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Serum metabolomic analysis reveals several novel metabolites in association with excessive alcohol use — an exploratory study

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Abstract

Appropriate screening tool for excessive alcohol use (EAU) is clinically important as it may help providers encourage early intervention and prevent adverse outcomes. We hypothesized

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AUTHOR CONTRIBUTIONS

DL, ZY, KC, DZ, MZ, and SL: study concept and design; KC, LH, AO, TZ, JM, PK, NH, and RAR: subject recruitment, specimen processing, sample preparation, and data entering; DL, DZ, MZ, and SL: acquisition of data and performed the analysis; KP, KT, and YJ: a critical review of the manuscript; DL, DZ, MZ, and SL: finalizing the manuscript. All authors have read and approved the manuscript for submission.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version at doi:10.1016/j.trsl.2021.10.008.

that patients with excessive alcohol use will have distinct serum metabolites when compared to healthy controls. Serum metabolic profiling of 22 healthy controls and 147 patients with a history of EAU was performed. We employed seemingly unrelated regression to identify the unique metabolites and found 67 metabolites (out of 556), which were differentially expressed in patients with EAU. Sixteen metabolites belong to the sphingolipid metabolism, 13 belong to phospholipid metabolism, and the remaining 38 were metabolites of 25 different pathways. We also found 93 serum metabolites that were significantly associated with the total quantity of alcohol consumption in the last 30 days. A total of 15 metabolites belong to the sphingolipid metabolism, 11 belong to phospholipid metabolism, and 7 metabolites belong to lysolipid. Using a Venn diagram approach, we found the top 10 metabolites with differentially expressed in EAU and significantly associated with the quantity of alcohol consumption, sphingomyelin (d18:2/18:1), sphingomyelin (d18:2/21:0,d16:2/23:0), guanosine, S-methylmethionine, 10-undecenoate (11:1n1), sphingomyelin (d18:1/20:1, d18:2/20:0), sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0), N-acetylasparagine, sphingomyelin (d18:1/19:0, d19:1/18:0), and 1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1). The diagnostic performance of the top 10 metabolites, using the area under the ROC curve, was significantly higher than that of commonly used markers. We have identified a unique metaboloic signature among patients with EAU. Future studies to validate and determine the kinetics of these markers as a function of alcohol consumption are needed.

INTRODUCTION

Excessive alcohol use (EAU) is a significant risk factor for health problems such as injuries, violence, liver diseases, and cancer.[1–3] According to the National Institute on Alcohol Abuse and Alcoholism (NIAAA), excessive drinking is defined as men who drink more than 4 standard drinks in a day (or more than 14 per week) and women who drink more than 3 drinks in a day (or more than 7 per week).[4] Effective intervention depends on the timely diagnosis of EAU. The conventional laboratory tests commonly used to screen for EAU are the levels of liver enzymes, such as gamma-glutamyl transpeptidase (GGT), the aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratio, the mean corpuscular volume of erythrocytes (MCV), and carbohydrate-deficient transferrin (CDT). However, our studies and others show that these tests are insufficiently sensitive and thus cannot be relied upon for EAU screening and diagnosis.[5,6]

Excessive alcohol use interferes with the absorption, metabolism, transport, and excretion of nutrients leading to an imbalance in serum electrolytes and metabolic changes.[7,8] We previously demonstrated that patients with alcohol-induced organ damage, such as alcohol-associated liver disease, have significant alterations in the level of serum metabolites from a wide range of metabolic pathways.[8] The study of metabolites is an emerging and potentially important area of basic and translational research. As a rapidly growing field, metabolomics focuses on the measurements of metabolite variations caused by pathological changes at a small molecular level.[9] Since metabolites are the final step of cellular processes and are highly sensitive to environmental changes, the study of metabolomics enables us to better understand the phenotypic changes.[9,10]

Identifying the potential unique metabolites to screen for EAU is clinically important as it may help providers encourage early intervention and prevent adverse health outcomes from the use of alcohol. In this study, we hypothesized that patients with excessive alcohol use will have distinct serum metabolites when compared to those without excessive alcohol use. Serum metabolic profiling of 22 healthy controls without a history of excessive alcohol use and 147 patients with a history of EAU was performed. One of the challenges in metabolomic studies is the limitation in analytical methodology to identify the unique metabolites of interest. To address this knowledge gap, we employed seemingly unrelated regression (SUR), a powerful method to identify the unique metabolites that are differentially expressed in patients with EAU.[11]

METHODS

Study cohort.

A total of 147 excessive drinkers (ED) were enrolled from Fairbanks Drug and Alcohol Treatment Center (Indianapolis, IN). All fulfilled the definition of excessive drinking as defined by the National Institutes of Health (NIH)/National Institute on Alcohol Abuse and Alcoholism, >4 standard drinks in a day (or >14 per week) in men and >3 drinks in a day (or >7 per week) in women.[12] To be eligible, all participants were at least 21 years old. Participants were ineligible if they had a history of liver disease, jaundice, or complications from portal hypertension, concurrent infection with viral hepatitis B or C, history of any infections within 4 weeks, or recent surgical procedures within 3 months before the enrollment[5,13] In addition, 22 healthy controls with no known history of excessive drinking were enrolled from the Roudebush Veterans Administration Medical Center (Indianapolis, IN). At enrollment, demographic information, clinical characteristics, and laboratory tests were obtained. Patients completed self–administered questionnaires on marital status, Alcohol Use Disorders Identification Test–Concise (AUDIT–C), and Timeline Follow-back (TLFB), to determine the quantity of alcohol consumed over the past 30 days before enrollment. The TLFB is a calendar method that rates high in validity and reliability for determining daily alcohol intake.[14–17] Blood was collected and centrifuged at 1500 g for 10 minutes at 4°C. The serum was stored at −80°C until analysis. The study design was approved by the institutional review board at Indiana University Purdue University Indianapolis, Fairbanks Alcohol Rehabilitation Center, and Roudebush Veterans Administration Medical Center.

Serum sample preparation and metabolomic analysis.

Serum preparation and analysis were performed at Metabolon (Durham, NC), as previously described.[8] The detailed methods were also provided in Supplementary materials. In brief, samples were prepared using the automated MicroLab STAR system (Hamilton, Reno, NV). For quality control purposes, several recovery standards were added prior to the first step in the extraction process. Small molecules bound to protein or trapped in the precipitated protein matrix were dissociated to remove protein. To recover chemically diverse metabolites, proteins were precipitated with methanol under vigorous shaking, followed by centrifugation. The resulting extract was divided into five fractions: two for analysis by two separate reverse phases (RP)/ultrahigh performance liquid chromatography–

tandem mass spectroscopy (UPLC–MS/MS) methods with positive ion mode electrospray ionization (ESI), one for analysis by RP/UPLC–MS/MS with negative ion mode ESI, one for analysis by hydrophilic interaction liquid chromatography (HILIC)/UPLC–MS/MS with negative ion mode ESI, and one sample reserved for backup. Samples were placed briefly on a TurboVap (Zymark) to remove the organic solvent. Sample extracts were stored overnight in liquid nitrogen before preparation for analysis. All methods used a Waters ACQUITY UPLC and a Thermo Scientific Q–Exactive high resolution/accurate mass spectrometer interfaced with a heated ESI–II source and Orbitrap mass analyzer operated at 35,000 mass resolution. The sample extract was dried and then reconstituted in solvents compatible with each of the four methods. Each reconstitution solvent contained a series of standards at fixed concentrations to ensure injection and chromatographic consistency. One aliquot was analyzed using acidic positive ion conditions and chromatographically optimized for more hydrophilic compounds. In this method, the extract was gradient eluted from a C18 column (Waters UPLC BEH C18–2.1 × 100 mm, 1.7 μm) using water and methanol, containing 0.05% perfluoropentanoic acid and 0.1% formic acid (FA). Another aliquot was also analyzed using acidic positive ion conditions but chromatographically optimized for more hydrophobic compounds. In this method, the extract was gradient eluted from the same aforementioned C18 column using methanol, acetonitrile, water, 0.05% perfluoropentanoic acid, and 0.01% FA and was operated at an overall higher organic content. Another aliquot was analyzed using basic negative ion–optimized conditions using a separate dedicated C18 column. The basic extracts were gradient eluted from the column using methanol and water but with 6.5 mM ammonium bicarbonate at pH 8. The fourth aliquot was analyzed by negative ionization following elution from an HILIC column (Waters UPLC BEH Amide 2.1 × 150 mm, 1.7 μm) using a gradient consisting of water and acetonitrile with 10 mM ammonium formate, pH 10.8. The MS analysis alternated between MS and data–dependent multistage scans using dynamic exclusion. The scan range varied slightly between methods but covered 70–1,000 m/z. Raw data files were archived and extracted. Metabolites were identified by automated comparison of ion features in the experimental samples to a reference library of chemical standard entries that included retention time, molecular weight (m/z), preferred adducts, and in–source fragments as well as associated MS spectra. Metabolites were curated by visual inspection for quality control.

Statistical analysis.

Basic statistical analysis: We presented basic characteristics as mean ± SD for continuous variables and as percentages for categorical variables. Appropriate comparisons, including χ^2-test, Student's t-test, and analysis of variance, were used. $P \leq 0.05$ was considered statistically significant.

Seemingly Unrelated Regressions (SUR): *A* SUR model consists of multiple linear regressions, each built for a response variable that corresponds to the level of a single metabolite in this study. With a total of p variables and m metabolites measured from n individuals, the i-th metabolite's level of the j-th individual can be modeled by the following multiple linear regression, $Y_{ij} = \beta_{i0} + \beta_{i1}X_{1j} + \beta_{i2}X_{2j} + \ldots + \beta_{ik}X_{kj} + \ldots + \beta_{ip}X_{pj} + \epsilon_{ij}$, $i = 1,2; \ldots, m; j = 1,2, \ldots, n; k = 1,2, \ldots p$; where Y_{ij} is the level of the i-th metabolite for the j-th individual, X_{kj} is the k-th covariate for the j-th individual, and ϵ_{ij} is the error term for

the *i*-th metabolite and j-th individual. We assume that there is no correlation across different individuals while allowing for potential correlation of multiple metabolites (modeled via correlated error terms) of the same individual.[18]

In this study, we considered the status of alcohol consumption as a categorical variable based on the NIH/NIAAA criteria (healthy controls vs ED) and then as a continuous variable using the data from timeline follow-back. For the latter, we examined the total number of drinks in the past 30 days before the enrollment and the average number of drinks per drinking day (derived from the total number of drinks divided by the number of drinking days in the past 30 days). A series of statistical models were applied to investigate the biomarkers associated with each type of alcohol consumption outcome. We identified factors that affected all metabolites with the SUR model, single metabolite analysis using multiple linear regression model, and SUR analysis of 73 biological pathways to incorporate correlations between groups of metabolites (Fig 1). We constructed the receiver operating characteristic curve to determine the diagnostic performance of selected metabolites in predicting EAU. The α-level of 0.05 was used as the cutoff for all significance tests. Detailed information on the statistical models, covariates, and interaction terms are described in the method section of Supplementary materials.

RESULTS

Baseline demographics and clinical characteristics of the study cohort.

The detailed demographic and clinical characteristics of the study cohort are shown in Supplementary Table I. There was no difference in sex (men, 68% vs 72%, $P = 0.73$), race (White, 86% vs 84%, $P = 0.81$), marital status (54.5% vs 41.5%, $P = 0.25$), and body mass index (27.7 vs 27.9, $P = 0.80$) between controls and excessive drinkers. Relative to healthy controls, excessive drinkers were older (38.6 vs 29.5 years, $P = 0.0001$) and had higher level of total bilirubin (0.7 vs 0.4 mg/dl, $P = 0.001$), AST (36.2 vs 18.6 U/L, $P = 0.02$), GGT (86.2 vs 25.2 U/L, $P = 0.0001$), %CDT (2.1 vs 1.5%, $P = 0.0001$), and AUDIT-C score (23.6 vs 2.7, $P < 0.01$). Among excessive drinkers, the average total drinks, total drinking days, average drinks per drinking day, and average drinks per day in the last 30 days were 241.1 drinks, 19.1 drinks, 11.8 drinks, and 8.0 drinks, respectively.

Significant differences in the level of serum metabolites between healthy controls and excessive drinkers.

We identified significant factors affecting all metabolites using backward elimination (Supplementary Table II). We first fitted a multiple linear regression (MLR) model including all of these significant factors for each metabolite to determine if the serum level of each metabolite was significantly different in healthy controls when compared to excessive drinkers. Among 67 metabolites (out of 556) whose levels were significantly different between groups after adjusting for multiple testing, 16 metabolites belong to the sphingolipid metabolism pathway, 13 belong to phospholipid metabolism, and the remaining 38 were metabolites involved in 25 different pathways. Detailed information on these metabolites is presented in Table I and Supplementary Table III.

Similarly, we constructed the SUR model including all of the significant factors in Supplementary Table II to identify the differential pathways between healthy controls and excessive drinkers. Using likelihood ratio tests, we identified 36 significant pathways that were uniquely different in excessive drinkers when compared to healthy controls. The top two pathways with significant differences in the levels of serum metabolites were sphingolipid metabolism (48 serum metabolites) and phospholipid metabolism (38 serum metabolites) with an adjusted *P*-value at $< 1 \times 10^{-15.}$ Detailed information regarding these pathways is shown in Supplementary Table IV.

Identification of serum metabolites associated with the total alcohol consumption in the last 30 days.

We next employed the analysis to determine the serum metabolites associated with the total quantity of alcohol consumption in the last 30 days before the enrollment. Using a multiple linear regression analysis including significant factors identified via backward elimination (Supplementary Table V), we found 93 serum metabolites (out of 556) that were significantly associated with the total quantity of alcohol consumption in the last 30 days after adjusting for multiple testing. A total of 15 significant metabolites belong to the sphingolipid metabolism pathway, 11 belong to phospholipid metabolism, and 7 metabolites belong to lysolipid (Table II and Fig 2). The detailed information of each metabolite is shown in Supplementary Table VI with the following top three most significant metabolites, guanosine (adjusted *P*-value 3.44×10^{-6}), sphingomyelin (d18:2/21:0, d16:2/23:0) (adjusted *P*-value 3.44×10^{-6}), and sphingomyelin (d18:2/18:1) (adjusted *P*-value 9.62×10^{-6}). The pathways with metabolites significantly associated with total alcohol consumption in the last 30 days from SUR analysis are shown in Supplementary Table VII.

Identification of serum metabolites associated with the average alcohol consumption in the last 30 days.

We implemented the multiple linear regression analysis (Supplementary Table VIII) to identify serum metabolites that were associated with an average number of drinks per drinking day in the last 30 days. The detailed information of these metabolites is illustrated in Table III, Fig III, and Supplementary Tables IX and X. Among 107 differentially significant metabolites, 21 and 11 metabolites belong to sphingolipid and phospholipid metabolism pathways, respectively (Table III). The top four most significant metabolites were sphingomyelin (d18:2/18:1) (adjusted *P*-value 4.28×10^{-6}), glutamate (adjusted *P*-value 4.66×10^{-6}), sphingomyelin (d18:2/21:0, d16:2/23:0) (adjusted *P*-value 4.66×10^{-6}), and guanosine (adjusted *P*-value 6.68×10^{-6}). Interestingly, these four metabolites were among the top metabolites in association with the total alcohol consumption in the last 30 days (Supplementary Table VI).

Identification of serum metabolites associated with the total quantity of alcohol consumption and an average number of drinks per drinking day in the last 30 days.

To translate our analysis to clinical application, we determined the common pathways and unique serum metabolites among excessive drinkers which were associated with the total quantity of alcohol consumption and an average number of drinks per drinking day in the last 30 days.

The Venn diagram was constructed based on the results in Tables I, II, and III. We found a total of 21 common pathways that were significantly different in excessive drinkers compared to healthy controls and the metabolites in these pathways were associated with the quantity and average number of drinks per drinking day (Fig 4A and Supplementary Table XI). The top two pathways belong to phospholipid (38 metabolites) and sphingolipid (48 metabolites) metabolism respectively. Using a similar approach, the Venn diagram was conducted using the uniquely significant metabolites as shown in Supplementary Tables III, VI, and IX. We found a total of 37 unique metabolites among excessive drinkers which were significantly associated with the quantity and the average number of drinks in the last 30 days (Fig 4B, Supplementary Fig 1, Supplementary Table XI and XII). The top 10 metabolites were sphingomyelin (d18:2/18:1), sphingomyelin (d18:2/21:0, d16:2/23:0), guanosine, S-methylmethionine, 10-undecenoate (11:1n1), sphingomyelin (d18:1/20:1, d18:2/20:0), sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0), N-acetylasparagine, sphingomyelin (d18:1/19:0, d19:1/18:0), and 1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1).

The performance of newly identified serum metabolites in differentiating excessive drinkers and its association with the quantity and an average number of drinks per drinking day in the last 30 days compared to commonly used laboratory tests to screen for excessive alcohol use.

To determine the diagnostic performance of the top 10 metabolites in discerning EAU, the ROC curve was constructed by fitting a logistic regression model using a binary response (ie, controls and EAU) (Supplementary Fig 2). We also compared the area under the ROC curve of the top 10 metabolites with that of commonly used markers (AST, ALT, AST:ALT ratio, MCV, GGT, and %CDT), and found the better diagnostic performance of these newly identified metabolites in differentiating excessive drinkers (Fig 5).

DISCUSSION

Excessive alcohol use is one of the major risk factors for mortality worldwide.[19] Numerous studies have shown the adverse effects of excessive alcohol use such as alcohol-associated liver disease, pancreatitis, infection, and cancer.[3,20–23] Screening for excessive alcohol use is important as it may lead to appropriate counseling and prompt intervention. The under detection of excessive alcohol use in the routine care of patients has been found with the use of a self-report questionnaire as a tool to estimate the quantity of alcohol consumption.[24] The commonly used identifiers for EAU such as AST, ALT, GGT, and MCV have limited use as especially in the setting of patients with underlying liver disease.[5] Levels of breath and blood alcohol concentrations or levels of the highly-specific direct markers ethyl glucuronide (EtG) and ethyl sulphate (EtS) in blood and/or urine have been used, but they are only specific to very recent alcohol ingestion; their use may not be useful in routine appointment during clinic visit.[25] Phosphatidylethanol (PEth) accumulates after repeated drinking and its level may be useful to detect recent alcohol use.[26,27] The management of EAU patients during clinical care can be significantly impacted by the identification of unique serum metabolites with a short window of assessment to detect alcohol consumption, better association with the quantity of recent alcohol consumption, and with better diagnostic performance.

Excessive alcohol use interferes with the absorption, metabolism, transport, and excretion of nutrients. We previously reported significant alterations in serum fatty acids and proteins in alcohol-fed mice and patients with excessive alcohol use.[28,29] The present study was conducted to comprehensively analyze the changes in the levels of metabolites in patients with excessive alcohol use using a metabolomic approach. We hypothesized that excessive alcohol drinking will lead to alterations in serum metabolome and that the changes in the metabolomic signature may be useful to screen for excessive alcohol use.

As in many discovery studies using the omic approaches, the classic statistical analyses, such as t-test and analysis of variance, are limited in value as they are primarily univariate models to determine each metabolite between/among groups.[30] Traditional linear regression methods are infeasible as the nature of the metabolomic data is a large number of metabolites over a limited number of samples.[31] Multivariate regression methods, especially principal component analysis (PCA) and partial least squares (PLS) regression, are widely applied in metabolomic studies, however, low statistical power is often an issue in multivariate techniques when dealing with a large number of metabolites and confounding variables.[31] To overcome these shortcomings, we employed the SUR model to resolve the challenges of confounding factors and the large metabolic dataset. We focused our analysis to identify the unique serum metabolites to distinguish excessive alcohol users from those of controls and those with a strong association with the quantity and an average number of drinks per drinking day in the last 30 days before the enrollment. Using the SUR method with and without likelihood ratio tests, we can identify unique metabolites primarily in the sphingolipid and phospholipid pathway with significant alterations in excessive alcohol users. Interestingly, the metabolites in these two pathways were also associated with the quantity of alcohol consumed and the average number of drinks per drinking day. Using the Venn diagrams, we identified the following metabolites (five in sphingolipid pathway and one each in phospholipid, purine metabolism, methionine metabolism, medium-chain fatty acid, and alanine/aspartate pathways), as novel biomarkers for excessive alcohol use, sphingomyelin (d18:2/18:1), sphingomyelin (d18:2/21:0, d16:2/23:0), guanosine, S-methylmethionine, 10-undecenoate (11:1n1), sphingomyelin (d18:1/20:1, d18:2/20:0), sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0), N-acetylasparagine, sphingomyelin (d18:1/19:0, d19:1/18:0), and 1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1). These metabolites have a better diagnostic performance in discerning those with EAU when compared to commonly used laboratory tests to screen for excessive alcohol use (Fig 5). Our study is not designed to explore the molecular mechanism underlying the changes in these markers upon excessive alcohol use. However, alcohol has been shown to interfere with sphingolipid, phospholipid, and fatty acid metabolism pathway.[28,32–34] Alcohol is known to inhibit methionine adenosyltransferase, a key enzyme in the methionine metabolism pathway.[35,36] Our observation is likely due to the effects of excessive alcohol use on these pathways in different tissues and organs leading to the alterations of these metabolites, as reflected in the serum.

Our study has several strengths. We employed the SUR model to analyze a comprehensive metabolomic dataset in a large cohort of patients with excessive alcohol use. However, we acknowledged a few limitations. First, there is no best way to determine the quantity of alcohol consumption. To overcome this issue, we chose the timeline follow-back, as the tool

to quantify the quantity of alcohol consumption over the last 30 days before enrollment. This questionnaire has been shown to have high validity and reliability for determining daily alcohol intake.[16] Second, we did not explore the molecular mechanism of how alcohol affects the level of metabolites which we identified. Third, this was an exploratory study. We did not have a validation cohort to validate the diagnostic performance of these unique metabolites to screen for excessive alcohol use.

In summary, in this exploratory study, we have identified a unique metabolomic signature among patients with excessive alcohol use. Future studies to validate our findings and to determine the kinetics of these markers as a function of alcohol consumption (ie, duration or rate of decline after alcohol abstinence) are needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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All authors have read the journal's authorship agreement.

Abbreviations:

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUDIT	Alcohol Use Disorders Identification Test
CDT	Carbohydrate deficient transferrin
EAU	Excessive alcohol use
GGT	Gamma glutamyl transpeptidase
MCV	Mean corpuscular volume of erythrocytes
NIAAA	National Institute on Alcohol Abuse and Alcoholism
SUR	Seemingly Unrelated Regressions

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AT A Glance Commentary

Liu D, et al.

Background

Identifying a screening tool to screen for excessive alcohol use (EAU) is clinically important as it may help providers encourage early intervention. The conventional laboratory tests commonly used to screen for EAU are insufficiently sensitive. We hypothesized that patients with excessive alcohol use will have distinct serum metabolites when compared to healthy controls.

Translational Significance

We identified the top 10 metabolites, primarily in the sphingolipid pathway, among patients with EAU. These metabolites have a better diagnostic performance for EAU than that of commonly used laboratory tests. Future studies to validate and determine the kinetics of these metabolites as a function of alcohol consumption are needed.

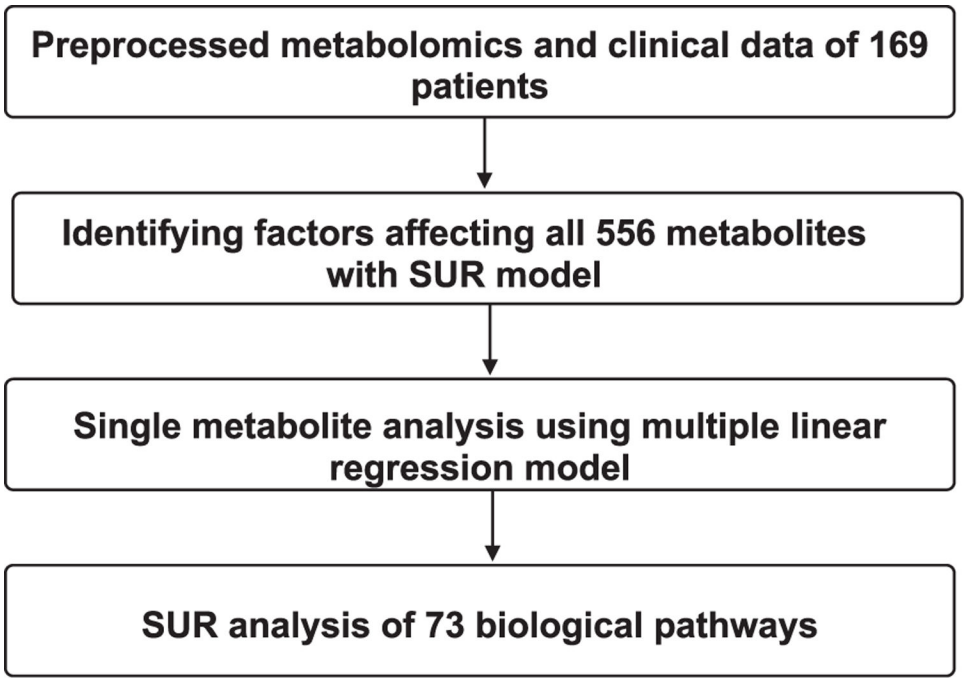

Fig 1.

Flowchart of the data analysis procedures for each of the three variables regarding drinking behaviors, ie, group indicating healthy controls vs excessive drinkers, total alcohol consumption in the last 30 days, and average alcohol consumption in the last 30 d.

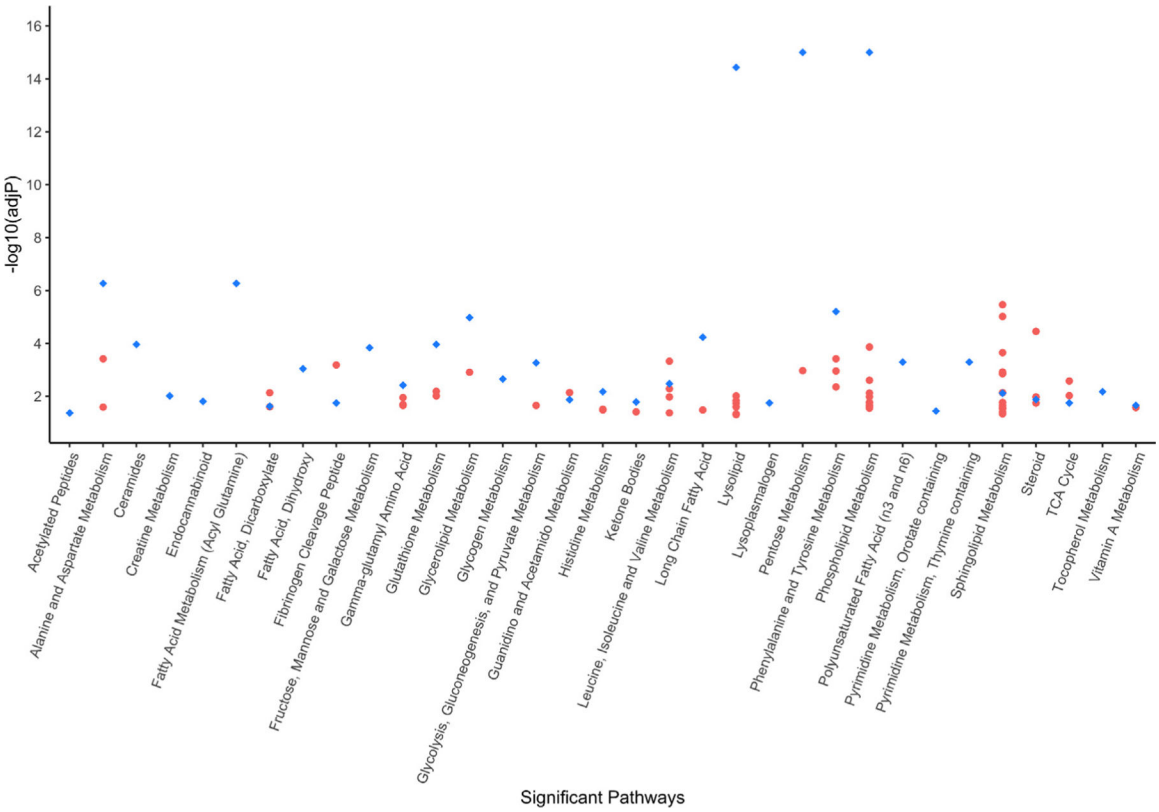

Fig 2.

Significant pathways containing significant metabolites in association with the quantity of alcohol consumption in the past 30 days.

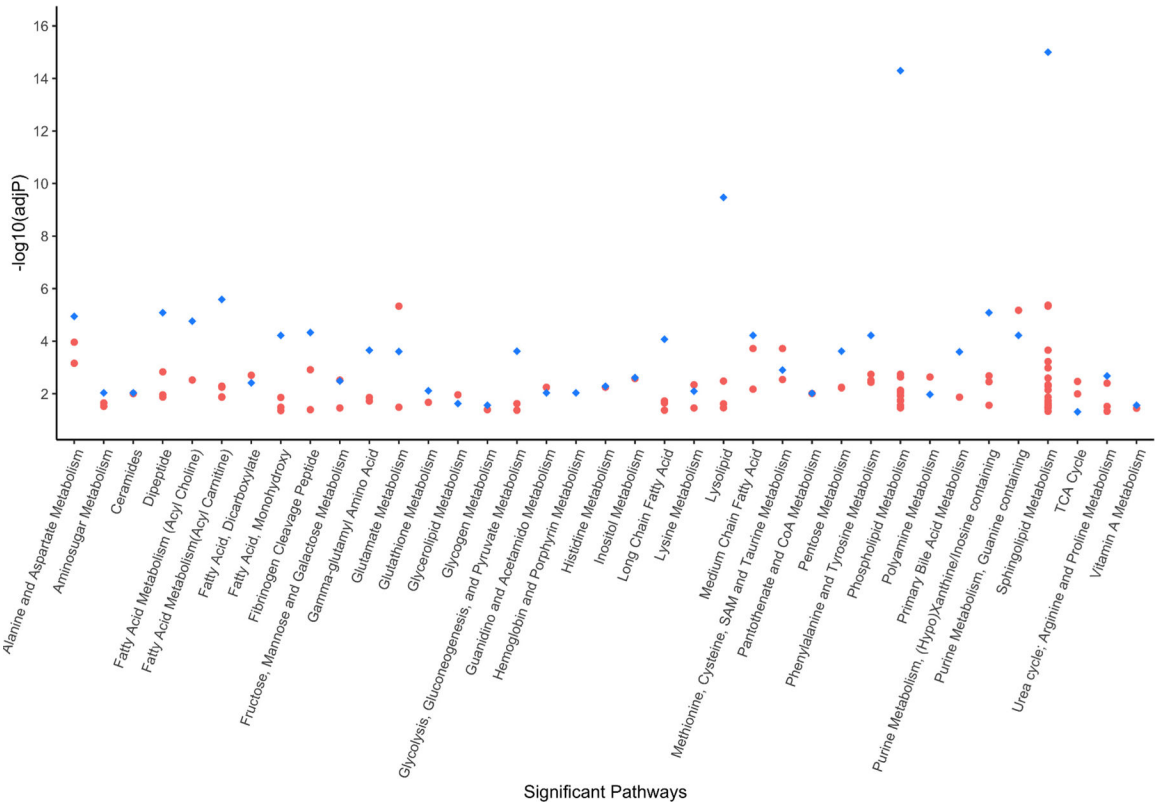

Fig 3.

Significant pathways containing significant metabolites in association with the average number of drinks per drinking day in the past 30 days.

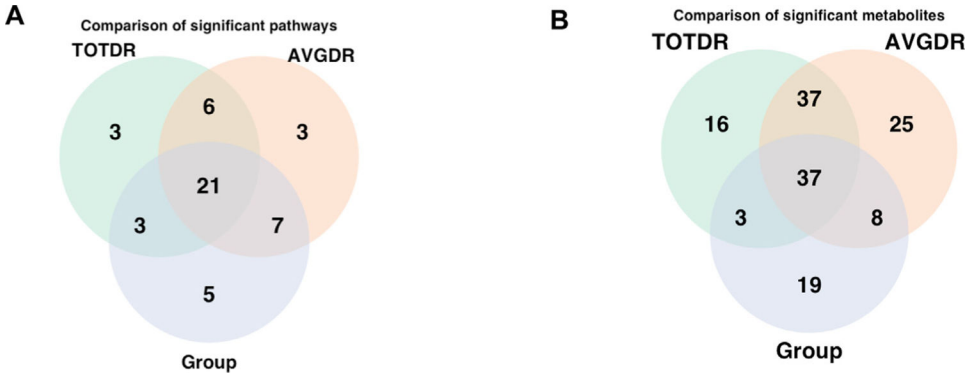

Fig 4.

Summary of the significant metabolites and metabolic pathways for three different alcohol consumption variables. Using binary *group*, the quantity of alcohol consumption and the average number of drinks per drinking day in the past 30 days. Fig 4A summarizes the number of significant metabolic pathways with SUR model. Fig 4B summarizes the number of significant metabolites identified with multiple linear regression model.

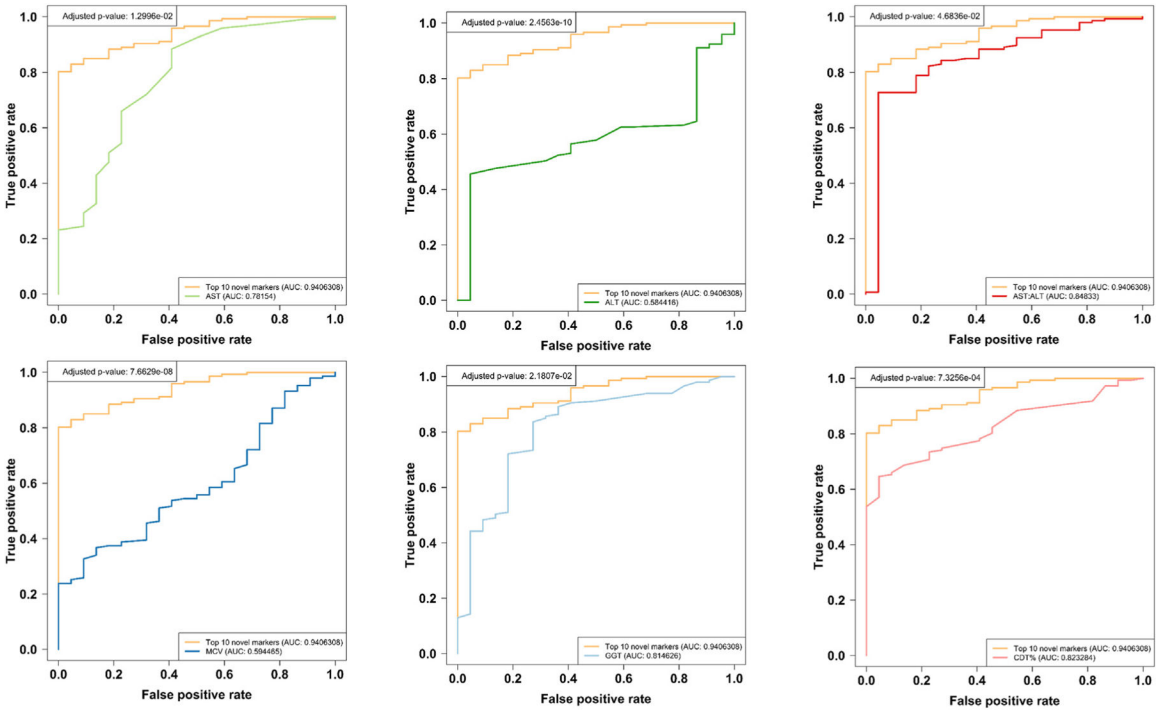

Fig 5.

ROC analysis on the diagnostic performance of the top 10 metabolites compared to commonly used biomarkers in differentiating patients with EAU.

Table I.

Pathways with significantly differential metabolites in healthy controls and excessive drinkers using a multiple linear regression model

Pathways with significant metabolites	Number of significant metabolites in this pathway
Sphingolipid Metabolism	16
Phospholipid Metabolism	13
Fatty Acid Metabolism (Acyl Carnitine)	4
Methionine, Cysteine, SAM and Taurine Metabolism	4
Lysolipid	3
Dipeptide	2
Fatty Acid Metabolism (also BCAA Metabolism)	2
Glutathione Metabolism	2
Monoacylglycerol	2
Pentose Metabolism	2
Alanine and Aspartate Metabolism	1
Ascorbate and Aldarate Metabolism	1
Endocannabinoid	1
Fatty Acid Metabolism (Acyl Choline)	1
Fatty Acid, Amino	1
Fatty Acid, Dicarboxylate	1
Glycerolipid Metabolism	1
Guanidino and Acetamido Metabolism	1
Histidine Metabolism	1
Inositol Metabolism	1
Leucine, Isoleucine and Valine Metabolism	1
Medium Chain Fatty Acid	1
Polyamine Metabolism	1
Primary Bile Acid Metabolism	1
Purine Metabolism, (Hypo)Xanthine/Inosine containing	1
Purine Metabolism, Guanine containing	1
Urea cycle; Arginine and Proline Metabolism	1

Table II.

Pathways with significantly differential metabolites associated with a total quantity of alcohol consumption in the last 30 days using a multiple linear regression model

Pathways with significant metabolites	Number of significant metabolites in this pathway
Sphingolipid Metabolism	15
Phospholipid Metabolism	11
Lysolipid	7
Leucine, Isoleucine and Valine Metabolism	4
Steroid	4
Purine Metabolism, (Hypo)Xanthine/Inosine containing	3
Phenylalanine and Tyro sine Metabolism	3
Gamma-glutamyl Amino Acid	3
Fatty Acid Metabolism (Acyl Carnitine)	3
Fatty Acid Metabolism (Acyl Choline)	2
Dipeptide	2
Glutathione Metabolism	2
Alanine and Aspartate Metabolism	2
Histidine Metabolism	2
Glutamate Metabolism	2
Methionine, Cysteine, SAM and Taurine Metabolism	2
TCA Cycle	2
Fatty Acid, Dicarboxylate	2
Pyrimidine Metabolism, Uracil containing	2
Urea cycle; Arginine and Proline Metabolism	2
Lysine Metabolism	2
Primary Bile Acid Metabolism	1
Long Chain Fatty Acid	1
Purine Metabolism, Guanine containing	1
Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	1
Fibrinogen Cleavage Peptide	1
Inositol Metabolism	1
Glycerolipid Metabolism	1
Pentose Metabolism	1
Guanidino and Acetamido Metabolism	1
Pantothenate and CoA Metabolism	1
Vitamin A Metabolism	1
Ketone Bodies	1
Aminosugar Metabolism	1
Hemoglobin and Porphyrin Metabolism	1
Medium Chain Fatty Acid	1
Polyamine Metabolism	1

Table III.

Pathways with significantly differential metabolites associated with an average number of drinks per drinking day in the last 30 days using a multiple linear regression model

Pathways with significant metabolites	Number of significant metabolites in this pathway
Sphingolipid Metabolism	21
Phospholipid Metabolism	11
Fatty Acid Metabolism (Acyl Carnitine)	6
Fatty Acid, Monohydroxy	4
Lysolipid	3
Purine Metabolism, (Hypo)Xanthine/Inosine containing	3
Dipeptide	3
Phenylalanine and Tyrosine Metabolism	3
Long Chain Fatty Acid	3
Urea cycle; Arginine and Proline Metabolism	3
Alanine and Aspartate Metabolism	2
Fibrinogen Cleavage Peptide	2
Medium Chain Fatty Acid	2
Gamma-glutamyl Amino Acid	2
Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	2
Pentose Metabolism	2
Glutamate Metabolism	2
Methionine, Cysteine, SAM and Taurine Metabolism	2
Fructose, Mannose and Galactose Metabolism	2
Lysine Metabolism	2
Aminosugar Metabolism	2
TCA Cycle	2
Steroid	2
Fatty Acid Metabolism (Acyl Choline)	1
Purine Metabolism, Guanine containing	1
Primary Bile Acid Metabolism	1
Inositol Metabolism	1
Fatty Acid, Dicarboxylate	1
Histidine Metabolism	1
Glutathione Metabolism	1
Guanidino and Acetamido Metabolism	1
Ceramides	1
	1
Pantothenate and CoA Metabolism	
Polyamine Metabolism	1
Glycerolipid Metabolism	1
Glycogen Metabolism	1
Vitamin A Metabolism	1

Pathways with significant metabolites	Number of significant metabolites in this pathway
Leucine, Isoleucine and Valine Metabolism	1
Carnitine Metabolism	1
Dipeptide Derivative	1