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Original Publication:

Stergios Kechagias, Dženeta Nezirević Dernroth, Anders Blomgren, Therese Hansson, Anders Isaksson, Lisa Walther, Robert Kronstrand, Bertil Kågedal and Fredrik H Nystrom, Phosphatidylethanol Compared with Other Blood Tests as a Biomarker of Moderate Alcohol Consumption in Healthy Volunteers: A Prospective Randomized Study, 2015, Alcohol and Alcoholism, (50), 4, 399-406.
http://dx.doi.org/10.1093/alcalc/agv038

Phosphatidylethanol compared with other blood tests as a biomarker of moderate alcohol consumption in healthy volunteers: A prospective randomized study

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Running title

PEth compared with other blood tests

Key words: Phosphatidylethanol, carbohydrate deficient transferrin, LC-MS/MS, moderate alcohol consumption, prospective randomized study

ABSTRACT

Aim: It is generally agreed that traditional alcohol biomarkers lack in sensitivity to detect hazardous alcohol consumption. The present study was undertaken to evaluate the ability of phosphatidylethanol (PEth) and traditional alcohol markers to detect moderate alcohol consumption and to distinguish between moderate alcohol consumption and abstinence.

Methods: Forty-four subjects, 32 females and 12 males, were included in the study. They were randomized to alcohol abstention or to alcohol consumption. Female participants consumed 150 mL of red wine (equivalent to 16 g of alcohol) per 24 h and the male participants double the amount. The study lasted for 3 months. Blood samples were drawn at the start and at the end of the study period. Blood samples were analysed for PEth, carbohydrate-deficient transferrin (CDT), mean corpuscular volume (MCV), γ-glutamyltransferase (GGT), aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Results: ROC curves for the various biochemical markers were plotted in order to assess their ability to discriminate between abstention and moderate daily consumption of alcohol. PEth and CDT were the only markers with AUROCs significantly higher than 0.5, and PEth was detected in all participants randomized to alcohol consumption.

Conclusion: PEth was the only marker that could detect moderate intake and the present results also indicate that PEth probably can distinguish moderate alcohol consumption from abstinence.

INTRODUCTION

Alcohol is an important cause of morbidity and mortality. Early identification and treatment of individuals at great risk of developing an alcohol use disorder represent major challenges for health care professionals. When e.g. liver disease occurs as a consequence of alcohol consumption serum liver enzymes and other parameters are used in order to evaluate the somatic status. Other negative consequences of alcohol intake include for example traffic accidents. The society has to combat such consequences by legislative measures. In Sweden individuals whose driving licences have been withdrawn because of driving under the influence have to prove a sober living by exhibiting normal levels of carbohydrate deficient transferrin (CDT) for several months before the driving licence can be renewed.

Alcohol may have an impact on social welfare (Bergman *et al*., 2013) and in moderate amounts it has some beneficial effects on physical welfare as well. In Sweden, ingestion of 14 or more drinks[1] for men and 9 or more drinks for women per week is considered risk drinking (Andreasson and Allebeck 2005). This corresponds to 24 g/day for men and 15.4 g/day for women. In their model Nichols *et al.* (Nichols *et al*., 2012) found that the optimal level of population alcohol consumption for chronic disease prevention in England is 5 g/day. Thus, public health targets should aim for a reduction in population alcohol consumption in order to reduce chronic disease mortality.

For estimation of alcohol consumption the questions of the AUDIT-C questionnaire (Bush *et al*., 1998) are often used. However, people with alcohol misuse will often inaccurately report they don't have a problem, which creates a need for more objective methods to investigate a person's drinking habits. Serum levels of liver enzymes and CDT are to be used when heavy drinkers are investigated. For social drinkers and risk drinkers more sensitive methods are needed. During the last two decades analysis of phosphatidylethanol (PEth) has

[1] One drink is equivalent to 12 g of ethanol

emerged as a more sensitive and specific method (Aradottir *et al.*, 2006; Gnann *et al.*, 2009; Helander and Zheng 2009; Gnann *et al.*, 2010; Nalesso *et al.*, 2011; Zheng *et al.*, 2011). PEth comprises a group of homologous phospholipids found in cell membranes (Isaksson *et al.*, 2011). Of these homologues the one containing palmitic acid and oleic acid (PEth 16:0/18:1) seems to be the most abundant in human blood, roughly 36 % (Zheng *et al.*, 2011) or 45 % (Marques *et al.*, 2011). This species therefore has been chosen in further analysis (Isaksson *et al.*, 2011).

The present prospective randomized study was designed to assess the diagnostic accuracy of PEth and more specifically PEth 16:0/18:1 in comparison to CDT as well as MCV and liver function tests in distinguishing moderate daily alcohol consumption from abstinence and to assess whether any of these markers can detect moderate consumption of alcohol.

METHODS

Subjects

By local advertisement we recruited 46 potential participants. They were all free from significant diseases as judged by medical check-up and history at recruitment. Only participants without a history of overconsumption of alcohol or psychiatric disease and also without alcohol abuse among first degree relatives were recruited and the lowest allowed age for participation was 25 years. The study design which implied that some individuals would be randomized to drink more than they usually did was discussed with the potential participants and accepted. One female subject withdrew her consent shortly after screening for personal reasons and one male potential participant was at inclusion found to have iron overload. Subsequent diagnostic work-up confirmed genetic hemochromatosis and he was consequently not allowed to participate in the study. The remaining 44 participants displayed no signs of relevant diseases, and were randomized to either of the groups by drawing lots.

The recruitment included three pairs of women and men living together and these couples were allowed to share the same randomization condition. The questions of the AUDIT-C questionnaire (Bush *et al.*, 1998) and interviews were used to assess habitual alcohol consumption at study entry. The interview focused on last past weeks´ consumption. No subject had recently changed alcohol consumption before inclusion.

Participants that were randomized to alcohol abstention were asked to avoid any sort of alcohol intake during the three study months (September to December of 2009). In order to increase adherence to study protocol participants were informed of monthly control of liver function tests and also that hair analysis of an ethanol metabolite (ethyl glucuronide) would be performed at the end of the study (Kronstrand *et al.*, 2012). Participants that were randomized to moderate consumption were asked to consume 150 mL of red wine daily for women and the double amount for men. They were asked not to drink any extra alcohol than the red wine which could be consumed at any time during the day. However a general recommendation was given to consume the wine in the evening since employers in general do not allow consumption of alcohol during working hours in Sweden. The red wines to be consumed were provided by the study organizers and had an alcohol content of 13.5%-14% v/v. Thus, daily alcohol intake was 16.0-16.5 g (1.3 standard drinks) for women and 32-33 g (2.7 standard drinks) for men, according to the protocol, among those who were randomized to alcohol ingestion. Subjects of the wine group were asked to drink the provided wines but were allowed to replace them occasionally with other wines that was offered, at for example dinners outside the home. They were asked to drink the prescribed amount of wine, no more nor less, also when drinking under such situations. The participants were instructed not to change eating and exercise habits during the trial. Blood for analysis of biomarkers was drawn in the fasting state at baseline, and after three months i.e. at the end of the study period. The participants were subjected to determination of body fat content with BOD POD (Life

Measurement Inc., Concord, CA) equipment (Fields *et al.*, 2002) at baseline and at the end of the trial. All participants were reimbursed with 1250 SEK (approximately $170) after completion of the study.

Analytical techniques

Determination of PEth 16:0/18:1 was performed at the Department of Clinical Chemistry, University Hospital, Lund, Sweden with a liquid chromatography tandem mass spectrometric (LC-MS/MS) method with increased analytical sensitivity only used for research purposes. This method was a modification of our validated method used for several years on clinical samples differing only in final volume for dissolving of the sample and in the column used for chromatographic separation. The limit of quantification (LOQ) was set to 0.005 μmol/L (3.5 ng/mL). Quality control (QC) samples consisting of pooled patient material showed CVs (coefficients of variation) of 8 % (n=10) and 4 % (n=30) at 0.005 μmol/L and 0.500 μmol/L (350 ng/mL), respectively.

Two hundred μL of whole blood were added to 1.4 mL of isopropanol (IPA) containing 57 nmol/L PEth 16:0/18:1(d31) from Avanti Polar Lipids (Alabaster, AL) as internal standard, followed by addition of 1.8 mL of hexane during mixing. The sample was centrifuged at 1500 *g* for 10 min, and the supernatant was transferred to a new tube and evaporated. The sample was then dissolved in 200 μL of methanol/IPA (30/70) and transferred to a HPLC glass vial.

For the analysis, Shimadzu LC-20ADXR pumps (Kyoto, Japan) were used together with a CTC HTC PAL autosampler (Zwingen, Switzerland) and a Sciex API 4000 masspectrometer (Concord, Ontario, Canada). Separation was performed on an Agilent Poroshell 120 Bonus-RP column (2.7 μm, 30x2.1 mm) (West Chester PA) which was held at 60°C. The flow was 0.400 mL/min and mobile phases were water/IPA/acetonitrile, 30/10/60 (A) and water/IPA/acetonitrile, 1/79/20 (B), both containing 4.8 mmol/L ammonium formate. HPLC-

grade methanol, iso-propanol, n-hexane and acetonitrile were purchased from Merck (Darmstadt, Germany) and ammonium formate was from Sigma-Aldrich (St. Louis, MO). The gradient was started at 15% B, raised to 40% B at 0.25 min, increase to 70% B until 2 min, directly increased to 100% B, held for 0.5 min and then equilibrated at 15% B until 3 min. Two μL of each sample were injected onto the column. Transitions of the MRM method for PEth 16:0/18:1 were m/z 701.5 to 281.2 and m/z 701.5 to 255.2. The reference compound for PEth 16:0/18:1 was bought from Biomol Research Laboratories (Plymouth Meeting, PA). Calibration samples and an internal control sample were analysed both before and after the subject samples in each run. Samples from same subjects were analysed in the same analytical run to avoid between run imprecision.

CDT was determined at the Department of Clinical Chemistry, University Hospital, Linköping, by high-performance liquid chromatography as previously described (Helander *et al*., 2003). CDT is expressed as a percentage of the disialoform of transferrin to total transferrin. The lower limits of determination (LOD) and quantification (LOQ) of the HPLC method are ~0.05 % and 0.1 %, respectively, of total serum transferrin. The intra- and interassay CV of the method for serum samples containing 1.0–5.6 % disialotransferrin are <5 % (Bergström and Helander, 2008). With this method serum reference values were collected from 132 healthy social drinkers in connection with a regular health examination. Only those who screened negative on the AUDIT (score <8 for men and <6 for women) and had no indication of excessive drinking were included (Helander *et al.*, 2003). The range for this population was 0.49 – 1.77 %; mean (SD), 1.16 (0.25) %; median, 1.17 %. The upper limit of the reference interval was reported to be 1.7 %, corresponding to the mean + 2 SD for control populations (Helander *et al.*, 2003). Sera for CDT were stored at -70° C until analysis. After thawing of the samples, analysis was performed batch-wise within a few days using the same analytical column and avoiding drift in the analysis. At a mean CDT level of 1.5 % the intra

assay CV was 2.5 % and for a 2-month period the inter assay data [mean ± SD (CV)] at different levels were 3.50 ± 0.14 (4.0 %) and 1.39 ± 0.11 (7.9 %), respectively.

AST, ALT, bilirubin, GGT, and ALP were determined on Advia 1200/1650/1800 (Siemens Healthcare Diagnostics, East Walpole, MA). Enzyme measurements were performed at 37° C according to IFCC. The reagents for AST and ALT included pyridoxal phosphate. Alkaline phosphatase assay was with AMP buffer. For details regarding enzyme measurements see Weykamp *et al.* (Weykamp *et al*., 2014) and references therein. MCV was determined by the Abbott CELL-DYN Sapphire haematology analyser (Lake Forest, IL) at the Department of Clinical Chemistry, University Hospital, Linköping, Sweden.

Statistics

Statistical calculations were done with PASW 18.0 software (SPSS Inc. Chicago, IL, USA). Linear correlations were calculated as stated in the text. Comparisons within and between groups were done with Student's paired and unpaired 2-tailed *t*-test or as stated in the results section. Since habitual alcohol consumption at study entry and PEth values were non-normally distributed, non-parametric tests were also used for calculations as stated (Wilcoxon Signed Rank test, Mann-Whitney test, and Spearman correlations (r_s). Statistical significance was considered at the 5% level ($p \leq 0.05$). Fat-free body mass was calculated as the difference between total body-weight and body fat content. Receiver-operating characteristics (ROC) curves were constructed to assess the overall accuracy of biomarkers and to identify optimal cut-offs. The ROC curve is a plot of sensitivity vs. specificity (1 – specificity) for all possible cut-off values. The most commonly used index of accuracy is the area under the ROC curve (AUROC), with values close to 1.0 indicating a high diagnostic accuracy.

Ethics

The study was approved by the Regional Ethics Committee of Linköping and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participating subjects. The study was registered at ClinicalTrials.gov (NCT00954434).

RESULTS

There were no dropouts during the three month study period. Due to missing samples, PEth was unfortunately not determined in two subjects of the wine group at follow up. Habitual alcohol consumption, according to AUDIT-C, at baseline did not differ between groups (13 g/week (range 4-68 g/week in the group randomized to red wine) vs. 13 g/week (range 4-100 g/week in the group randomized to abstention; p=0.85 by Mann-Whitney). Compared with reported alcohol consumption, all subjects randomized to the wine group markedly increased their alcohol consumption (range 1.63-56 times). Table 1 shows anthropometrics and laboratory variables before and at the end of the study period.

Levels of all studied biomarkers were similar in both randomization groups at baseline. At baseline, there was a significant correlation between PEth and reported habitual alcohol consumption expressed both in absolute quantities as well as correlated for body weight and body composition (Table 2A). There were no significant correlations between the other biomarkers studied and reported habitual alcohol consumption (Table 2A). Neither were there any significant correlations between biomarkers (data not shown) with AST and ALT (r_s=0.36, p=0.02) as the only exceptions. During the intervention PEth was significantly reduced in the group randomized to abstention (p<0.001 analysed with Wilcoxon Signed Rank test; Table 1) while the remaining biomarkers including CDT were unaffected. In the group randomized to daily consumption of red wine PEth was not significantly changed after three months. However, a statistically significant increase of CDT and AST was noted in the

group randomized to consumption of red wine (Table 1). During the study period no subject developed levels of aminotransferases exceeding the upper limit of normal. In all subjects CDT as well as PEth were well below the limits (1.9 % and 0.30 µmol/L, respectively) currently considered to indicate overconsumption of alcohol. After three months of daily consumption of red wine all subjects in this group had detectable PEth in blood and levels were significantly correlated with alcohol consumption (Table 2B). There were no significant correlations between the other biomarkers and alcohol consumption (Table 2B), but there was a significant correlation between PEth and CDT (r_s=0.42, p=0.007) and between AST and ALT (r_s=0.48, p=0.001) at the end of the study.

ROC curves of the various biochemical markers were plotted in order to assess their ability to discriminate between abstention and moderate daily consumption of red wine at study end. The areas under the ROC curves (AUROC) are shown in Table 3. PEth and CDT were the only markers with AUROCs significantly higher than 0.5 (p<0.0001 and p=0.001, respectively). The ROC curves for PEth and CDT are shown in Figure 1. AUROC for PEth was higher than for CDT but the difference was not statistically significant since confidence intervals overlapped (Table 3). A cut-off of 0.009 µmol/L (6.3 ng/mL) for PEth yielded sensitivity 84 % and specificity 83 %. For CDT a cut-off of 0.86 % yielded sensitivity 85 % and specificity 71 %. The corresponding values for PEth 0.006 µmol/L (4.2 ng/mL) were sensitivity 100 % and specificity 78 %. Only at the level 0.04 µmol/L (28 ng/mL) did the specificity reach 100 % (at the expense of sensitivity 28 %) meaning that at this level there are no false positives. Similar results of 100 % specificity and 28 % sensitivity was reached with CDT at a cut-off of 1.2 %. However, when 100 % sensitivity was reached for CDT at a cut-off of 0.65 % specificity was only 33 %. All individual values for PEth in the two randomization groups are shown in Figure 2.

We also tested the diagnostic accuracy of various combinations of biomarkers in distinguishing moderate daily alcohol consumption from abstinence. Multiplication of PEth with CDT resulted in AUROC 0.94 (0.86-1) which was not statistically significant from the AUROC for PEth alone 0.92 (0.82-1). Otherwise, addition of or multiplication with other biomarkers to the PEth values did not result in higher AUROCs (data not shown).

DISCUSSION

Comparison of PEth results to self-reported alcohol consumption among moderate drinkers have been reported previously (Bajunirwe *et al*., 2014; Jain *et al*., 2014). In the present study we found similar correlations between PEth and alcohol consumption with those reported previously but to our knowledge this is the first study using a randomized and prospective design. Moreover, we extend previous findings by showing that PEth could be detected in all moderate drinkers, i.e. subjects randomized to the wine group, using a sensitive analytical method.

Interestingly, our AUROCs and ROC curves (Table 3; Figure 1) on sensitivity-specificity pairs between wine drinkers and abstainers show great similarities with the results shown by Hartmann *et al.* (Hartmann *et al.*, 2007). They calculated ROC curves for 56 alcohol-dependent drinkers admitted to hospital for detoxification against 35 sober patients, with PEth, CDT, MCV and GGT as test variables. The resulting AUROC was 0.974 [P<0.0001, confidence interval (CI) 0.932–1.016] for PEth. At a cut-off of 0.36 µmol/L (253 ng/mL) for total PEth, the sensitivity was 94.5 % and specificity 100 %. The AUROCs were for CDT 0.931 (P<0.0001, CI 0.866–0.955), for GGT 0.894 (P<0.0001, CI 0.815–0.972), and for MCV 0.883 (P<0.0001, CI 0.801–0.965). For CDT, the sensitivity was 77.1 % and the specificity 88%. For GGT, the sensitivity and specificity were 94 % and 72 %, respectively. MCV reached a sensitivity of 40 % and a specificity of 96 %.

The clinical use of CDT has mainly addressed the question how to diagnose heavy alcohol consumption. However, a very intriguing finding and one not addressed completely in the literature, is whether CDT can also detect moderate alcohol consumption when compared with abstinence. In our study a cut-off of 0.86 % for CDT gave a sensitivity of 85 % and a specificity of 71 %, and CDT gave a pretty good detection by ROC analysis. This is in line

with the findings by Schellenberg *et al*. (Schellenberg *et al.*, 2005) who found a proportional dose–response effect of daily ethanol intake on %CDT values in the range of 0–70 g per day.

Our study subjects were not alcohol dependent and it seems reasonable that the traditional liver function tests were not significantly different between our groups. However, both PEth and CDT showed significant results (Table 3) with AUROCs of 0.92 (CI 0.82-1.0) and 0.82 (CI 0.68-0.96) respectively. It should be noted that we used a much more sensitive method for determination of PEth than Hartmann *et al.* (Hartmann *et al.*, 2007), but also that our wine drinkers had a much lower intake of alcohol, which might be a more demanding task when it comes to separation of the two groups. Yet all participants randomized to consumption showed positive results for PEth, i.e. the sensitivity was 100 %. However, PEth was not significantly changed in subjects randomized to consumption. This is probably a consequence of the fact that all participants were social drinkers at baseline and the increase of alcohol consumption that the intervention caused was too modest to increase PEth.

Varga *et al*. (Varga *et al.*, 1998) could not demonstrate an increase in PEth after consumption of a single dose of ethanol (even as much as 50 g) using an HPLC method for total PEth with a quantification limit of 0.8 µmol/L (562 ng/mL). In a more recent study using an LC-MS/MS method with substantially higher analytical sensitivity, single doses (49.3 – 108.8 g) of ethanol yielded values for PEth 16:0/18:1 ranging from 0.04 to 0.10 µmol/L (Gnann *et al.*, 2012). However, risk drinking has also been defined as regular consumption of moderate amounts of ethanol i.e. ≥ 168 g/week for men and ≥108 g/week for women (Andreasson and Allebeck, 2005). In the present prospective and randomized study we evaluated if biomarkers can be used to detect regular moderate alcohol consumption fulfilling risk drinking criteria according to the definition used the Public Health Agency of Sweden.

We found that PEth was the only biomarker that correlated to reported habitual low alcohol consumption at baseline. In the group randomized to daily intake of red wine PEth reflected

alcohol consumption better than CDT. The association between PEth and alcohol consumption was even better than previously reported (Aradottir *et al.*, 2006). There are several possible explanations for this. These include differences in measurement methods for PEth and that our study was prospective using a pre-defined consumption of alcohol in contrast to other studies where alcohol intake was estimated retrospectively. Moreover, we correlated alcohol intake with body composition. The volume of distribution of ethanol is related to the total body water and thus the same dose of ethanol per unit of body weight produces widely different blood-alcohol concentrations (Arthur *et al.*, 1984). Although measurements of total body water were not undertaken, participants were subjected to determination of body fat and we were able to correct for total body fat content, which should better mirror distribution of alcohol than correcting for total body weight. However, correction for body weight and body composition only increased correlation coefficients slightly which indicates that the used analytical method for determination of PEth is the most plausible explanation for the high correlation coefficients noted in this study.

Especially interesting is the question how much ethanol has to be ingested for a certain time to obtain a positive biomarker result. The superiority of PEth compared to other markers was shown after repeated intake of 48-102 g ethanol/day for three weeks (Varga *et al.*, 1998). In another study on 18 active alcoholic patients undergoing detoxification, PEth was the only biomarker in blood that was detected in all subjects (Wurst *et al.*, 2004). In the present study we extend these findings and show that PEth determined with a sensitive analytical method is superior to other biomarkers and can be used in the clinical setting to distinguish moderate alcohol consumption from abstinence. Moderate drinking is important to detect in several circumstances. In subjects with established alcoholic liver disease abstinence is of crucial importance. Even moderate alcohol consumption worsens portal hypertension in patients with alcohol-induced cirrhosis (Luca *et al.*, 1997), and continued drinking is associated with

increased mortality (Borowsky *et al.*, 1981). Other situations where biochemical markers may be needed to confirm abstention from alcohol are treatment of recovering alcoholics and during pregnancy. The assigned level of drinking in the wine group was chosen in order to be very close to what is considered risk drinking by the Public Health Agency of Sweden. However, the performance of PEth in distinguishing moderate alcohol consumption from abstinence may be inferior to that reported in the present study in a more representative population including individuals with lower alcohol consumption.

So far, no false positive PEth values have been recorded in blood from humans, neither as a consequence of endogenous molecules nor as a consequence of drugs (Varga *et al.*, 1998; Wurst *et al.*, 2003). The clinical specificity of PEth as an alcohol marker is in practice 100 %. In our study, the abstention group was asked to avoid any sort of alcohol intake during three months. Sixteen out of 23 subjects in the group of abstainers had PEth results <0.005 µmol/L (<3.5 ng/mL) (limit of quantification) after three months. However, 7 subjects (30 %) still had measurable PEth levels, with one value as high as 0.035 µmol/L (25 ng/mL) and the others had values of 0.005 µmol/L, 0.005 µmol/L (3.5 ng/mL), 0.008 µmol/L (5.6 ng/mL), 0.014 µmol/L (9.8 ng/mL), 0.025 µmol/L, and 0.025 µmol/L (18 ng/mL) (Figure 2).

In theory there are four possible explanations for this: 1) remaining PEth concentrations in blood after three months of abstention 2) contamination of samples or unspecific analytical method, 3) alcohol drinking during the abstention period and 4) formation from endogenously produced ethanol.

The mean half-life of PEth ranged from 4.5 to 10.1 days in the first week and from 5.0 to 12.0 days in the second week after initiation of abstention from alcohol by social drinkers (Gnann *et al*., 2012). We therefore think it is highly unlikely that detectable amounts of PEth (≥ 0.005 µmol/L) should remain after 3 months of abstention and therefore we can rule out this first possibility. The second possibility is also ruled out since the analytical specificity is

well controlled. In particular the chromatography MS/MS result of 0.035 µmol/L (25 ng/mL) is so clear cut that the result is obviously true. Formation of PEth from endogenous production of ethanol for obvious reasons can also be excluded. This leaves the third alternative to discuss.

Nalesso *et al.* using liquid chromatography-high resolution mass spectrometry (LC-HRMS) analysed PEth in blood from eleven heavy drinking patients, eight social drinkers and eleven teetotallers (Nalesso *et al.*, 2011). The total blood PEth concentrations of the heavy drinking patients were 0.89-5.25 µmol/L (630-3700 ng/mL). Of the social drinkers five subjects had PEth concentrations of 0.006-0.085 µmol/L (4.2-60 ng/mL). All teetotallers had PEth values below detection limit (0.001 µmol/L). Our abstention group had explicit instructions to avoid any alcohol intake during the study period. However, they were all social drinkers before intervention and we cannot rule out the possibility that some of them at least temporarily during the study period violated the instructions. The group of teetotallers in the study by Nalesso *et al.* would seem to be more reliably abstinent than our group of primarily social drinkers.

The criteria for the two different study groups, e.g. to avoid any alcohol intake or to consume a strictly specified daily amount of alcohol (no more and no less) for a period of time as long as three months is challenging and may well be a source of error. Probably, all participants have not been able to completely adhere to the criteria and this limits the interpretation of the data. A consequence of violation of the study protocol would be an increased scattering of biomarker results. However, mean or median values for the biomarkers, i.e. PEth, may still be fairly representative for the actual consumption levels in this study. Other reasons for the scatter in the wine group would probably be interindividual differences in the rate of formation and elimination of PEth. Another consequence of non-

adherence is that the clinical specificity of PEth according to ROC-analysis will be too low, since PEth will detect even a low intake of alcohol occurring in the group of abstainers.

In conclusion, we found that PEth was the only marker that could detect moderate alcohol intake and the present results also indicate that PEth probably can distinguish moderate alcohol consumption from abstention.

Funding

This work was supported by University Hospital of Linköping Research Funds, Linköping University, Gamla Tjänarinnor, ALF grants from Östergötland County, Alcohol Research Council of the Swedish Alcohol Retailing Monopoly (SRA), Skåne county council´s research and development foundation and the Gyllenstierna Krapperup´s Foundation.

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Table 1. Anthropometric and laboratory data before and after randomization to total abstention from alcohol or consumption of 150 mL red wine/day for women and 300 mL/day for men. Numbers are means (SD) except for habitual alcohol consumption and PEth where median and range are given. Gender distribution is given in absolute values.

	Abstention				Consumption of red wine		
Variable	Baseline	After three months	*P*		Baseline	After three months	*P*
Age (yr)	34 (9)				33 (9)		
Sex (M/F)	5/18				7/14		
Weight (kg)	68.8 (15)	68.4 (15)	0.20		73.5 (9.0)	73.3 (9.6)	0.82
Body-mass index (kg/m^2)	23.3 (4.2)	23.2 (4.3)	0.20		25.0 (3.4)	24.9 (3.7)	0.87
Habitual alcohol consumption (g/week)	13 (4-68)				13 (4-100)		
ALT (U/L)	21 (8)	19 (8)	0.061		21 (9)	24 (10)	0.16
AST (U/L)	25 (6)	24 (4)	0.29		23 (4)	26 (6)	0.005
AST/ALT	1.2 (0.4)	1.3 (0.3)	0.16		1.2 (0.4)	1.2 (0.4)	0.60
ALP (U/L)	47 (11)	49 (15)	0.48		56 (13)	53 (19)	0.41
GGT (U/L)	18 (7)	23 (12)	0.45		22 (11)	21 (13)	0.75
Bilirubin (mg/dL)	0.7 (0.3)	0.7 (0.3)	0.62		0.7 (0.2)	0.6 (0.3)	0.31
MCV (fL)	90 (5)	90 (3)	0.43		89 (4)	88 (4)	0.04
CDT (%)	0.88 (0.23)	0.77 (0.23)	0.089		0.90 (0.21)	1.02 (0.21)	0.024
PEth (µmol/L)	0.020 (<0.005-0.24)	<0.005 (<0.005-0.035)	0.001		0.018 (<0.005-0.12)	0.022 (0.007-0.17)	0.91

P values shown in the table denote comparisons between baseline and after three months within each randomization group.

P value >0.05 for all comparisons between the two groups at baseline. Values not shown.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase, CDT, carbohydrate deficient transferrin, GGT, gamma-glutamyl transferase, MCV, mean corpuscular volume, PEth, phosphatidylethanol.

Conversion: PEth (µmol/L) x 703 = ng/mL

Table 2A. Spearman´s rank correlation (r_s) between biomarkers and habitual alcohol consumption (expressed as g/week, g/kg body mass/week, and g/kg fat free body mass/week, respectively) at baseline.

Biomarker	Alcohol consumption (g/w)	Alcohol consumption (g/kg/w)	Alcohol consumption (g/kg fat free body mass/w)
	r_s (P)	*r_s (P)*	r_s *(P)*
PEth	0.56 (0.01)	0.57 (0.001)	0.62 (0.001)
CDT	0.05 (ns)	0.08 (ns)	0.06 (ns)
GGT	0.05 (ns)	-0.03 (ns)	0.05 (ns)
MCV	0.05 (ns)	0.06 (ns)	0.04 (ns)
Bilirubin	0.05 (ns)	0.06 (ns)	0.04 (ns)
AST	0.18 (ns)	0.18 (ns)	0.14 (ns)
ALT	0.05 (ns)	0.04 (ns)	0.08 (ns)
AST/ALT-ratio	0.08 (ns)	0.12 (ns)	0.04 (ns)
ALP	-0.05 (ns)	-0.05 (ns)	0.02 (ns)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase, CDT, carbohydrate deficient transferrin, GGT, gamma-glutamyl transferase, MCV, mean corpuscular volume, PEth, phosphatidylethanol.

Table 2B. Spearman´s rank correlation (r_s) between biomarkers and alcohol consumption (expressed as g/week, g/kg body mass/week, and g/kg fat free body mass/week, respectively) among subjects randomized to daily consumption of red wine for 3 months.

Biomarker	Alcohol consumption (g/w)	Alcohol consumption (g/kg/w)	Alcohol consumption (g/kg fat free body mass/w)
	r_s (*P*)	r_s (*P*)	r_s (*P*)
PEth	0.61 (0.005)	0.54 (0.02)	0.69 (0.001)
CDT	-0.06 (ns)	0.14 (ns)	0.30 (ns)
GGT	0.15 (ns)	0.11 (ns)	0.13 (ns)
MCV	-0.05 (ns)	-0.08 (ns)	-0.18 (ns)
Bilirubin	-0.20 (ns)	-0.26 (ns)	-0.28 (ns)
AST	0.19 (ns)	0.21 (ns)	0.24 (ns)
ALT	0.17 (ns)	0.21 (ns)	0.25 (ns)
AST/ALT-ratio	-0.16 (ns)	-0.06 (ns)	-0.20 (ns)
ALP	0.10 (ns)	0.13 (ns)	0.12 (ns)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase, CDT, carbohydrate deficient transferrin, GGT, gamma-glutamyl transferase, MCV, mean corpuscular volume, ns, not significant PEth, phosphatidylethanol.

Table 3. Ability of biomarkers, denoted as area under receiver-operating characteristics curves (AUROCs), to discriminate between abstention and moderate daily consumption of red wine for 3 months.

Biomarker	AUROC	95% CI
PEth	0.92	0.82-1
CDT	0.82	0.68-0.96
GGT	0.54	0.35-0.72
MCV	0.38	0.19-0.56
Bilirubin	0.42	0.23-0.60
AST	0.56	0.36-0.75
ALT	0.61	0.43-0.80
AST/ALT-ratio	0.39	0.20-0.53
ALP	0.65	0.47-0.83

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase, CDT, carbohydrate deficient transferrin, GGT, gamma-glutamyl transferase, MCV, mean corpuscular volume, PEth, phosphatidylethanol.

Figures

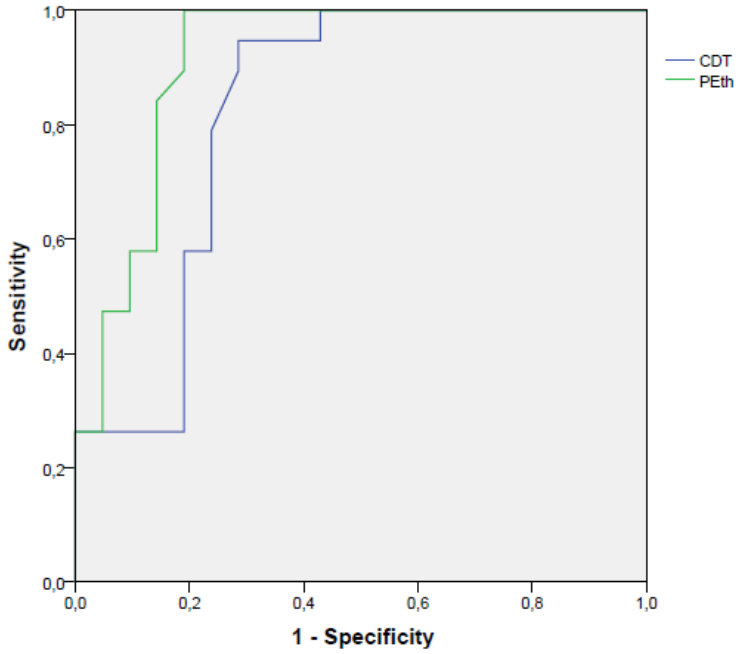

Fig. 1. Receiver-operating characteristic (ROC) curves for PEth and CDT, respectively, for abstention vs. daily consumption of 1 or 2 glasses of red wine (16-33 g ethanol) for three months.

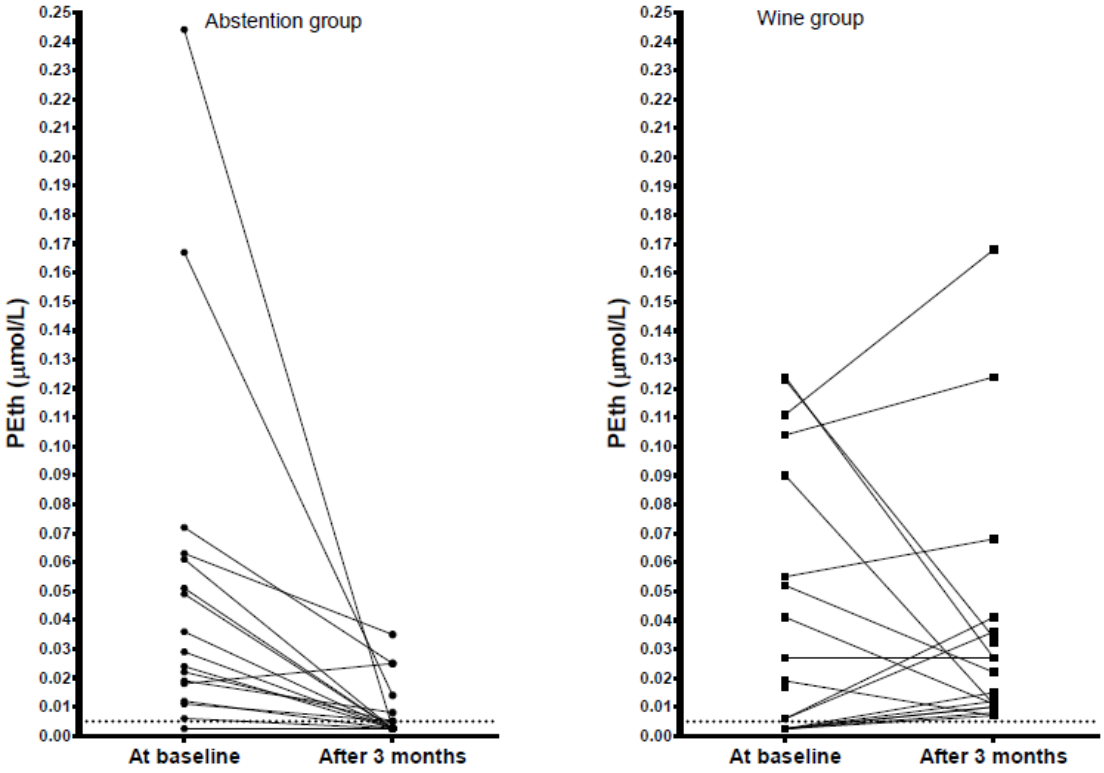

Fig. 2. Distribution of PEth (µmol/L) at baseline and after 3 months in 23 subjects abstaining from alcohol for 3 months (left panel) and in 20 subjects consuming 1 or 2 glasses of red wine (16-33 g ethanol) daily for 3 months (right panel). The horizontal dotted line depicts the limit of quantification 0.005 µmol/L (3.5 ng/mL). Conversion: PEth (µmol/L) x 703 = ng/mL