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Phosphatidylethanol Reliably and Objectively Quantifies Alcohol Consumption in Adolescents and Young Adults

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Background: Alcohol contributes to numerous annual deaths and various societal problems not just in adult, but also in adolescent, populations. Therefore, it is vital to find methods for reliably detecting alcohol use for early preventative measures. Research has shown phosphatidylethanol (PEth) to be superior to self-report instruments and indirect biomarkers for alcohol consumption in adult populations. However, the transferability onto an adolescent population has not yet been investigated.

Methods: $N = 106$ adolescents and young adults aged between 13 and 21 years were included. PEth analysis using high-pressure liquid chromatography–tandem mass spectrometry was performed on dried blood spot samples. Self-report questionnaires for alcohol consumption (Alcohol Use Disorders Identification Test—Consumption, AUDIT-C, and Timeline Followback, TLFB) and drug and alcohol consumption (Detection of Alcohol and Drug Problems in Adolescents, DEP-ADO) were completed by each participant.

Results: AUDIT-C scores showed large correlations with PEth 16:0/18:1 ($r_s = 0.732$) and PEth 16:0/18:2 ($r_s = 0.661$) concentrations. AUDIT-C with a cutoff value ≥3 was largely correlated with PEth 16:0/18:1 ($\eta = 0.411$) and showed a medium-sized correlation with PEth 16:0/18:2 ($\eta = 0.397$) concentrations. Using an AUDIT-C cutoff value ≥5 showed large correlations with both PEth 16:0/18:1 ($\eta = 0.510$) and PEth 16:0/18:2 ($\eta = 0.497$) concentrations, respectively. ROC curves indicated higher PEth concentrations are a good model for detecting positive AUDIT-C cutoff values (AUROC range: 0.800 to 0.849). PEth concentrations showed medium to large correlations with DEP-ADO and TLFB subscales (range $r_s = 0.469$ to 0.746).

Conclusion: The results suggest that PEth is a reliable and objective marker for quantifying alcohol consumption in adolescents and young adults. This could be of importance for early preventative measures against hazardous alcohol consumption, which is increasingly common at younger ages.

Key Words: Alcohol Use Disorders Identification Test—Consumption, Ethyl Glucuronide, Phosphatidylethanol, Self-Reported Alcohol Consumption.

ALCOHOL IS ONE of the most widely used addictive substances worldwide: In 2018, 43% of the population aged 15 years and older reported consumption in the past 12 months. Specifically for younger populations, 27% of 15- to 19-year-olds worldwide reported to be current drinkers (World Health Organization; WHO, 2018). Between 2000 and 2014, the rates of acute alcohol poisoning requiring hospitalization in Germany have increased for 10- to 19-year-olds by 162 and 120% for females and males, respectively (despite a small decline between 2012 and 2014; Rabenberg et al., 2016). Since alcohol abuse contributes to the development of a vast spectrum of illnesses and societal problems (Karriker-Jaffe et al., 2018; Laslett et al., 2010), it is important to find methods whereby hazardous alcohol use can be reliably detected, thus allowing for early preventative measures.

Recent findings suggest that direct biomarkers of alcohol consumption—in particular phosphatidylethanol (PEth) and ethyl glucuronide (EtG)—are superior to the indirect biomarkers γ-glutamyl transferase, carbohydrate-deficient transferrin, and mean corpuscular volume (for a comparison of direct versus indirect biomarkers, see, for example, Isaksson et al., 2011; Mann et al., 2016; Wurst et al., 2015).

PEth is an abnormal phospholipid, consisting of a glycerol backbone with fatty acid chains at positions sn-1 and sn-2, and phosphoethanol via an ester bond at position sn-3. It is only synthesized in the presence of alcohol in a reaction with phosphatidylcholine, catalyzed by the phospholipase D, even

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when only minimal amounts of ethanol (EtOH) are present in the blood (Alling et al., 1983; Gnann et al., 2010; Gustavsson and Alling, 1987; Lundqvist et al., 1994). Currently, there are 48 known homologues of PEth; the 2 most common homologues of PEth are 16:0/18:1 and 16:0/18:2 (Gnann et al., 2010). As a biomarker for alcohol consumption, PEth has a half-life of 4 to 10 days (Varga et al., 2000; Weinmann et al., 2016). As a result of this variance, a precise time frame for PEth quantifiability in the blood is difficult to predict, but estimations range from 3 to 12 days after a single drinking event (Schröck et al., 2017a) and 9 to 10 days (Schröck et al., 2014) to 21 to 29 days (Gnann et al., 2010; Wurst et al., 2015) after prolonged use. Various studies have also shown a positive correlation between the amount of alcohol consumed and the blood PEth concentration in adults (Hartmann et al., 2006; Wurst et al., 2010).

A second direct biomarker, EtG, is a metabolite of EtOH formed through the activity of the enzyme UDP-glucuronosyltransferase (Wurst et al., 2015). Only minimal amounts of EtOH (1 g) need to be consumed to render a measurable EtG value (Wurst et al., 2015). EtG is detectable in blood for around 36 hours; in urine for around 5 days (if only a small amount, i.e., <1 g EtOH is consumed, this is reduced to 11 to 27 hours); and in hair for 3 to 6 months (Thon et al., 2013; Wurst et al., 2015). In contrast to PEth, a retrospective determination of alcohol consumption patterns is feasible using EtG in hair, but only to a limited extent in blood and urine (Wurst et al., 2015).

Recent research also suggests these direct biomarkers to be superior to self-report instruments, such as the Alcohol Use Disorder Identification Test (AUDIT) and Timeline Followback (TLFB), for precise quantification of alcohol consumption (for self-reporting vs. direct biomarkers, see, for example, Hahn et al., 2012; Schröck et al., 2017b). Self-report measures are subject to underreporting of alcohol consumption in sample populations with a social desirability bias. This has been reported for HIV-infected patients (Hahn et al., 2012b). Underreporting also occurs in antenatal populations (May et al., 2018).

Notwithstanding certain limitations (see "Discussion" section), the bulk of current research reiterates the recommendation of the 2014 German evidence- and consensus-based S3 guidelines for the use of PEth in blood and EtG in hair as indicators for chronic alcohol consumption in adult populations (Mann et al., 2016, 2017).

However, the transferability of these findings onto an adolescent population has not been investigated thus far. Adolescents and young adults tend to show heavy episodic patterns of alcohol consumption (Piano et al., 2017), which may have an impact on PEth and EtG concentrations. To the best of our knowledge, this is the first study explicitly examining the correlation between direct biomarkers (PEth and EtG) and self-reporting instruments (Alcohol Use Disorders Identification Test—Consumption [AUDIT-C] and TLFB) in a clinical adolescent population.

Establishing PEth as a reliable and objective marker of alcohol consumption in adolescents would be beneficial for sensitive circumstances with a potential social desirability bias, such as binge-drinking youths or those with a prior criminal record (Richter and Johnson, 2001). PEth could also be useful during alcohol withdrawal treatment and relapse monitoring, as suggested for adult populations (Luginbühl et al., 2019b). We hypothesize PEth to be a feasible direct biomarker for these purposes.

Purpose of the Study

The primary goal of the present study was to investigate the correlation between self-reported alcohol consumption, using the AUDIT-C, and a direct biomarker for alcohol consumption, PEth, in a clinical population of adolescents and young adults.

Secondary goals were to investigate: (i) correlations between PEth concentrations, TLFB scores, and results of a brief assessment of at-risk alcohol and drug use in adolescents, the DEP-ADO (Detection of Alcohol and Drug Problems in Adolescents), and (ii) correlations between EtG in urine, AUDIT-C, DEP-ADO, and TLFB.

MATERIALS AND METHODS

Participants

One hundred and six adolescents and young adults between 13 and 21 years old were subsequently included in the present study. The primary inclusion criterion was current treatment at the German Center for Addiction Research in Childhood and Adolescence of the Department of Child and Adolescent Psychiatry and Psychotherapy at the University Medical Center Hamburg-Eppendorf in Hamburg, Germany.

Exclusion criteria were as follows: (i) mental disability and (ii) insufficient command of the German language. Lack of substance abuse was not an exclusion criterion, nor was readmittance within the recruitment period.

Study Design

In this cross-sectional, diagnostic study, inpatients from the youth addiction ward and outpatients from the youth addiction day-care ward and drug and alcohol treatment center at the University Medical Center Hamburg-Eppendorf were subsequently included over a 7-month period from October 2018 until April 2019.

Participants on the youth addiction ward and youth addiction day-care ward underwent routine venous blood collection on the day of admittance or shortly after. PEth analysis was performed on dried blood spot (DBS) samples to ensure PEth stability (Faller et al., 2013; Schröck et al., 2016). DBS was generated using DBS bioanalysis cards on the day of collection from lithium-heparinized venous blood samples (v-DBS). After DBS generation according to a standard operation procedure (Luginbühl et al., 2019b), the cards were folded shut and placed in a ziplock bag along with a silica gel drying agent. These were stored at −20°C for a maximum of 8 days, before being shipped at room temperature to the cooperating laboratory (Institute of Forensic Medicine, University of Bern, Switzerland). All of the questionnaires were completed within 3 days of v-DBS generation.

For the participants from the drug and alcohol treatment center, capillary blood was used and DBS was immediately generated (c-DBS), prior to storage under the same conditions as the v-DBS and shipment to the cooperating laboratory (Luginbühl et al., 2019b). All of the questionnaires were completed on the same day as c-DBS generation.

AUDIT-C, TLFB, DEP-ADO

In the short form of the AUDIT, the AUDIT-C, only the first 3 items (focusing on alcohol consumption: quantity, frequency, and binge drinking, i.e., ≥6 drinks on one occasion) are used as a brief screening tool (Bush et al., 1998; German translation Babor et al., 2001). For means of comparison in the present study, a "standard drink" was defined in accordance with the guidelines suggested by the National Institute on Alcohol Abuse and Alcoholism (Babor et al., 2001; Johnston et al., 2011) as containing 14 g of pure EtOH, hence approximated as 330 ml beer (alcohol by volume approx. 5%), 150 ml wine (alcohol by volume approx. 12%), or 40 ml spirits (alcohol by volume approx. 40%). For male and female participants, the cutoff value at which the screening is considered positive for heavy drinking and/or active alcohol abuse or dependence (Bush et al., 1998) has been suggested as 3 or higher (Liskola et al., 2018). Elsewhere, slightly higher cutoff values of ≥4 and ≥5 (Schröck et al., 2017b) or ≥5 and ≥7 (DeMartini and Carey, 2012) have been suggested for females and males, respectively. Due to this discrepancy in the literature, we conducted our analyses for 2 cutoff values separately, namely ≥3 and ≥5.

The TLFB is a calendar-based drinking assessment tool that allows retrospective specifications on the amount and frequency of alcohol consumption (Sobell et al., 1979). A modified version, mirroring the consumption time frame detected by PEth, was used for the present study. After the precise amount of alcohol consumed during this period is determined (using the same "standard drink" convention, see above), 3 questions focus on whether the consumed amount reported for the past 7 days were typical for the past month and year, respectively, and whether the amount of alcohol consumed a year ago was approximately the same as currently. If not, that is, if more or less alcohol was consumed in the past 7 days than in the past month or year, then a second (and third) score will be calculated for the total amount consumed in the past month (TLFB-2) or year (TLFB-3) as an average per week.

The DEP-ADO is a 7-question screening tool for detecting problematic alcohol or drug use (Germain et al., 2007; Landry et al., 2016). Questions primarily address the frequency of substance use in both the past 30 days and 12 months, and the age of initiation, prior injection drug use, severity of drug use, and possible negative effects associated with substance abuse. DEP-ADO-a, the first subscale assessing alcohol consumption, is of particular interest for the present study. The English version 3.2 was used in the present study, translated into the German language according to standard practice.

The AUDIT-C has been validated for children and adolescents (Liskola et al., 2018; Rumpf et al., 2013) and young adults (Bush et al., 1998). Only the French version of the DEP-ADO has been validated for ages 14 to 17 years (pertinent for use with 12- to 13-year-olds; Germain et al., 2007). In the present study, it was also used for slightly older participants because we expected the psychological developmental age of the study population to be lower than the respective biological age, as a result of delayed maturation contingent on emotional stress. The TLFB-modified version has not been validated for a German population, but content validity can be assumed by literature as it is often used as the gold standard (Johnson et al., 2013).

Determination of PEth and UEtG (EtG in Urine)

Whole blood concentrations of the 2 most common PEth homologues, 16:0/18:1 and 16:0/18:2 (Gnann et al., 2010), were measured using high-pressure liquid chromatography–tandem mass spectrometry (HPLC-MS/MS; Luginbühl et al., 2019a) at the Institute of Forensic Medicine, University of Bern, Switzerland.

For both PEth homologues, there was a discrepancy between the level of detection (LOD) and level of quantification (LOQ; Ulwelling and Smith, 2018), which was 6 and 20 μg/l, respectively. For values between the LOD and LOQ, reported by the laboratory as <20 μg/l, we estimated the median value, which was 13 μg/l.

EtG was quantified by immunoassay in urine (in mg/l), as a clinical routine on the 2 wards and the treatment center, at the Department of Legal Medicine at the University Medical Center Hamburg-Eppendorf.

Statistics

Correlations were analyzed using Spearman's rank correlation coefficient (r_s), as all variables were significantly nonnormal (tested using the Kolmogorov–Smirnov test; K-S test, D). Further, eta correlation (η) was used to assess the association between categorical and metric variables, as was the case for AUDIT-C cutoff scores compared with PEth and EtG concentrations. Spearman's r_s correlation coefficients of ±0.1, ±0.3, and ±0.5 are interpreted as small, medium, and large, respectively (Cohen, 1992). Eta correlations of ±0.1, ±0.25, and ±0.4 are interpreted as small, medium, and large, respectively.

A theoretically desired level of statistical significance was defined at $\alpha \leq 0.05$. For PEth 16:0/18:1 and PEth 16:0/18:2 concentrations, respectively, a maximum of $k = 8$ independent significance tests were conducted. After the Bonferroni adjustment, an empirical $p \leq 0.006$ therefore indicates the desired 5% level. For UEtG concentrations and $k = 10$ significance tests, this would hold true given an empirical $p \leq 0.005$.

Receiver operating characteristic (ROC) curves were plotted for PEth 16:0/18:1 and 16:0/18:2 concentrations against AUDIT-C cutoff values. An area under the ROC curve (AUROC) of 0.6, 0.7, 0.8, and 0.9 is interpreted as of weak or poor, moderate or fair, good, and excellent statistical property, respectively (Carter et al., 2016; Greiner et al., 2000). For better comparability, sensitivity and specificity values are reported in tables, but as usual 1–specificity values are given in diagrams.

All statistical analysis was performed using SPSS Statistics (version 25, IBM Corp., https://www.ibm.com/de-de/products/spss-statistics).

Ethics

The study was approved by the ethics committee of Hamburg's Chamber of Physicians (*Ethik-Kommission der Ärztekammer Hamburg*) on 16.07.2018 (file number PV5809). Further, an amendment was approved on 28.01.2019. Informed, written consent was obtained from each participant (and for those under 18 years old additionally from a parent, carer, or legal guardian) prior to their inclusion in the study.

RESULTS

Sample Characteristics

The sample consisted of $N = 106$ participants with a mean age of 17.15 years (SD 1.47). More than 3 quarters of the sample (77%) belonged to the adolescent age range between

14 and 17. One third (35%) was female. On average, the participants had received 9 full years of preuniversity education prior to hospital admittance and study participation. Table 1 reports the detailed sample characteristics and patient demographics.

Table 1. Sample Characteristics (N = 106)

	M (SD) or Mdn (IQR)/n (%)[a]
Age in years[b]	17.15 (SD 1.47)
Child (<14 years)	3 (2.8%)
Adolescents (14 to 17 years)	82 (77.4%)
Young adults (≥18 years)	21 (19.8%)
Sex (male/female)	69 (65.1%)/37 (34.9%)
DBS method	
v-DBS	74 (69.8%)
c-DBS	32 (30.2%)
Years of preuniversity education (n = 102)	9.10 (SD 1.18)
ICD-10 diagnoses[c]	
F0 (Organic, including symptomatic)	0 (0.0%)
F1 (Psychoactive substance-related disorder)	75 (70.7%)
F10 (Alcohol)	30 (28.3%)
F11 (Opioid)	13 (12.3%)
F12 (Cannabinoids)	64 (60.4%)
F13 (Sedatives or hypnotics)	10 (9.4%)
F14 (Cocaine)	21 (19.8%)
F15 (Other stimulants)	22 (20.8%)
F16 (Hallucinogens)	7 (6.6%)
F17 (Tobacco)	51 (48.1%)
F18 (Volatile solvents/inhalants)	0 (0.0%)
F19 (Multiple drug use/other)	3 (2.8%)
F2 (Schizophrenia, schizotypal)	4 (3.7%)
F3 (Mood, affective)	69 (65.1%)
F4 (Neurotic, stress-related, somatoform)	34 (32.1%)
F5 (Behavioral syndromes)	3 (2.8%)
F6 (Personality)	34 (32.0%)
F7 (Mental retardation)	0 (0.0%)
F8 (Psychological development)	5 (4.7%)
F9 (Onset in childhood/ adolescence)	85 (80.2%)
AUDIT-C (n = 101)	4 (IQR 6)
Negative (<3 points)	37 (36.6%)
Positive (≥3 points)	64 (63.4%)
Negative (<5 points)	56 (55.4%)
Positive (≥5 points)	45 (44.6%)
DEP-ADO (n = 100)	21 (IQR 26)
Green light (no obvious problem)	38 (38.0%)
Yellow light (developing problem)	9 (9.0%)
Red light (obvious problem)	53 (53.0%)
TLFB (n = 101) in g EtOH	
TLFB-1 (last week)	0 (IQR 52.4)
TLFB-2 (average per week in last month)	19.8 (IQR 79.6)
TLFB-3 (average per week in last year)	26.4 (IQR 99.4)
PEth (n = 102) in μg/l	
PEth 16:0/18:1	13 (IQR 36)
Not detected (<6 μg/l)	48 (47.1%)
PEth 16:0/18:2	6.5 (IQR 27)
Not detected (<6 μg/l)	51 (50.0%)
UEtG (n = 79) in mg/l	4 (IQR 6)

AUDIT/-C, Alcohol Use Disorders Identification Test—Consumption; c-DBS/v-DBS, capillary/venous dried blood spot; DEP-ADO, Detection of Alcohol and Drug Problems in Adolescents; ICD-10, International Statistical Classification of Diseases and Related Health Problems—Version 10; PEth, phosphatidylethanol in blood; TLFB, Timeline Followback; UEtG, ethyl glucuronide in urine.

[a]Data are means (M) with standard deviations (SD), medians (Mdn) with interquartile ranges (IQR) or frequencies (n) with percentages (%)

[b]Age groups according to the Protection of Young Persons Act in Germany (*JuSchG* §1.1)

[c]Names given in short form for orientation purposes only.

All test variables (PEth, AUDIT-C, DEP-ADO, TLFB, and UEtG) showed a skewed distribution; hence, nonparametric correlational measures were used and medians (with interquartile range) reported. As shown in Table 2, correlations between these measures were significant (all p-values < 0.001), at least medium-sized and predominantly large. In summary, PEth concentrations showed high concordance with self-reported alcohol consumption, in particular with AUDIT-C, TLFB, and DEP-ADO alcohol subscale scores.

Correlation Between AUDIT-C and PEth Concentrations

Correlation analysis with Spearman's rank showed large correlations between AUDIT-C scores and PEth 16:0/18:1 (r_s = 0.732) and PEth 16:0/18:2 (r_s = 0.661) concentrations, respectively (details given in Table 2, Figs 1 and 2).

Comparison of DEP-ADO and PEth Concentrations

Also reported in detail in Table 2, the DEP-ADO alcohol subscale and total score were investigated with regard to PEth. The alcohol subscale largely correlated (r_s = 0.668 and 0.620), and the total score showed a large or medium correlation (r_s = 0.536 and 0.469) with PEth 16:0/18:1 and PEth 16:0/18:2 concentrations, respectively.

Comparison of TLFB and PEth Concentrations

As shown in Table 2, Spearman's rank showed large correlations between PEth homologues and all TLFB subscales: for TLFB-1 (r_s = 0.730 and r_s = 0.746), TLFB-2 (r_s = 0.732 and r_s = 0.704), and TLFB-3 (r_s = 0.742 and r_s = 0.685) with PEth 16:0/18:1 and PEth 16:0/18:2 concentrations, respectively.

Exploratory Analysis With UEtG

Correlation analysis with Spearman's rank showed large correlations between UEtG and PEth 16:0/18:1 (r_s = 0.594) and PEth 16:0/18:2 (r_s = 0.602) concentrations, as well as between UEtG and TLFB-1 and TLFB-2 scores (r_s = 0.639 and r_s = 0.531, respectively, Table 3).

Further, medium correlations were found between UEtG concentrations and AUDIT-C scores (r_s = 0.483), DEP-ADO alcohol subscale scores (r_s = 0.466), and TLFB-3 scores (r_s = 0.487).

ROC Analysis for AUDIT-C and PEth

To evaluate PEth concentrations with regard to a binary screening cutoff value, in this case AUDIT-C scores, we analyzed area under receiver operating characteristic curve (AUROC; see Table 4), sensitivity, and specificity for cutoff values ≥3 and ≥5 with respect to increasing PEth

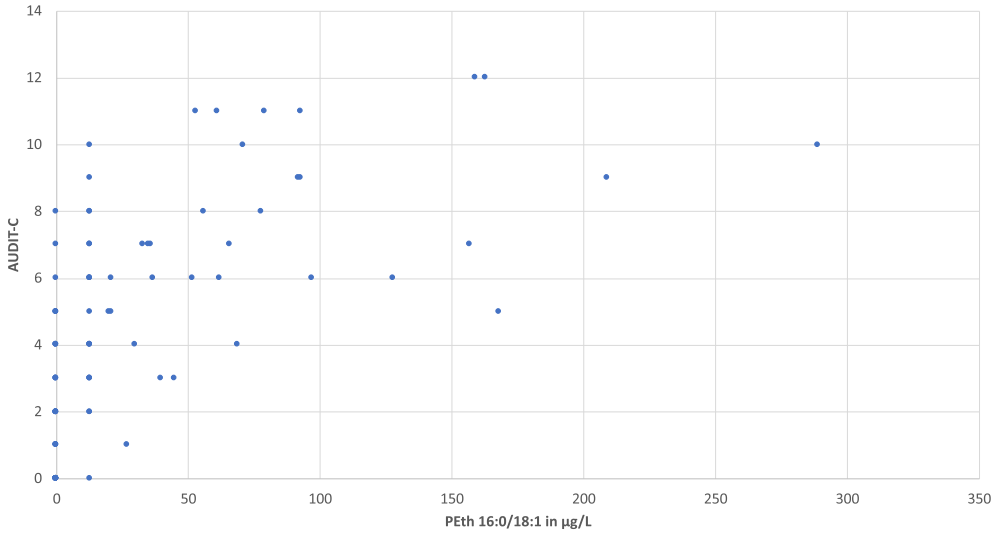

Fig. 1. Scatter diagram for PEth 16:0/18:1 concentrations and AUDIT-C scores ($N = 100$). [Color figure can be viewed at wileyonlinelibrary.com]

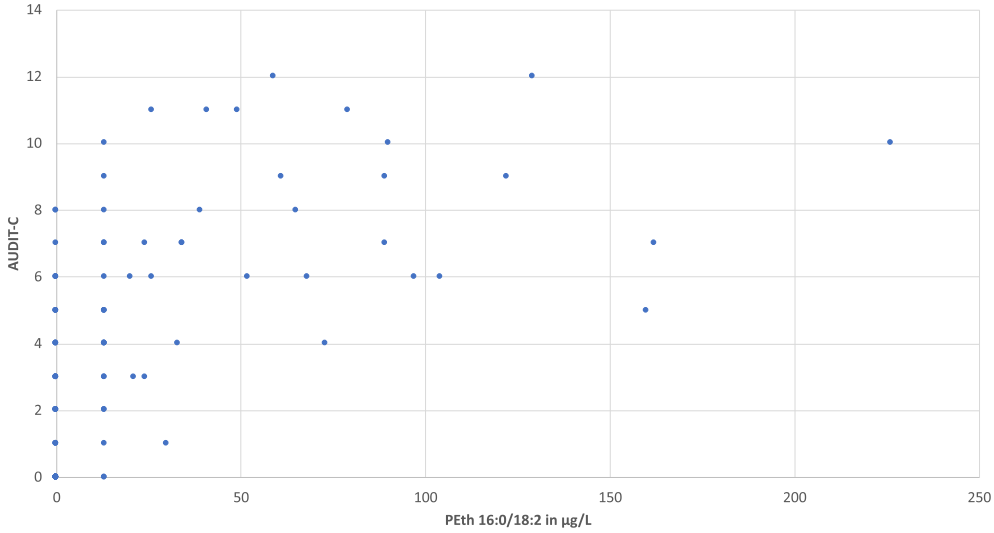

Fig. 2. Scatter diagram for PEth 16:0/18:2 concentrations and AUDIT-C scores ($N = 100$). [Color figure can be viewed at wileyonlinelibrary.com]

Table 2. Spearman Rank Correlations (r_s) Between PEth 16:0/18:1 and PEth 16:0/18:2 Concentrations (in µg/l) and Self-Report Instruments*

	AUDIT-C	AUDIT-C (≥3)[a]	AUDIT-C (≥5)[a]	DEP-ADO	DEP-ADO-a	TLFB-1	TLFB-2	TLFB-3
PEth 16:0/18:1	0.732	0.411	0.510	0.536	0.668	0.730	0.732	0.742
	$n = 100$	$n = 100$	$n = 100$	$n = 99$	$n = 99$	$n = 100$	$n = 100$	$n = 100$
PEth 16:0/18:2	0.661	0.397	0.497	0.469	0.620	0.746	0.704	0.685
	$n = 100$	$n = 100$	$n = 100$	$n = 99$	$n = 99$	$n = 100$	$n = 100$	$n = 100$

AUDIT/-C, Alcohol Use Disorders Identification Test—Consumption (≥3/≥ 5): respective cutoff value; DEP-ADO, Detection of Alcohol and Drug Problems in Adolescents—a, alcohol subscale; PEth, phosphatidylethanol in blood; TLFB, Timeline Followback: −1, −2, −3: last week, last month, last year, respectively.

[a]Eta correlation (η).

*All p-values < 0.001 indicating statistical significance at 5% level after the Bonferroni adjustment.

concentrations (see Table 5, Figs 3 and 4). ROC curves show both PEth 16:0/18:1 and PEth 16:0/18:2 concentrations to be a good model for detecting positive AUDIT-C cutoff values (AUROC: 0.800 to 0.849 and 0.806 to 0.845, respectively), with no superiority of one of these. But according to Table 5 results—using cutoff values generated by the statistic

Table 3. Spearman Rank Correlations (r_s) Between UEtG Concentrations (in mg/l), PEth 16:0/18:1 and PEth 16:0/18:2 Concentrations (in μg/l), and Self-Report Instruments

	PEth 16:0/18:1	PEth 16:0/18:2	AUDIT-C	AUDIT-C (≥3)*	AUDIT-C (≥5)*	DEP-ADO	DEP-ADO-a	TLFB-1	TLFB-2	TLFB-3
UEtG	0.594 $p < 0.001$, $n = 79$	0.602 $p < 0.001$, $n = 79$	0.483 $p < 0.001$, $n = 77$	0.241 $p = 0.042$, $n = 77$	0.256 $p = 0.023$ $n = 77$	0.305 $p = 0.063$, $n = 76$	0.466 $p < 0.001$, $n = 76$	0.639 $p < 0.001$, $n = 77$	0.531 $p < 0.001$, $n = 77$	0.487 $p < 0.001$, $n = 77$

AUDIT/-C, Alcohol Use Disorders Identification Test—Consumption (≥3/≥5): respective cutoff value; DEP-ADO, Detection of Alcohol and Drug Problems in Adolescents—a, alcohol subscale; PEth, phosphatidylethanol in blood; TLFB, Timeline Followback: −1, −2, −3, last week, last month, last year, respectively; UEtG, ethyl glucuronide in urine

*Eta correlation (η) not statistically significant at 5% level after the Bonferroni adjustment.

Table 4. AUROC ($n = 100$) for PEth Homologues and AUDIT-C Cutoff Values*

	AUDIT-C cutoff ≥3	AUDIT-C cutoff ≥5
PEth 16:0/18:1		
AUROC	0.849	0.845
95% CI	0.773 to 0.924	0.765 to 0.926
PEth 16:0/18:2		
AUROC	0.800	0.806
95% CI	0.715 to 0.885	0.715 to 0.896

AUROC, area under receiver operating characteristic curve; AUDIT/-C, Alcohol Use Disorders Identification Test—Consumption; CI, confidence interval; PEth, phosphatidylethanol in blood (in μg/l); ROC, receiver operating characteristic.

*All p-values < 0.001.

Table 5. ROC Curve ($n = 100$) Sensitivity and Specificity for PEth Homologue Concentrations at AUDIT-C Cutoff Values ≥3/≥5

	Cutoff (in μg/l)	AUDIT-C cutoff ≥3		AUDIT-C cutoff ≥5	
		Sensitivity	Specificity	Sensitivity	Specificity
PEth 16:0/18:1	6.5	0.762	0.892	0.841	0.732
	16.5	0.492	0.973	0.614	0.911
	20.5	0.476	0.973	0.591	0.911
	24.0	0.444	0.973	0.545	0.911
	28.5	0.444	1	0.545	0.929
	31.5	0.429	1	0.545	0.946
PEth 16:0/18:2	6.5	0.698	0.838	0.773	0.714
	16.5	0.460	0.973	0.568	0.911
	20.5	0.444	0.973	0.545	0.911
	22.5	0.429	0.973	0.545	0.929
	25.0	0.397	0.973	0.523	0.946
	31.5	0.365	1	0.477	0.964

AUDIT/-C, Alcohol Use Disorders Identification Test—Consumption; PEth, phosphatidylethanol in blood; ROC, receiver operating characteristic.

program which followed the empirical data distribution—both PEth concentrations revealed better predictions of the AUDIT-C cutoff value ≥5.

DISCUSSION

The purpose of this study was to investigate the correlation between self-reported alcohol consumption and the direct biomarker PEth in a population of adolescents and young adults. Our main findings were as follows:

1. a strong correlation between self-reported and PEth-quantified alcohol consumption,
2. higher PEth concentrations to be a good model for detecting positive screening results, that is, AUDIT-C cutoff values ≥3 or ≥5 (AUROC range: 0.800 to 0.849), and
3. moderate correlation between self-reported and UEtG-quantified alcohol consumption.

This is in general accordance with recent research on PEth in adult populations and supports our hypothesis, suggesting the feasibility of using PEth as an objective and valid alcohol consumption marker in adolescents.

As a direct biomarker of alcohol consumption, PEth seems to have certain advantages over indirect biomarkers and self-report instruments: Indirect biomarkers may be contingent upon non–alcohol-related factors, while accuracy of self-reporting instruments has shown to decline in situations with social desirability for lower consumption patterns (Hahn et al., 2012b; Richter and Johnson, 2001)—although as Figs 1 and 2 show, underreporting alcohol consumption in the AUDIT-C was not a significant concern in the present study. There are also limitations associated with PEth. As previous studies have discussed, these include a possible influence of nutrition, in vitro neoformation, blood alcohol concentration (BAC), and interindividual variation in phospholipase activity on PEth concentrations (e.g., Schröck et al., 2017a; Weinmann et al., 2016). Further research is required to investigate the confounding influence of such factors, which are as yet merely hypothetical (preliminary data is available for the impact of BAC, see Schröck et al., 2018).

More specific to the present study, an important difference between measures is the consumption time frame they quantify: PEth is detectable in blood for around 2 to 3 weeks (Schröck et al., 2017a; Wurst et al., 2015), while EtG is detectable in urine for around 5 days (Thon et al., 2013; Wurst et al., 2015), although both are dependent on the amount of alcohol consumed (see Introduction). Among the self-report instruments, the TLFB quantifies consumption for the past 7 days, 30 days, and 12 months; likewise, the DEP-ADO quantifies consumption for the past 30 days and

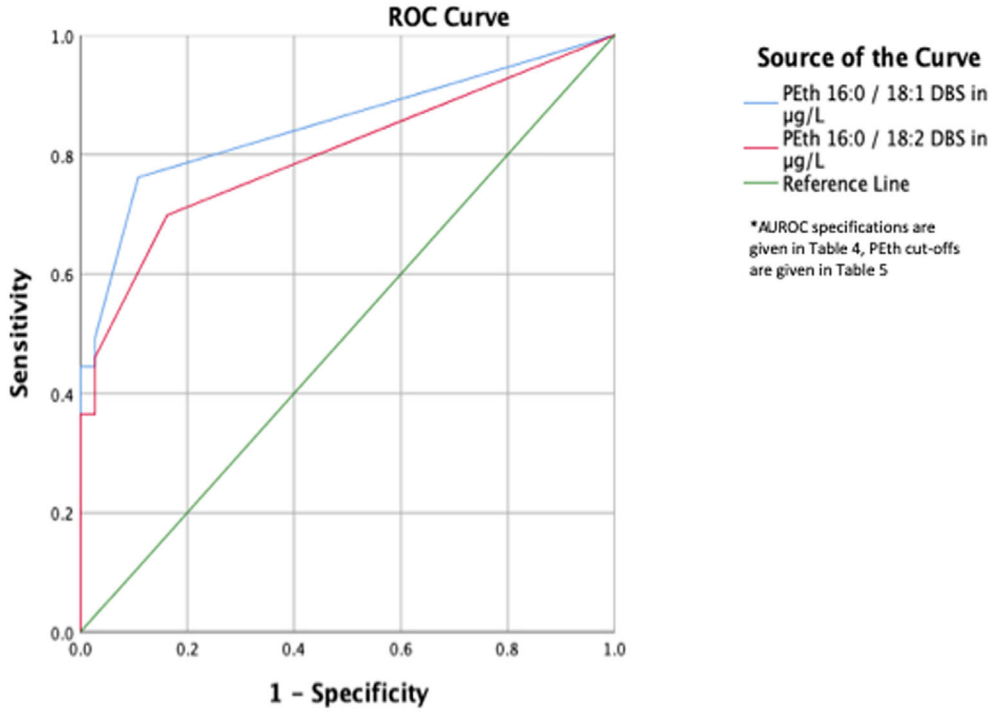

Fig. 3. ROC analysis for PEth homologues and AUDIT-C cutoff value ≥3. Source of the curve (printed in figure). [Color figure can be viewed at wileyonlinelibrary.com]

12 months, while the AUDIT-C only quantifies consumption for the past 12 months.

Hence, although there is a certain overlap in detection time frames between biomarker and self-report instruments, in particular for consumption in the past 1 to 4 weeks detectable by PEth, UEtG, TLFB-1/-2, and DEP-ADO-a, there is a discrepancy between others, notably PEth concentrations and AUDIT-C scores. However, this discrepancy is not unique to the present study and has been considered and reported elsewhere: Across various studies, regardless of whether the AUDIT-C time frame was adjusted to the past month (Jain et al., 2014) or the past 3 months (Asiimwe et al., 2015), maintained at 1 year (Schröck et al., 2017b) or taking only reports of abstinence for the analysis (Eyawo et al., 2017), correlations with PEth concentrations remained fairly constant. The strength of correlations was further corroborated by additional self-report instruments (other than AUDIT-C) which quantified similar time frames to PEth in the respective studies. It can consequently be assumed that in adult populations, consumption patterns on the whole (accounting for individual differences) can be considered to be relatively stable, that is, a consumption pattern reported over a period of 1 to 3 months is unlikely to change significantly over the course of a year, hence the significant results comparing PEth concentrations and AUDIT-C scores.

Based on the results from the present study which show large correlations between overlapping and nonoverlapping time frames for biomarker and self-report instruments, respectively, a similar deduction for adolescents can be made: Although PEth is only quantifiable in blood for approximately 2 to 3 weeks, it appears to be a reliable indicator of alcohol consumption within this time frame and beyond, possibly as a result of reasonably stable consumption patterns. Notably, this is in contrast to the anticipated limitation of consumption patterns in an adolescent population interrupted by many more days of abstinence, thus being of a heavy, episodic rather than continuous nature. It would be instructive for future research to assess interindividual consumption patterns and to compare PEth results to this variable. As an alternative conclusion, it is also plausible that PEth concentrations are indeed stable across different consumption patterns. This will need to be subject to further research. Further, although PEth concentrations will not be available immediately upon sampling, it would be instructive to compare PEth levels with real-time BAC to investigate a possible underreporting of longer-term alcohol consumption during acute intoxication.

With respect to the AUDIT-C screening, our ROC analysis results show that both PEth homologues are a good model for detecting positive screening results, regardless of whether AUDIT-C cutoff values of ≥3 or ≥5 were tested. However, as the ≥3 cutoff leads to a large rate of false positives, overall the result for the ≥5 cutoff value is better and should thus be preferred.

Inversely, however, based on our results it is difficult to make accurate recommendations for an optimal PEth concentration cutoff value to predict a positive AUDIT-C screening. The ROC curves indicate an optimal PEth

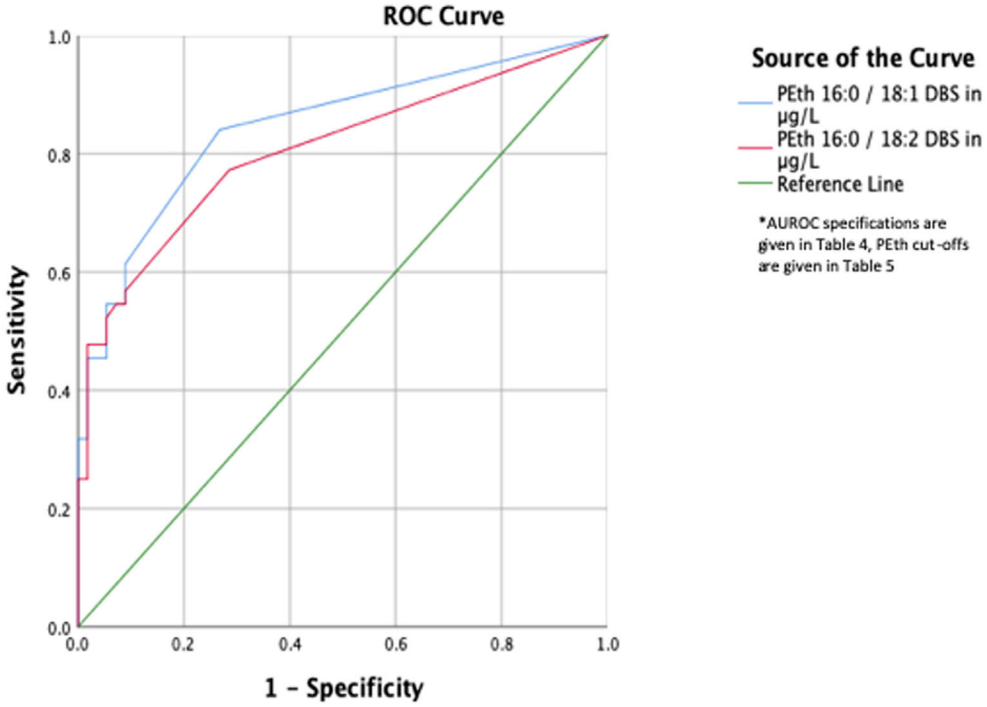

Fig. 4. ROC analysis for PEth homologues and AUDIT-C cutoff value ≥5. Source of the curve (printed in figure). [Color figure can be viewed at wileyonlinelibrary.com]

concentration of 6.5 µg/l. For PEth 16:0/18:1, this optimal point has sensitivity of 84% and specificity of 73% for AUDIT-C ≥ 5. Likewise, for PEth 16:0/18:2, optimal point has sensitivity of 77% and specificity of 71% for ≥5 cutoff value, respectively (see Table 5). As a PEth concentration of 6.5 µg/l lies between the LOD (6 µg/l) and LOQ (20 µg/l), such a cutoff value would cease to be more than an approximation, not directly measurable.

Overall, the results for the ROC curves are promising, but not final. Study replication, especially with participants with clinical diagnoses, and employing other validation criteria, would be advantageous. In particular, diagnoses could become more reliable in populations prone to self-report bias, such as young men with a prior criminal record (Richter and Johnson, 2001). More reliable diagnoses would, in turn, enable expedient therapeutic planning.

Limitations

In addition to the limitations associated with PEth yet to be conclusively resolved, it cannot be excluded that the 2 different blood sampling methods, v-DBS and c-DBS, influenced results. Further, the modified TLFB version and the DEP-ADO German translation used in this study have only content validity to date, and an intoxicated state during study participation cannot be excluded for some participants, all of which may have influenced the self-reported data.

CONCLUSIONS

In a population of adolescents and young adults, we found PEth to be a reliable and objective biomarker for quantifying alcohol consumption. Notwithstanding further research, establishing PEth as a routine test could prove helpful in various clinical settings: as a binary measure when any pattern of alcohol consumption is considered harmful (e.g., during pregnancy or for critically ill patients); for a range of issues in legal medicine, such as the allocation of transplant organs; in situations susceptible to social desirability bias where self-reporting instruments are inherently unsuitable; and finally, as a tool for contingency management aimed at reducing hazardous alcohol consumption, by using direct biomarkers such as PEth to augment self-report measures (McDonell et al., 2017).

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CONFLICT OF INTEREST

None.

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