide (uEtG), a direct alcohol metabolite that reflects recent (prior 1 to 3 days) as well as heavy drinking, can be used to rule in or out recent drinking (Anton, 2014). UEtG measured by MS or immunoassay is available at commercial laboratories, or via a rapid qualitative immunoassay that can be administered by dipstick on site (Leickly et al., 2015). A positive uEtG test (at either a cut-off of 100 ng/ml for research or clinical use or a higher, more conservative, cut-off of 500 ng/ml for forensic use) can indicate recent drinking, which may indicate continued or relapse drinking in alcohol treatment settings. A negative uEtG test combined with a negative PEth test would rule out heavy drinking over several weeks. For a longer assessment period, EtG can also be extracted from hair, indicating chronic drinking over several months, with the time of drinking detected dependent on the distance from the scalp of the hair sample analyzed (Crunelle et al., 2014). Carbohydrate deficient transferrin (CDT), an indirect biomarker with low sensitivity but high specificity for heavy drinking over several weeks may also be combined with PEth for detecting moderate to higher levels of drinking (Winkler et

al., 2013). A recent publication highlighted the benefits of using all four of these biomarkers together to obtain a more definitive picture on the level and time frame of drinking (Kummer et al., 2016). While using four markers might have limited availability and use, strategic pairs of biomarkers can be chosen depending on the desired level or duration of drinking to be detected. For example, uEtG combined with PEth could distinguish recent (prior 2-3 days) drinking from longer-term (prior 2-3 weeks) drinking.

Summary and future directions.

PEth is a very useful objective measure for detecting heavy alcohol consumption and ruling out abstinence. In addition, changes in PEth may be particularly useful for obtaining objective estimates of changes in drinking in intervention trials. However the variability in PEth formation and elimination makes it less definitive for determining low-risk and at-risk drinking, as highlighted by Papas et al. Future research should investigate PEth precursor molecules, patterns of PEth homologue formation, and integration of other biological measurements reflecting PEth formation and elimination that explain variation in PEth concentration. In addition, focus on decreasing the LOQ (8 ng/ml) towards the LOD may increase the sensitivity for detecting low-risk and at-risk drinking. In the meantime, combinations of biomarkers and self-report may improve the usefulness of the tests in some situations. Studies comparing PEth to self-report should include information on drinking patterns that might affect PEth formation, such as when the last drink was consumed and the rate of drinking. Further studies that examine the formation and degradation of PEth in real world drinking scenarios, leveraging wearable alcohol biosensors and ecological momentary assessment data, can provide rich data to further the interpretation of PEth results. With such improvements, we are optimistic that alcohol biomarkers such as PEth can improve the objective measurement of alcohol consumption. This could lead to more valid research data as well as improve clinical treatment for those with all levels of drinking.

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