Phosphatidylethanol Levels Are Elevated and Correlate Strongly with AUDIT Scores in Young Adult Binge Drinkers

Mariann R. Piano1,*, Stephanie Tiwari1, Lauren Nevoral2, and Shane A. Phillips2

1Department of Biobehavioral Health Science, University of Illinois at Chicago, Chicago, IL, USA, and 2Department of Physical Therapy, University of Illinois at Chicago, Chicago, IL, USA

*Corresponding author: Department of Biobehavioral Health Science, University of Illinois at Chicago, 845 S. Damen Ave. (MC 802), Chicago, IL 60612, USA. Tel.: +1-312-413-7908; Fax: +1-312-996-7934; E-mail: piano@uic.edu

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Abstract
Aims: To compare levels of phosphatidylethanol (PEth) to self-reported alcohol intake among young adult binge drinkers (18–30 years).

Methods: Abstainers (n = 23), moderate (n = 22), and binge drinkers (n = 58) completed an alcohol consumption questionnaire and the AUDIT. PEth was measured in whole blood and dried blood spots via high-performance liquid chromatography with tandem mass spectrometry. Also measured was mean corpuscular volume (MCV) and gamma glutamyl transpeptidase (GGT).

Results: Most subjects were female (65%) and Caucasian (73%). Among binge drinkers, past-month average number of binge episodes was 7.2 ± 4; average duration of binge drinking behavior was 4.3 ± 3 years. AUDIT scores and PEth levels (ng/ml) in whole blood or dried blood spots were significantly (P < 0.001) greater in binge drinkers (13 ± 4, 186 ± 170, and 65 ± 53, respectively) compared to moderate drinkers (6 ± 3, 24 ± 29, and 11 ± 13, respectively) and abstainers (0.6 ± 0.89, 0, and 0, respectively). No differences were found in MCV and GGT among groups. There were significant correlations between whole blood and dried blood spot PEth levels and AUDIT scores (Spearman’s r = 0.745 and 0.738, P < 0.0001, respectively), and whole blood and dried blood spot PEth levels were significantly correlated (0.899, P < 0.0001).

Conclusions: PEth levels measured in whole blood and dried blood spots were significantly greater in binge drinkers compared to abstainers and moderate drinkers, and these levels were positively correlated with AUDIT scores.

INTRODUCTION
Phosphatidylethanol (PEth) is an emerging biomarker of moderate and heavy alcohol consumption and may be useful to corroborate self-report of alcohol use (Viel et al., 2012). PEth has been measured in whole blood using different analytic methods, including high-performance liquid chromatography with tandem mass spectrometry (HPLC LC/MS/MS). Recently, methods have been developed to measure PEth in dried blood spots (DBS). The DBS collection method allows for noninvasive and cost-effective approaches for the measurement of biomarkers in clinical and population studies (Oxler et al., 2014). Others recently reported that DBS PEth in combination with other measures (e.g. self-report) increased the accuracy of detecting prenatal alcohol exposure (Bakhireva et al., 2014) and corroborating alcohol use among young injection drug users (Jain et al., 2014). Studies often use self-report to detect unhealthy and excessive alcohol use, such as binge drinking.

Over the past several years, there have been several reports examining the reliability and validity of various alcohol questionnaires to
detect alcohol misuse and high-risk drinking in college-aged populations. There are several well-established tools, such as the Alcohol Use Disorders Identification Test (AUDIT), CAGE (cut down, annoyed, guilty or eye opener) and TWEAK screen (tolerance, worried, eye opener, amnesia, cut down). However, in terms of detecting a binge drinking pattern, the CAGE and TWEAK have limited information or dimensions that reflect drinking patterns, quantity/frequency, mean number of drinks per occasion, and binge drinking duration. The AUDIT, however, covers the conceptual domains of alcohol consumption (frequency and amount) and has been used to identify alcohol dependence and at-risk heavy drinking (i.e., binge drinking) among college students (Kokotailo et al., 2004). Using a biomarker of heavy alcohol consumption such as PEth along with self-report could provide an objective measure for use in research, screening, and treatment of hazardous alcohol use among young adults.

Binge drinking is pervasive on college campuses and among young adults. More alarming though, is the regularity of binge drinking episodes: one in five students report three or more binge drinking episodes in the prior 2 weeks (Centers for Disease Control and Prevention, 2012). Cross-sectional studies have also found that male and female college students report 9–10 drinking days per month with an average of 4–6 drinks per drinking episode (Mundt et al., 2009). There is also evidence of more extreme forms of drinking and drinking ‘games’ (Rutledge et al., 2008). These data highlight the seriousness and scope of the binge drinking problem.

To the best of our knowledge, there are no studies examining PEth levels with self-reported alcohol consumption among young adults. Therefore, the aims of this study were to: (a) prospectively compare PEth levels and self-reported alcohol intake (via an alcohol intake questionnaire and AUDIT scores) in young adult (18–30 years) abstainers, moderate drinkers, and binge drinkers and (b) determine the correlation between PEth levels measured in whole blood and DBS.

METHODS

Study design and subjects
Subjects were part of a larger ongoing prospective cross-sectional study designed to examine the cardiovascular effects of binge drinking. A convenience sample of young adult (18–30 years) abstainers, moderate drinkers (MODs), and binge drinkers (BDs) were recruited from two large Midwestern university campuses. Young adults were recruited via flyers and email communication, and two cohorts were enrolled between June 2014 and October 2014. Inclusion criteria were history of alcohol abstention or drinking. Exclusion criteria were obesity (BMI >35), current or past history of cigarette smoking, inflammatory disease (e.g., rheumatoid), diagnosed cardiovascular or renal disease, active infection (2 months prior), or pregnancy.

Study group allocation and self-reported alcohol use
The study was approved by the Office of Protection of Research Subjects and Institutional Review Board (#2014-0017), and written consent was obtained from all subjects. The initial screening included informed consent and 2–3 questions about alcohol consumption in the prior 30 days and when alcoholic beverages were last consumed. Demographic and other medical information was collected by self-administration of a medical history questionnaire. Alcohol consumption and drinking patterns were determined by an alcohol intake questionnaire (AIQ) and the AUDIT, and data from these tools were used to corroborate group assignment. Questionnaires were completed by all subjects and the order of questionnaire administration was randomly assigned. We previously used the AIQ to screen and categorize young adults for history of binge drinking (Goslawski et al., 2013).

The AIQ is a 20-item tool and includes a modified version of the National Institute of Alcoholism and Alcohol Abuse (NIAAA) 6-item set of questions on binge drinking (Task Force on Recommended Alcohol Questions, 2003). These quantity, frequency, and binge drinking questions have good psychometric properties to detect high-risk or binge drinking and have been used in a research setting (Esser et al., 2012). The AIQ included ‘time qualifier’ questions (e.g., ‘During your last drinking episode how fast did you consume the alcoholic beverage?’ 2–3 drinks/h, 4–5 drinks/h, 6–7 drinks/h, 8–9 drinks/h, >10 drinks/h) and questions without a ‘time qualifier’ (e.g., ‘How many drinks on average do you consume in one occasion [sitting]?’). The latter questions were used because others reported a combination of measures and questions may be required to capture heavy binge drinking (Corbin et al., 2014). Additional questions were added to the AIQ to determine the type of alcohol consumed, history of familial alcohol abuse, duration of binge drinking (e.g., years), maximal/largest number of drinks consumed by the subject in the last 30 days and history of blackouts as well as other information such as membership in fraternity or sorority (Esser et al., 2012). Total AUDIT and AUDIT-C scores were calculated. The AUDIT is a 10-item questionnaire and has been used extensively in different populations (Reinert and Allen, 2007). The AUDIT has been validated in young adult populations and a total score of >6 has high sensitivity (91%) and specificity (60.0%) for detecting high-risk drinking among young adults (Kokotailo et al., 2004). The AUDIT-C (the first three questions of the AUDIT) has been used as a three-item screening test for at-risk heavy drinking in college populations (a score of >7 for males and >5 for female indicates at-risk drinking; Demartini and Carey, 2012).

Before completing alcohol questionnaires, each subject was provided a visual calendar along with a written display of standard drink conversions. A standard drink was defined as one containing 12 g of alcohol and was equivalent to 12 ounces of beer, 5 ounces of wine, 1.5 ounces of 80-proof spirits, or 8–9 ounces of malt liquor.

Alcohol abstainers were defined as those that consumed no more than one standard drink per month in the last 2–3 years (and abstinence cannot be due to a medical illness or prior alcohol abuse). Moderate or social drinkers were defined as follows: for males, the consumption of no more than three standard drinks per sitting with no more than 1–2 times per week, and for females the consumption of no more than two standard drinks per sitting with no more than 1–2 times in a given week in the last 5 years (Maurage et al., 2012). Binge drinkers were defined as those consuming five or more standard drinks either on one occasion or within a 2-h period in the last 30 days if male, and four or more standard drinks on one occasion or in a 2-h period in the last 30 days if female. Binge drinkers must have had a history of at least two binge drinking episodes in the last month.

Sample collection and laboratory measures
After completion of questionnaires, 10 ml of venous blood was obtained for the measurement of blood alcohol levels (BAL), PEth, mean corpuscular volume (MCV) and gamma glutamyl transpeptidase (GGT). Venous blood for MCV and GGT was only collected in the second cohort of subjects. Blood alcohol levels were also measured because others have shown that BALs greater than 0.1 g/l (10 mg%) may generate false PEth values (Aradottir et al., 2006; Isaksson et al., 2011). Venous blood for whole blood PEth levels
was collected into tubes containing ethylenediaminetetraacetic acid. Immediately after blood collection, five blood spots were placed onto a DBS card (U.S. Drug Testing Lab [USDTL], Des Plaines, IL, USA). Whole blood specimens were sent to Labcorp Clinical Trials (Cincinnati, OH, USA) for GGT, MCV, BAL and PEth levels (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanol [16:0/18:1]) measurements by HPLC LC/MS/MS analysis. DBS for PEth were sent to (USDTL) for analysis (by HPLC LC/MS/MS). All whole blood specimens (stored at 4°C) and DBS (stored at room temperature) were shipped to respective laboratories within 24 h of collection. The within-batch coefficient of variation (CV) was 3.3% and the between-batch CV was 3.6%. The limit of quantification for whole blood PEth levels was 20 ng/ml and levels in excess of >8 ng/ml were considered positive and evidence of moderate to heavy drinking (Labcorp Clinical Trials). The within-batch coefficient of variation (CV) was 3.3% and the between-batch CV was 3.6%. The limit of quantification for DBS PEth was 8 ng/ml and levels in excess of >8 ng/ml were considered positive and evidence of moderate to heavy drinking (USDTL). At the cutoff of 8 ng/ml, the within-batch (n = 5, 4 days) CV ranged between 2.3% and 10.5% and the between-batch CV was 10.2%.

Statistical analysis

Descriptive statistics (means, medians, and percentagess) were calculated for demographic and other continuous variables (SAS Inc., Chicago, IL, USA). PEth levels and AUDIT scores were compared among groups using Kruskal–Wallis one-way analysis of variance (ANOVA). Spearman’s rho was used to determine the relationships between whole blood and DBS PEth levels and AUDIT scores and whole blood and DBS PEth levels.

RESULTS

A total of 103 subjects were recruited and based upon inclusion criteria subjects were classified as abstainers (n = 22), moderate (n = 23) and/or binge (n = 58) drinkers. Of the 103, we were unable to obtain blood or there was blood processing issues with five subjects. Among all subjects (N = 103), the mean age was 22 ± 3 years (interquartile range [IQR]; 20–24); most were female (65%) and Caucasian (73%). There were no significant differences in age or sex among the three groups (Table 1). However, in terms of race, significantly more MODs and BDs were Caucasian, and among the abstainer group significantly more were of Asian ethnicity.

Whole blood and DBS PEth levels were negative (or 0) in all abstainers. Whole blood and DBS PEth levels were significantly greater in BDs compared to MODs and abstainers (P < 0.0001), whereas no differences were found between the MOD and abstainer group (Table 1).

In the combined sample of MODs and BDs (n = 98) AUDIT scores were significantly correlated with whole blood and DBS PEth levels (Spearman’s r = 0.745 and 0.738, P < 0.0001, respectively; Figs 2 and 3). Whole blood values were greater than DBS values, which is expected because the cutoff values (20 and 8 ng/ml, respectively) are different; however, whole blood and DBS PEth levels were significantly and positively correlated (n = 98; Spearman’s r = 0.899, P < 0.0001; Fig. 4).

Among the BD group, past-month average number of binge episodes was 7.2 ± 4, and the average duration of binge drinking behavior was 4.3 ± 3 years. The average number of drinks consumed during the last binge episode was 8.3 ± 2, with a range of 4–13.

Table 1. Demographic characteristics, biomarker and AUDIT scores

<table>
<thead>
<tr>
<th></th>
<th>Abstainers</th>
<th>Moderate drinkers</th>
<th>Binge drinkers</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22 ± 2.5</td>
<td>23 ± 2.7</td>
<td>22 ± 2.6</td>
<td>0.139</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11 (48%)</td>
<td>8 (36%)</td>
<td>17 (29%)</td>
<td>0.289</td>
</tr>
<tr>
<td>Female</td>
<td>12 (52%)</td>
<td>14 (64%)</td>
<td>41 (71%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>8 (35%)</td>
<td>13 (59%)</td>
<td>52 (90%)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>2 (3%)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>14 (60%)</td>
<td>9 (41%)</td>
<td>4 (7%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1 (4%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Blood alcohol level (mg %)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Grains of ethanol consumed/week</td>
<td>0</td>
<td>58 ± 42</td>
<td>231 ± 128&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Grains of ethanol consumed/kg/week</td>
<td>0</td>
<td>0.95 ± 0.57</td>
<td>3.27 ± 1.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PEth (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood</td>
<td>0</td>
<td>24 ± 29</td>
<td>186 ± 170&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBS</td>
<td>0</td>
<td>11 ± 13</td>
<td>65 ± 53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MCV (Fl)</td>
<td>86 ± 4</td>
<td>85 ± 3</td>
<td>87 ± 4</td>
<td>0.573</td>
</tr>
<tr>
<td>GGT (IU/l)</td>
<td>14 ± 6</td>
<td>12 ± 6</td>
<td>17 ± 9</td>
<td>0.136</td>
</tr>
<tr>
<td>AUDIT Score</td>
<td>0.6 ± 0.89</td>
<td>6 ± 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13 ± 4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AUDIT-C</td>
<td>0.43 ± 0.5</td>
<td>4.2 ± 1.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.98 ± 1.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean ± SD or percent.

<sup>a</sup>Indicates significantly greater than moderate and binge drinking group.

<sup>b</sup>Indicates greater than abstainer group. Mean corpuscular volume (MCV; normal range for values 79–97 Fl) and gamma glutamyl transpeptidase (GGT; normal range 0–60 IU/l) were measured in the second cohort of subjects (N = 69, abstainers = 20, moderate drinkers = 13 and binge drinkers = 36).
Based upon the number of drinks consumed per week we determined grams of ethanol consumed per week and grams of ethanol consumed per kilogram (kg) per week (Table 1). Among all drinkers, we found both whole blood and DBS PEth levels significantly correlated with grams of ethanol consumed per week (whole blood \( r = 0.327, P = 0.003 \); DBS \( r = 0.457, P < 0.0001 \)). Similarly, both whole blood and DBS PEth levels significantly correlated with the maximum number of drinks consumed in the last 30 days (whole blood \( r = 0.637, P < 0.0001 \); DBS \( r = 0.58 \) and \( P \leq 0.0001 \)). There was also a significant correlation between whole blood and DBS PEth levels and the number of times subjects consumed 4–5 drinks in one sitting within the last 30 days (whole blood \( r = 0.718, P < 0.0001 \); DBS \( r = 0.68, P \leq 0.0001 \)).

### Table 2. Detection of PEth in DBS and whole blood samples among moderate and binge drinkers

<table>
<thead>
<tr>
<th></th>
<th>Moderate drinkers ((n = 22))</th>
<th>Binge drinkers ((n = 54))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBS (&gt;8 ng/ml)</td>
<td>11</td>
<td>51</td>
</tr>
<tr>
<td>Whole blood (&gt;20 ng/ml)</td>
<td>10</td>
<td>52</td>
</tr>
<tr>
<td>Incidence (%) of positive samples</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBS</td>
<td>50</td>
<td>94</td>
</tr>
<tr>
<td>Whole blood</td>
<td>45</td>
<td>96</td>
</tr>
<tr>
<td>Range of positive samples</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBS (ng/ml)</td>
<td>13.2–29.4</td>
<td>12.7–313</td>
</tr>
<tr>
<td>Whole blood (ng/ml)</td>
<td>26–89</td>
<td>43–731</td>
</tr>
<tr>
<td>Median of positive samples</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBS (ng/ml)</td>
<td>16</td>
<td>54</td>
</tr>
<tr>
<td>Whole Blood (ng/ml)</td>
<td>42.5</td>
<td>124</td>
</tr>
</tbody>
</table>

Fig. 1. Box plot of DBS (top) and whole blood (bottom) PEth concentrations by drinking category. The boundaries of the box are the 25th and 75th percentile, the hatched line is the mean, and the thin solid line is the median. Error bars are the 90th and 10th percentile, and solid circles are outlying points.

Fig. 2. Scatter plot of correlation between whole blood PEth levels and total AUDIT scores among all moderate and binge drinkers. \( r = \) Spearman rank correlation coefficient.

Fig. 3. Scatter plot of correlation between dried blood spot PEth levels and total AUDIT scores among moderate and binge drinkers. \( r = \) Spearman rank correlation coefficient.

Fig. 4. Scatter plot of correlation between whole blood and DBS PEth values. \( r = \) Spearman rank correlation coefficient. One outlying paired subject data point for corresponding PEth whole blood (596 ng/ml) and DBS (313 ng/ml) was eliminated to facilitate presentation of the results.
There was not a significant difference among groups in the percent of subjects reporting a family history of alcohol abuse (abstainers 44%, MOD drinkers 14%, BD 30%, $p = 0.332$). BDs experienced a significant greater number of ‘ever’ blackouts (76%) compared to MODs (52%) and abstainers (4%), whereas there was no difference between abstainers and MODs.

**DISCUSSION**

To the best of our knowledge, this is the first study to examine PEth levels in young adult BDs. The major findings are: (a) PEth levels measured in whole blood were significantly greater in BDs compared to abstainers and MODs and these levels were positively correlated with AUDIT scores and (b) whole blood and DBS values were significantly correlated, suggesting that either one could be used to corroborate self-reported measures of alcohol consumption in young adults.

PEth is an abnormal phospholipid that originates from phosphatidylcholine in the presence of alcohol (Isaksson et al., 2011). In the absence of alcohol but presence of water, phosphatidylcholine is hydrolyzed by phospholipase D to phosphatic acid and choline. Because PEth is formed only in the presence of alcohol, PEth has emerged as a potential direct biomarker of moderate and heavy alcohol consumption. PEth is not a single molecular species but a group of glycerophospholipid homologues with a common nonpolar phosphoethanol head group on which two fatty acid moieties are present with chain lengths of 16–18 carbons (Helander and Zheng, 2009).

The most common molecular PEth species found in human blood after alcohol consumption are the 16:0/18:1 and 18:1/18:1 species. In studies evaluating alcohol consumption, investigators have reported total PEth levels (i.e. several PEth homologues) or one of the commonly occurring homologues (most often the 16:0/18:1 homologue). Analytic methods to measure PEth have evolved; among mass spectrometric methods, HPLC LC/MS/MS has high analytic sensitivity (Nalesso et al., 2011). In this study, PEth was measured using HPLC LC/MS/MS, and both commercial laboratories measured the 16:0/18:1 homologue.

In BDs in our study, whole blood PEth levels ranged from 0 to 731 ng/ml and in DBS from 0 to 313 ng/ml. Other studies have reported among alcohol-dependent subjects undergoing detoxification treatment a high PEth sensitivity and specificity for determining the presence of alcohol consumption (Aradottir et al., 2006; Helander and Zheng, 2009; Wurst et al., 2010). Among those studies, PEth values ranged between 3.4 and 16.3 μmol/l (approximately 2386–11,583 ng/ml; Aradottir et al., 2006; Helander and Zheng, 2009; Wurst et al., 2010). All of those studies used HPLC with evaporative light-scattering detection and reported ‘total’ PEth homologue values (Aradottir et al., 2006; Helander and Zheng, 2009; Wurst et al., 2010). Stewart et al. (2009) reported a median PEth value of 63 ng/ml (HPLC MSMS, homologue 16:0/18:1) in subjects with liver disease ($n = 21$), 16 of whom had the diagnosis of alcoholic liver disease. Faller et al. using HPLC LC/MS/MS reported PEth (16:0/18:1) values from subjects undergoing alcohol detoxification ($N = 40$) that ranged 922–21,000 ng/ml for whole blood (mean 23,375 ng/ml) and 900–213,000 ng/ml for DBS (mean 23,470 ng/ml; Faller et al., 2011). Faller et al. (2011) enrolled subjects undergoing alcohol detoxification; thus, it is possible that subjects also had elevated BALs at the time of PEth measurement, which may generate *in vitro* formation of PEth confounding interpretation of the PEth levels attributable to long-term heavy alcohol consumption (Aradottir et al., 2006). In our study, BALs were negative in all BDs. Others have also excluded individuals with elevated BALs or obtained blood more than 24–48 h after study enrollment (Aradottir et al., 2006; Helander and Zheng, 2009; Wurst et al., 2010). Considering that, along with analytic technique, source of PEth (whole blood vs. DBS), PEth homologues measured, and timing of PEth measurement with respect to last drinking episode, it is difficult to compare PEth values among studies.

Another variable affecting absolute PEth values is the amount and duration of alcohol consumption. Though there is no established PEth cutoff value for distinguishing social or moderate drinking from at-risk drinking, there is evidence of a dose relationship. Others have shown that a single dose of alcohol (46 g in males and 32 g in women, $N = 5$) was not associated with an increase in whole blood total PEth (Varga et al., 1998). However, following 21 days of daily alcohol consumption, whole blood PEth levels were increased (1.01–2.12 μmol/l) in 8 of the 12 subjects (age range 19–31 years) and in particular in those reporting mean daily alcohol intake of 48–102 g (Varga et al., 1998). In those consuming the lower limit of 30–47 g daily ($n = 4$), PEth values were negative (Varga et al., 1998). Among moderate/social female drinkers ($n = 80$, mean age 26 years), Stewart et al. (2010) reported that median PEth (16:01/18:1) values were 45 ng/ml (range 0–565 ng/ml). In the 14 days preceding the blood sampling, subjects averaged ~1.6 drinks per day; among these subjects, 29 did not have quantifiable PEth levels (Stewart et al., 2010).

Our whole blood median PEth value for MODs was 13 ng/ml. We did not estimate daily alcohol consumption among our MODs. However, based on answers to two AIQ questions (one that reflects how many days per week alcohol is consumed and another that reflects how many drinks are consumed on a typical drinking day), we determined that MODs consumed a median of 45 g of alcohol (mean of 58 g) and BDs a median of 195 g (mean of 231 g) of alcohol per week. Similar to Stewart et al. (2010), we also found that, among all drinkers, whole blood and DBS PEth levels were significantly correlated with grams of ethanol consumed per week and the maximum number of drinks consumed in the last 30 days, suggesting that PEth levels may be reflective of the quantity of alcohol consumed. Interestingly, in our study the correlation between PEth values and quantity of alcohol consumption were stronger when using the longer time frame of 30 days versus 1 week of alcohol consumption.

We also found a significant correlation between PEth levels (whole blood and DBS) and the number of times subjects consumed 4–5 drinks in one sitting within the last 30 days. Gnann et al., in a study simulating a 5-day binge drinking episode, examined the effects of once daily vodka (40%) consumption (i.e. an amount bringing the blood alcohol level to 1 g/kg or 23 μmol/l within 1 h) in adults (mean age 30 years, $n = 11$; Gnann et al., 2012). HPLC LC/MS/MS was used for whole blood PEth (16:0/18:1) analysis, with a 20 ng/ml cutoff value (Gnann et al., 2012). During the first days following the binge sessions, PEth values ranged 74–235 ng/ml. PEth values ranged up to 731 ng/ml in our BD group. In alcohol-dependent people undergoing treatment for alcohol-related problems others have reported PEth values that range from 351 to 4212 ng/ml (Helander and Zheng, 2009). Our findings and those of others in alcohol-dependent individuals may reflect a higher level of alcohol consumption.

Similar to our findings, among patients in an emergency room, Kip et al. (2008) found significant correlations between PEth and AUDIT scores ($r = 0.48$). In patients with AUDIT scores greater than 8 ($n = 22$), Kip et al. (2008) found whole blood PEth levels (determined by HPLC with evaporative light-scattering detection) were 0.33 μmol/l (median values; equivalent to about 231 ng/ml). In the latter study, the mean age of subjects with AUDIT scores >8 was 52 years (Kip et al., 2008). In young adults, AUDIT scores...
>6 have high sensitivity (91%) and specificity (60.0%) for detecting high-risk drinking among young adults (Kokotailo et al., 2004). Mean AUDIT-C scores in our BD group were 13 ± 4, indicating risky or hazardous drinking. The mean AUDIT score for MODs was 6 ± 3, indicating low-risk drinking. Importantly, we found that PEth levels were significantly correlated with total AUDIT and AUDIT-C scores, suggesting that AUDIT scores along with PEth levels could be used to categorize and distinguish moderate and binge drinking in young adults.

DBS represent an easy method of blood collection, does not require refrigeration and/or freezing, and are a low-cost method for PEth analysis. We found an excellent correlation between DBS and whole blood PEth levels. Others recently reported that DBS PEth in combination with other measures (e.g. self-report) increased the accuracy of detecting PEth levels. Also, in specimens obtained from alcohol-dependent patients, Faller et al. (2011) found that differences between whole blood and DBS PEth specimens were distributed evenly across the concentration ranges and normally distributed. Our results along with others (Faller et al., 2011) support the use of DBS for PEth determination.

Increased GGT, MCV and carbohydrate-deficient transferrin (CDT) have been used as indirect markers of long-term alcohol use; however, all three have moderate clinical sensitivity and specificity (Schrock et al., 2014). Others have reported that MCV and GGT have low sensitivity in younger individuals (Conigrave et al., 2003). We did not measure CDT, but we did not find any changes or increases in GGT or MCV in any of the BDs. As others have demonstrated, whole blood PEth had greater specificity for the detection of heavy alcohol consumption, and (unlike the aforementioned markers) is not influenced by age, gender, other ingested substances, or other diseases such as hypertension and kidney and/or liver disease (Stewart et al., 2009; Wurst et al., 2010).

Among the BDs, a large percentage reported experiencing a blackout after drinking, and past-month average number of binge episodes was 7.2 ± 4. Further, the maximum number of drinks consumed on any occasion during the past 30 days averaged 11 drinks, supporting the CDC report indicating that those defined as ‘BDs’ consume more than the 4/5 drinks per binge drinking episode (Centers for Disease Control and Prevention, 2012).

CONCLUSIONS

Our findings support the use of PEth in identifying abstinent young adults and differentiating between moderate drinking and binge drinking young adults. It is recommended that researchers use PEth levels along with the AUDIT and other questionnaires that specifically assess the duration and frequency of binge drinking episodes to distinguish between moderate drinking and binge drinking young adults. Whole blood and DBS PEth values were significantly correlated, suggesting that either one could be used to validate self-reported measures of alcohol consumption in young adults. Our data underscore the importance of elucidating the adverse health effects associated with repeated binge drinking with the goal of raising population awareness about the dangers of repeated binge drinking and formulating health care messages that discourage binge drinking.

AUTHOR CONTRIBUTIONS

M.R.P. and S.A.P. were responsible for study concept and M.R.P., S.A., P., S.T., and L.N. implemented the study and collected data. M.R.P. and S.T. performed statistical analysis. M.R.P. and S.A.P. drafted the manuscript, and all authors provided critical feedback.

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CONFLICT OF INTEREST STATEMENT

None declared.

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