Assessing Phosphatidylethanol (PEth) Levels Reflecting Different Drinking Habits in Comparison to the Alcohol Use Disorders Identification Test – C (AUDIT-C)

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Abstract

In addition to monitoring problematic or harmful alcohol consumption, drinking experiments indicated the potential of phosphatidylethanols (PEth) in abstinence monitoring. To date no profound evaluation of thresholds for the differentiation of abstinence from moderate drinking and for detection of excessive consumption based on PEth homologues exists. Investigations with a large group of healthy volunteers (n=300) were performed to establish PEth reference values reflecting different drinking habits.

Blood samples were analyzed for PEth 16:0/18:1 and 16:0/18:2 by online-SPE-LC-MS/MS method. Results were compared to AUDIT-C questionnaires, to the amounts of alcohol consumed during the two-weeks prior to blood sampling, and were statistically evaluated.

PEth concentrations were significantly correlated with self-reported alcohol consumption ($r>0.69$) and with AUDIT-C scores ($r>0.65$). 4.0% of 300 volunteers reported abstinence (AUDIT-C score: 0), no PEth was detectable in their blood. PEth 16:0/18:1 concentrations below the limit of detection of 10.0ng/mL match with abstinence and light drinking habits (≤10g pure alcohol/day). However, some volunteers classified as "excessive alcohol consumers" had negative PEth results.

In the group of volunteers classified as "moderate drinkers" (AUDIT-C score: 1–3 (women) and 1–4 (men)), 95% of the test persons had PEth 16:0/18:1 ranging from not detected to 112ng/mL, and PEth 16:0/18:2 ranging from not detected to 67.0ng/mL.

Combination of self-reported alcohol consumption and AUDIT-C score showed that negative PEth results match with abstinence or light drinking. Moderate alcohol consumption resulted in PEth 16:0/18:1 from 0 to 112ng/mL and for PEth 16:0/18:2 ranged from 0 to 67.0ng/mL. Higher PEth concentrations indicated excessive alcohol consumption.

Keywords: Phosphatidylethanol (PEth), Alcohol Use Disorders Identification Test – C (AUDIT-C), Online-SPE-LC-MS/MS, Abstinence, Moderate drinking

Introduction

Alcohol, as one of the most commonly used recreational drugs, is an important cause of morbidity and mortality (Kechagias et al., 2015). To gain information about a person's alcohol consumption habits, clinicians often rely on questionnaires such as Alcohol Use Disorders Identification Test (AUDIT) or its short form, the AUDIT-C (SAMHSA, WHO, 2001). As these may be influenced by memory bias and under-reporting by the desirability to report less alcohol ingestion (Bajunirwe et al., 2014), also biomarkers of alcohol intake are highly useful to identify drinking patterns and alcohol-related disorders, even at an early stage (Schröck et al., 2014, Wurst et al., 2015, Mann K et al., 2016).

Very recent alcohol ingestion can be verified by breath alcohol testing, determination of the blood alcohol concentration (BAC) or by the highly specific direct markers ethyl glucuronide (EtG) and ethyl sulphate (EtS) in blood and/or urine (Halter et al., 2008, Dresen et al., 2004). Besides hair analysis for EtG, which is used in forensic applications (Kharbouche et al., 2012, SOHT, 2014, Boscolo-Berto et al., 2013), for the identification of excessive alcohol consumption the traditional indirect markers carbohydrate deficient transferrin (CDT) and γ-glutamyltranspeptidase (GGT) are in clinical use. However, these markers lack in specificity as they are not only influenced by alcohol intake, but also by age, gender, smoking, and various diseases (Hannuksela et al., 2007, Thierauf et al., 2011). Additionally, these markers are suboptimal with regard to sensitivity for the detection of moderate alcohol consumption. For persons with moderate drinking habits or binge drinkers more sensitive markers are needed, such as phosphatidylethanol (PEth), which is a more specific and sensitive alcohol biomarker concerning this matter (Kechagias et al., 2015). PEth represents a group of phospholipids present in cell membranes, which are formed directly after alcohol intake (Gnann et al., 2009, Gnann et al., 2012) via the enzyme phospholipase D (PL D) from phosphatidylcholine (PC), as long as ethanol is present (Kobayashi and Kanfer, 1987). The

homologues PEth 16:0/18:1 and PEth 16:0/18:2 are the most abundant homologues in human blood (Helander and Zheng, 2009, Zheng et al., 2011, Nalesso et al., 2011).

Due to an elimination half-life of approximately four days (Varga et al., 2000), which however can vary strongly between individuals (Gnann et al., 2012), PEth accumulates in the body after repeated drinking, and is therefore suitable for the differentiation of problematic excessive alcohol consumption and moderate drinking (Aradottir et al., 2006, Viel et al., 2012, Winkler et al., 2013, Gnann et al., 2009).

Recently, drinking studies with moderate alcohol consumption have shown that PEth concentrations correlate with the consumed amounts of alcohol, and that PEth has a potential in abstinence monitoring as it is formed directly after alcohol intake (Gnann, 2011, Gnann et al., 2012, Schröck et al., 2016c). Another pilot study showed that combining EtG and EtS testing in urine with PEth analysis in blood seems to be effective in providing additional information on recent drinking (Skipper et al., 2013).

PEth also has a potential in driving aptitude assessment (DAA) as additional marker to EtG in hair, which is the most frequently routinely used direct marker for the detection of excessive alcohol consumption and also for abstinence monitoring in this context (Kharbouche et al., 2012). In a recent study, the classification into drinking groups (abstainers, moderate drinkers and excessive drinkers) according to EtG concentrations in hair corresponded to the detected PEth values in blood in 68 % of the tested samples (Schröck et al., 2016b).

So far, several PEth thresholds, such as 210 ng/mL (Helander and Hansson, 2013), 700 ng/mL (Schröck et al., 2016a) , and 800 ng/mL (Gnann, 2011) were proposed for the differentiation of moderate and excessive alcohol consumption, which were obtained either from alcoholics starting withdrawal or which were obtained during drinking studies.

By now, there are no reference levels established for PEth homologues allowing the differentiation of abstinence, moderate alcohol consumption and excessive consumption in addition to AUDIT-C or self-reports. On this background, our study was performed with 300

volunteers to investigate the potential of PEth in reflecting different drinking habits, such as abstinence, moderate and excessive alcohol consumption, and to assess reference values for setting-up a database of PEth 16:0/18:1 and PEth 16:0/18:2 concentrations, which can be expected after consumption of alcohol in a harmless way (≤ 20 g/day females, ≤ 40 g/day males, (WHO, 2000)). The PEth concentrations were statistically evaluated in comparison with AUDIT-C questionnaires and with self-reported amounts of ingested alcohol of the last two weeks prior to blood sampling.

Material and methods

Chemicals and materials

PEth 16:0/18:1, PEth 16:0/18:2 were purchased from Avanti Polar Lipids (Alabaster, USA). Acetonitrile was supplied by Agros Organics (New Jersey, USA), 2-propanol was obtained from Fisher Scientific (Loughborough, UK), and formic acid (HCOOH) was from Sigma Aldrich (Buchs, Switzerland). Ammonium acetate was provided by Merck (Darmstadt, Germany). Deionized H_2O was produced in-house with a Milli-Q water system from Millipore (Billerica, USA).

Deuterated internal standards were synthesized in our laboratory from phosphatidylcholine 16:0/18:1 and phosphatidylcholine 16:0/18:2 and D_6-ethanol catalyzed by phospholipase D (Schröck et al., 2016a). Heparin Vacuettes (volume: 9 mL) were obtained from Greiner Bio-one (Kremsmünster, Austria). Lithium-heparinized whole blood, which was used as blank blood, was obtained from volunteers who were abstinent from alcohol for at least 4 weeks.

Study design

To establish thresholds for PEth as diagnostic marker for the differentiation of abstinence and moderate drinking habits, as well as excessive drinking habits, reference values were

determined from 300 volunteers. Blood samples were collected after blood donation at the blood donation centers in Burgdorf, Bern and Thun (Switzerland). To prevent contamination with ethanol during veno-puncture, Ethanol-free swabs were used for disinfection, and the blood samples were stored at 4 °C directly after sampling and were analyzed for PEth within three days to prevent in-vitro formation of PEth. The volunteers additionally answered the AUDIT-C questionnaire and retrospectively documented their alcohol consumption of the last two weeks prior to blood sampling. The concentrations of the PEth homologues 16:0/18:1 and 16:0/18:2 in blood were compared to the AUDIT-C scores, to the self-reported amounts of consumed alcohol, and these results were statistically evaluated. This study has been approved by the Cantonal Ethics Commission Bern (064/13) on December 10, 2015.

AUDIT-C

The 10-item Alcohol Use Disorders Identification Test (AUDIT) is a screening questionnaire developed by the World Health Organization (WHO) to identify harmful or hazardous alcohol consumption. Response options for each item range from 0 to 4, resulting in a total possible score of 40.

The AUDIT-C is a shortened version of the 10-items AUDIT, which was developed as an even briefer and easier screening method of the alcohol consumption habits of the last year (SAMHSA, WHO, 2001). It consists of the first three questions concerning alcohol consumption habits such as quantity, frequency and binge drinking (Bush et al., 1998). An AUDIT-C score equal to zero indicates abstinence. For women an AUDIT-C score ≥ 4, and for men a score ≥ 5 indicates excessive alcohol consumption in a German speaking population according to the recent evidence- and consensus-based guidelines of the grade S3 (Mann K et al., 2016).

Self-reported alcohol consumption

A modified form of the time line follow back questionnaire, originally developed by Sobell and Sobell restricted to the last two weeks prior to the study was used to obtain retrospective estimates of drinking (Sobell and Sobell 1992). This questionnaire contained a choice of different alcoholic beverages with typical percent alcohol by volume (e.g. beer 5 %, wine 12 %, liquors 40 %). The volunteers were asked to retrospectively fill in the type of consumed alcoholic beverages and consumed amounts (volumes/sizes of units, and numbers of units per week)- for each of the two weeks prior to blood sampling. Participants with an alcohol consumption below ≤ 10 g/day were classified as light drinkers.

Determination of PEth

PEth 16:0/18:1 and PEth 16:0/18:2 were analyzed in 200 µL of whole blood with D_5-PEth 16:0/18:1 and D_5-PEth 16:0/18:2 as internal standards. The analysis was performed by online-SPE-LC-MS/MS with a QTrap 3200 mass spectrometer (Sciex, Toronto, Canada). The two homologues were chromatographically separated on a Luna RP-C5 column, 50 mm x 2 mm, 5 µm particle size (Phenomenex, Brechbühler, Schlieren, Switzerland). The limit of detection (LOD) was 10.0 ng/mL and the limit of quantification (LOQ) was 20.0 ng/mL for both PEth homologues. The precise details about sample preparation and the validated online-SPE-LC-MS/MS method are described in (Schröck et al., 2016c).

Statistic evaluation

Statistical analysis was performed with GraphPad Prism 6 (GraphPad Software Inc., La Jolla, California, USA). For the Kruskall-Wallis-Test, a non-parametric test used to detect differences between three or more groups, the results of this study were classified into three groups (group A: AUDIT-C score 0: abstainers; group B: AUDIT-C score 1 – 3 (women) and

1 – 4 (men): moderate alcohol consumption; group C: AUDIT-C score ≥ 4 (women) and ≥ 5 (men): excessive alcohol consumption) depending on the achieved points of the AUDIT-C (Mann K et al., 2016).

For receiver operating characteristics (ROC) analysis, the results were classified into two groups (group A and B: abstainers/moderate consumers versus group C: excessive consumers). The AUDIT-C scores served as primary reference standard for the evaluation of cut-offs for PEth 16:0/18:1 and 16:0/18:2 for differentiation of these populations with high sensitivity and specificity. Furthermore, the daily average of self-reported amount of alcohol consumed over the last 2 weeks prior to blood sampling was compared to PEth 16:0/18:1 and 16:0/18:2 concentrations using Spearman's rank correlation coefficient.

Results

Comparison of PEth concentrations to AUDIT-C scores

The 300 volunteers (203 male, 94 female, 3 not specified; 18 – 78 years, mean: 47 ± 13 years) were divided into the three groups A, B and C (see above: statistic evaluation; figures 1 and 2, table 1). Overall, male participants tended to have slightly higher alcohol consumption.

Group A: Four percent (6 female and 6 male) of the 300 participating volunteers reported abstinence (AUDIT-C score 0) and PEth was not detectable in any of these samples (with a limit of detection (LOD) of 10 ng/mL for PEth 16:0/18:1 and 16:0/18:2).

Group B: 71 percent (214 volunteers: 143 male, 68 female, 3 not specified) showed moderate alcohol consumption (AUDIT-C scores 1 – 3 for women, 1 – 4 for men). PEth 16:0/18:1 values ranged from not detected to 619 ng/mL (mean 31.8 ng/mL). PEth 16:0/18:2 values ranged from not detected to 286 ng/mL (mean 18.4 ng/mL). Among those “moderate drinkers” 61.7 % (n = 132) had positive PEth results (PEth ≥ 10.0 ng/mL (LOD)).

Twelve persons (8 female and 4 male, 31 – 67 years, mean 50 ± 12 years) reported "moderate drinking habits" (AUDIT-C scores representing moderate alcohol consumption), but did not report having consumed alcohol in the two weeks prior to blood sampling for this study. Only one of these 12 persons had PEth 16:0/18:1 (> 10.0 ng/mL) above the LOD (10.0 ng/mL) of the online-SPE-LC-MS/MS method. PEth 16:0/18:2 was not detectable in any of these persons. Another 9 persons (all with AUDIT-C scores representing moderate alcohol consumption) reported no alcohol ingestion in the week prior to blood sampling. Among them, four persons had PEth 16:0/18:1 concentrations above the LOD, but no PEth 16:0/18:2 was detectable. Another person had 28.3 ng/mL PEth16:0/18:1 and 17.0 ng/mL PEth 16:0/18:2. This means that 44.4 % (4 of 9) of the test persons being abstinent for one week prior to blood sampling were PEth negative (PEth < 10.0 ng/mL).

Group C: 25 percent (74 volunteers: 54 male and 20 female) showed excessive alcohol consumption (AUDIT scores ≥ 4 for women, ≥ 5 for men). PEth 16:0/18:1 values ranged from not detected to 937 ng/mL (mean 110 ng/mL). PEth 16:0/18:2 values ranged from not detected to 472 ng/mL (mean 63.2 ng/mL). Among those persons with excessive alcohol consumption 95.9 % (n = 71) had positive PEth results (PEth ≥ 10.0 ng/mL (LOD)). Detailed information can be found in table 1 and figure 2.

Five data points of figure 2 (PEth 16:0/18:1 and PEth 16:0/18:2, respectively) were outside the axis limit in the column of moderate and excessive alcohol consumption (moderate: 1. PEth 16:0/18:1: 414 ng/mL, PEth 16:0/18:2: 224 ng/mL; 2. PEth 16:0/18:1: 619 ng/mL, PEth 16:0/18:2: 286 ng/mL; excessive: 3. PEth 16:0/18:1: 506 ng/mL, whereas PEth 16:0/18:2: 168 ng/mL is still included; 4. PEth 16:0/18:1: 524 ng/mL, PEth 16:0/18:2: 376 ng/mL; 5. PEth 16:0/18:1: 937 ng/mL, PEth 16:0/18:2: 472 ng/mL).

Comparison of PEth concentrations to self-reported alcohol consumption two weeks prior to blood sampling

12 persons (6 female and 6 male) of the 300 participating volunteers had not-detectable PEth concentrations (< 10 ng/mL for both PEth 16:0/18:1 and 16:0/18:2) and reported abstinence.

A total of 85 test persons had (PEth not detected) but reported drinking in the last two weeks. These persons were mainly light drinkers (35 female, 49 male, 1 not specified) with AUDIT-C scores were between 1 - 4, and averaged amounts of consumed alcohol in the past two weeks ranged from 0 – 10 g/day. Concerning PEth 16:0/18:2 concentrations, 121 test persons (47 female, 71 male, 3 not specified) had PEth 16:0/18:2 < 10.0 ng/mL, with AUDIT-C scores between 1 - 5, and averaged amounts of consumed alcohol in the past two weeks ranged from 0 – 10 g/day.

161 test persons had PEth 16:0/18:1 concentrations between 10.0 and 100 ng/mL (44 female, 115 male, 2 not specified). AUDIT-C scores were between 1-7, and averaged amounts of consumed alcohol in the past two weeks ranged from 0 – 50 g/day. Concerning concentrations, 150 test persons had PEth 16:0/18:2 between 10.0 and 100 ng/mL (38 female, 112 male). AUDIT-C scores were between 1-8, and averaged amounts of consumed alcohol in the past two weeks ranged from 0 – 50 g/day. According to WHO (WHO, 2000), these ranges are still in the region of medium risk consumption (41 to 60 g) for men, but for women this amount lies in the range of high risk consumption (41 to 60 g).

42 test persons had PEth 16:0/18:1 concentrations > 100 ng/mL (9 female, 33 male), whereas only 17 test persons had PEth 16:0/18:2 concentrations > 100 ng/mL (3 female, 14 male). AUDIT-C scores were between 2-8, and averaged amounts of consumed alcohol in the past two weeks ranged from 2.0 – 33 g/day.

Statistic evaluation

We found statistically significant correlations of PEth 16:0/18:1, as well as of PEth 16:0/18:2 concentrations, with the measures of alcohol consumption (AUDIT-C and self-reported amount of consumed alcohol in the last two weeks prior to blood sampling, respectively) (table 2). The correlation of PEth 16:0/18:1 with the measures of alcohol consumption led to Spearman correlation coefficients of $r > 0.68$ (AUDIT-C) and $r > 0.70$ (self-reported amount of consumed alcohol)); the respective correlation of PEth 16:0/18:2 led to Spearman correlation coefficients of $r > 0.65$ (AUDIT-C) and $r > 0.69$ (self-reported amount of consumed alcohol)). There was a slightly higher significance of PEth to self-reported amounts of consumed alcohol than to the AUDIT-C score.

The Kruskall-Wallis-Test showed significant differences between the three alcohol consumption groups (abstinence, moderate and excessive alcohol consumption) with significantly varying P values (PEth 16:0/18:1: P value < 0.0001; PEth 16:0/18:2: P value < 0.0001).

For the extrapolation of reference values of PEth 16:0/18:1 and PEth 16:0/18:2 for moderate alcohol consumption, the group of 214 volunteers (71 % of 300 participants) was evaluated, as these participants reported AUDIT-C scores corresponding to moderate alcohol consumption. 95 % of the samples ranged between “not detected” and 112 ng/mL for PEth 16:0/18:1, and between “not detected” and 67.0 ng/mL for PEth 16:0/18:2 (figure 2).

Applying the suggested PEth 16:0/18:1 range of “not detected” to 112 ng/mL, which indicates abstinence or moderate alcohol consumption, to all participants of this study (n = 300), 87.7 % of the test persons (n = 263) were within this range, with average amounts of consumed alcohol of 0 – 50 g/day and a mean amount of consumed alcohol of 5.7 g/day.

However, only 36.5 % of those persons with AUDIT-C scores ≥ 4 (women) and ≥ 5 (men), respectively, had PEth 16:0/18:1 concentrations above 112 ng/mL and PEth 16:0/18:2 concentrations above 67.0 ng/mL.

Discussion

The aim of this study was to establish reference levels of PEth 16:0/18:1 and PEth 16:0/18.2 in comparison to AUDIT-C and self-reports reflecting different drinking habits, such as abstinence, moderate and excessive alcohol consumption in 300 volunteers. As this study was anonymous (only information about age and sex was available) and without consequences for the volunteers whatever resulted from the AUDIT-C, self-reports or PEth analysis, the answers to the questionnaires were supposed to be correct with no significant over- or under-reporting. However, there were two cases of clear under-reporting of alcohol consumption habits. For one female participant, AUDIT-C score was 2 (moderate alcohol consumption) and self-reported mean alcohol consumption was 2.1 g/day, whereas high PEth concentrations were found (PEth 16:0/18:1: 414 ng/mL, PEth 16:0/18:2: 224 ng/mL). For one male participant with an AUDIT-C score of 3 (moderate alcohol consumption) and self-reported mean alcohol consumption of 7.8 g/day, PEth concentrations were even higher (PEth 16:0/18:1: 619 ng/mL, PEth 16:0/18:2: 286 ng/mL). In both cases, PEth concentrations clearly exceeded the above mentioned thresholds for both PEth homologues for differentiation of moderate and excessive consumption.

All tested persons who declared to be abstainers were PEth negative, resulting in an accordance of 100 % between AUDIT-C score and PEth regarding abstinence, meaning that no “endogenous” PEth levels have been detected in the blood of abstainers. 91.7 % (n = 11) of the test persons (n = 12), who did not consume alcohol in the two weeks prior to blood sampling for this study, but were classified as moderate drinkers according to AUDIT-C score, were PEth negative (PEth < 10.0 ng/mL). In addition, 85 test persons, who declared drinking in the last two weeks (mean alcohol amount ranging from 0 – 10 g/day), had negative PEth 16:0/18:1 results. As a consequence, persons who are negative for PEth do not necessarily have to be abstainers, but might belong to the group of moderate consumers.

However, there were also some PEth negative volunteers in the group classified as excessive consumers (PEth 16:0:18:1 < 10.0 ng/mL: n = 9; PEth 16:0:18:2 < 10.0 ng/mL: n = 30).

PEth levels reflecting moderate alcohol consumption (AUDIT-C score 1 – 3 (women) and 1 – 4 (men)) ranged from not detected to 112 ng/mL for PEth 16:0/18:1, and from not detected to 67.0 ng/mL for PEth 16:0/18:2, corresponding to 95 % of the tested persons in the group with AUDIT-C scores representing moderate alcohol consumption.

This cut-off concentration for PEth 16:0/18:1 (112 ng/mL) for the differentiation of moderate and excessive alcohol consumption was higher than the findings from a recently published study among a group of persons with hepatic diseases, in which a threshold of 80.0 ng/mL PEth 16:0/18:1 for averaging at least 4 drinks/day was identified, which would impair or cause liver disease (Stewart et al., 2014).

After evaluating the suggested PEth 16:0/18:1 cut-off level of 112 ng/mL by ROC analysis (figure 3) (comparing PEth to AUDIT-C scores), 95 % of the group of moderate consumers and abstainers had PEth 16:0/18:1 concentrations ranging between not detected and 112 ng/mL, but only 36.5 % of the participants with AUDIT-C scores representing excessive alcohol consumption had PEth 16:0/18:1 concentrations above 112 ng/mL.

Comparing PEth 16:0/18:2 to AUDIT-C scores by ROC analysis (figure 4) to evaluate the suggested PEth 16:0/18:2 cut-off level of 67.0 ng/mL, 95 % of the group of “social consumers” and abstainers had PEth 16:0/18:2 concentrations ranging between not detected and 67.0 ng/mL, and 36.5 % of the participants with AUDIT-C scores representing excessive alcohol consumption had PEth 16:0/18:2 concentrations above 67.0 ng/mL.

Using the PEth 16:0/18:1 cut-off of 80.0 ng/mL from the literature (Stewart et al., 2014) for our test persons, only 93.3 % of the participants would be identified as “social consumers” and abstainers, but 46.0 % of the participants with AUDIT-C scores representing excessive alcohol consumption would be classified correctly for excessive drinking habits.

A limitation of our study is that excessive alcohol consumption is underrepresented in the participants of this study (only 74 of 300 test persons). Therefore, it was not our aim to evaluate a threshold for the detection of excessive alcohol consumption with high sensitivity. We found overlapping ranges of PEth concentrations between the groups with moderate and excessive alcohol consumption, respectively. This is mainly due to the fact, that AUDIT-C covers a rather long time period (1 year), whereas PEth can only be detected for some days to weeks after excessive drinking behavior.

The correlations of PEth 16:0/18:1 and PEth 16:0/18:2 to AUDIT-C score and to self-reports (amount of consumed alcohol in the last two weeks prior to blood sampling) were statistically significant – with slightly higher correlations of PEth to the amount of consumed alcohol than to AUDIT-C scores, as PEth can only cover the alcohol consumption of the last days to weeks. This is in agreement with other studies among moderate drinkers (Stewart et al., 2014, Bajunirwe et al., 2014, Jain et al., 2014, Kechagias et al., 2015).

Under-reporting of alcohol consumption might lead to unexpectedly (high) PEth concentrations – however, “false-positive” PEth results might be another source of discrepancies. To the best of our knowledge, with the exception of post-sampling in-vitro formation of PEth in the presence of ethanol in blood, no other sources for false-positive PEth results have been detected yet, but relevant amounts of hidden alcohol in nutrition or medication (which might not be reported in a questionnaire) as well as inter-individual variations of enzyme activities for the formation of PEth might cause unexpected elevated concentration levels of PEth.

We conclude, that the alcohol biomarker PEth can be applied to confirm or to disprove self-reported alcohol consumption. In the present study a high correlation between PEth values and self-reports was found. PEth 16:0/18:1 values below the LOD of 10.0 ng/mL are strongly correlated with light drinking habits (≤ 10 g pure alcohol/day) or abstinence.

As PEth 16:0/18:1 concentrations ranging from not detected to 112 ng/mL (for PEth 16:0/18:2 values from not detected to 67.0 ng/mL) correspond to 95 % of the tested persons in the group with AUDIT-C scores representing moderate alcohol consumption, we suggest to employ these reference ranges as optimal indicators for alcohol consumption in a harmless way (≤ 20 g/day females, ≤ 40 g/day males, (WHO, 2000)). PEth values above this range point to recent excessive alcohol consumption (AUDIT-C score ≥ 4 females, AUDIT-C score ≥ 5 males).

Tables

Table 1. Descriptive statistics for the volunteers regarding drinking types according to AUDIT-C

Table 2. Correlations between the measures of alcohol consumption and the PEth 16:0/18:1 and PEth 16:0/18:2 values

Figure Legends

Figure 1. Distribution of the AUDIT-C (AUDIT: Alcohol Use Disorders Identification Test) scores.

Figure 2. Classification of PEth 16:0/18:1 and 16:0/18:2 values in comparison to consumption according to AUDIT-C score. The crosses represent the mean, the boxes show the interquartile range, the line within the boxes is the median, and the tails represent the 5 – 95 percentiles with the dots as outliers.

Five data points (PEth 16:0/18:1 and PEth 16:0/18:2, respectively) are outside the axis limit in the column of moderate and excessive alcohol consumption (moderate: 1. PEth 16:0/18:1: 414 ng/mL, PEth 16:0/18:2: 224 ng/mL; 2. PEth 16:0/18:1: 619 ng/mL, PEth 16:0/18:2: 286 ng/mL; excessive: 3. PEth 16:0/18:1: 506 ng/mL, whereas PEth 16:0/18:2: 168 ng/mL is still included; 4. PEth 16:0/18:1: 524 ng/mL, PEth 16:0/18:2: 376 ng/mL; 5. PEth 16:0/18:1: 937 ng/mL, PEth 16:0/18:2: 472 ng/mL).

Figure 3. ROC analysis for PEth 16:0/18:1: For the threshold of 112 ng/mL, a specificity of 96.6 % and a sensitivity of 22.9 % were determined

Figure 4. ROC analysis for PEth 16:0/18:2: For the threshold of 67 ng/mL, a specificity of 96.6 % and a sensitivity of 22.1 % were determined

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