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TITLE: Phospholipids as Biomarkers for Excessive Alcohol Use

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14. ABSTRACT

Our proposal is to determine whether a panel of phospholipids, sphingomyelin (SM) and lysophosphatidylcholine (LPC), can be used as the markers for excessive alcohol use (EAU). The *central objective* of this proposal is to determine the diagnostic values of SM and LPC as the potential biomarkers for EAU. The study was conducted to determine the relationship between the serum levels of SM and LPC and the amount of alcohol consumption during the past month. The proposal has both scientific and practical significance in accordance with the research mission of the DOD in the area of drug and alcohol use.

15. SUBJECT TERMS
Excessive alcohol use, lipidomic

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1. INTRODUCTION:

The research proposal responds directly to the Department of Defense – Peer Reviewed Medical Research Program (PRMRP) which calls for scientific research on drug and alcohol abuse. This research area is relevant and designated as one of the FY11 PRMRP topic areas as directed by Congress. Our proposal is to determine whether a panel of phospholipids can be used as the markers for excessive alcohol use (EAU). Drinking becomes excessive when it causes or elevates the risk for alcohol-related problems or complicates the management of other health problems. 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). Negative life stress from combat operations in Afghanistan (Operation Enduring Freedom, OEF) and Iraq (Operation Iraqi Freedom, OIF) is a major contributor to the onset and exacerbation of EAU, a rising epidemic reported to be as high as 40% among returning veterans. The impact of EAU is significant when we observed the rising in the complications related to alcohol (such as alcoholic liver disease, alcoholic hepatitis, and alcoholic pancreatitis) among returning veterans at the younger age (~31 years old), compared to previously reported cases in the non-veteran populations. Efforts have been made to use the routine labs such as serum AST, ALT, and MCV to screen for ongoing alcohol use; however, these tests are not sensitive and their levels can be persistently elevated despite alcohol abstinence. In alcohol treatment setting, frequent monitoring of biomarker levels may provide the clinician a means of early recognition of relapse which, in turn, may suggest the need to intensify or redirect efforts to prevent further drinking. *Because of these clinical implications, a panel of biomarker(s) to detect EAU with improved sensitivity and specificity is needed.* This study to use the lipidomic approach to determine markers for EAU is innovative. The *central objective* of this proposal is to determine the diagnostic values of certain phospholipids as the potential biomarkers for EAU. The study was conducted in a well characterized cohort of subjects with excessive alcohol use to determine the relationship between the serum levels of certain lipids and the quantity of alcohol consumption during the past month.

2. KEYWORDS:

Excessive alcohol use, lipidomics, timeline follow back

3. ACCOMPLISHMENTS:

3.1 **What were the major goals of the project?**

The followings were stated in our statement of work. This is a clinical human research study with the objective to determine the diagnostic values of the certain serum phospholipids, as the potential biomarkers for excessive alcohol use.

Outlined of timeframe and milestones

Task 1. Establish the infrastructure to initiate the study
Task 2. Subject recruitment
Task 3. Data Collection
Task 4. Continuing review and IRB approval
Task 5. Specimen and data analyses
Task 6. Final Analyses and Report Writing

We are able to enroll subjects into the study as we planned. We submitted the quarterly as well as annual reports since the study inception to inform about the progression of the study.

3.2 What was accomplished under these goals?

We have accomplished all the goals as listed in the applications. The support from the DOD results in publications as outlined in the section below.

3.3 What opportunities for training and professional development has the project provided?

Nothing to report

3.4 **How were the results disseminated to communities of interest?**

Nothing to report

3.5 **What do you plan to do during the next reporting period to accomplish the goals?**

Nothing to report

4. IMPACT

4.1 What was the impact on the development of the principal discipline(s) of the project?

Nothing to report

4.2 **What was the impact on other disciplines?**

Nothing to report

4.3 **What was the impact on technology transfer?**

Nothing to report

4.4 **What was the impact on society beyond science and technology?**

Nothing to report

5. **CHANGES/PROBLEMS:**

We did not experience any problems in recruitment or changes in the protocol

6. PRODUCTS:

Nothing to report

7. **Publications, conference papers, and presentations**

Because of the support from the DoD, the following publications are partly supported by this grant (through the PI effort)

1. Tu W, Chu C, Li S, Liangpunsakul S. Development and validation of a composite score for excessive alcohol use screening. J Investig Med 2016;64:1006-1011.

2. Liangpunsakul S, Agarwal R. Altered circadian hemodynamic and renal function in cirrhosis. Nephrol Dial Transplant 2016.

3. Liangpunsakul S, Haber P, McCaughan GW. Alcoholic Liver Disease in Asia, Europe, and North America. Gastroenterology 2016;150:1786-1797.

4. Shen H, Liangpunsakul S. Histamine H2-Receptor Antagonist Use Is Associated With Lower Prevalence of Nonalcoholic Fatty Liver Disease: A Population-based Study From the National Health and Nutrition Examination Survey, 2001-2006. J Clin Gastroenterol 2016.

5. Liangpunsakul S, Crabb DW. Early Detection of Alcoholic Liver Disease: Are We a Step Closer? Gastroenterology 2016;150:29-31.

6. Patel S, Jinjuvadia R, Patel R, Liangpunsakul S. Insulin Resistance is Associated With Significant Liver Fibrosis in Chronic Hepatitis C Patients: A Systemic Review and Meta-Analysis. J Clin Gastroenterol 2016;50:80-84.

7. Gough G, Heathers L, Puckett D, Westerhold C, Ren X, Yu Z, Crabb DW, Liangpunsakul S. The Utility of Commonly Used Laboratory Tests to Screen for Excessive Alcohol Use in Clinical Practice. Alcohol Clin Exp Res 2015.

8. Liangpunsakul S, Lai X, Ross RA, Yu Z, Modlik E, Westerhold C, Heathers L, Paul R, O'Connor S, Crabb DW, Witzmann F. Novel serum biomarkers for detection of excessive alcohol use. Alcohol Clin Exp Res 2015;39:556-565.

9. Jinjuvadia R, Liangpunsakul S. Trends in Alcoholic Hepatitis-related Hospitalizations, Financial Burden, and Mortality in the United States. J Clin Gastroenterol 2015;49:506-511.

10. Zhou P, Werner JH, Lee D, Sheppard AD, Liangpunsakul S, Duffield GE. Dissociation between diurnal cycles in locomotor activity, feeding behavior and hepatic PERIOD2 expression in chronic alcohol-fed mice. Alcohol 2015;49:399-408.

11. Seitz HK, Mueller S, Hellerbrand C, Liangpunsakul S. Effect of chronic alcohol consumption on the development and progression of non-alcoholic fatty liver disease (NAFLD). Hepatobiliary Surg Nutr 2015;4:147-151.

12. Liangpunsakul S. Carbohydrate-responsive element-binding protein, Sirtuin 1, and ethanol metabolism: a complicated network in alcohol-induced hepatic steatosis. Hepatology 2015;62:994-996.

13. Lai X, Liangpunsakul S, Li K, Witzmann FA. Proteomic profiling of human sera for discovery of potential biomarkers to monitor abstinence from alcohol abuse. Electrophoresis 2015;36:556-563.

14. Chayanupatkul M, Liangpunsakul S. Alcoholic hepatitis: a comprehensive review of pathogenesis and treatment. World J Gastroenterol 2014;20:6279-6286.

15. Kang X, Petyaykina K, Tao R, Xiong X, Dong XC, Liangpunsakul S. The inhibitory effect of ethanol on Sestrin3 in the pathogenesis of ethanol-induced liver injury. Am J Physiol Gastrointest Liver Physiol 2014;307:G58-G65.

16. Liangpunsakul S, Bennett R, Westerhold C, Ross RA, Crabb DW, Lai X, Witzmann FA. Increasing serum pre-adipocyte factor-1 (Pref-1) correlates with decreased body fat, increased free fatty acids, and level of recent alcohol consumption in excessive alcohol drinkers. Alcohol 2014;48:795-800.

17. Zhou P, Ross RA, Pywell CM, Liangpunsakul S, Duffield GE. Disturbances in the murine hepatic circadian clock in alcohol-induced hepatic steatosis. Sci Rep 2014;4:3725.

Summary of the overall projects during the funding period

1) Provide supporting data regarding the primary goal of this project (the sphingomyelin and lysophosphatidylcholine as markers)

: The support from the DOD has allowed us to further expand what we previously proposed in the grant application into study the whole metabolomics profiles in human serum with excessive

alcohol use. Further, we also expand our study to determine whether any of the unique changes in metabolites are unique to alcoholic liver disease (one of the most common health consequences from excessive alcohol use). As shown in detail in the subsequent section, we believe that this will be the largest human study to date in this topic. We had identified not only the sub-species of sphingomyelin – we in fact identified several metabolites in bile acids, heme degradation, and glycolysis pathway which were significantly changed in subjects with excessive drinkers and those with alcoholic liver disease when compared to controls (see full report below)

2) Please provide a status or update or data regarding the finger-stick blood spot assay.
: We collected the finger-stick blood spot in all subjects. Unfortunately, the post doc who initially was planned to perform the experiments in this aim had left to pursue future career in China during the 2nd-3rd year of grant funding. Since we expand the scope of our studies to not only study the specific lipids (as outlined below) but also other metabolites; we believe that despite we would not be able to pursue this aim, we have rich dataset for future studies to pursue mechanistically as well as biomarker discovery.

3) A cohesive summary of what you accomplished with this funding over its period of study/performance.
: As mentioned, we believe that we have performed the largest of metabolomics profiling in human subjects with excessive drinkers to date. The in-depth analyses (in addition to what we presented below) are underway (expected to be done in 2-3 months).

4) Update of any products [patents, licenses, papers] from this work.
: We published 17 papers which are partly supported by the DOD grant (as outlined above).

Preliminary results on the metabolomics profiling in subjects with excessive alcohol use and alcoholic liver disease

In addition to the papers which were published; the followings are the results on metabolic profiling as we proposed to do in our DOD grant. We are in the process of preparing the manuscript. The goal of this study is to determine metabolomics profiling utilizing a cross-sectional approach between controls and excessive drinkers. The samples consisted of 22 controls and 147 excessive drinkers. Among excessive drinkers, 57 were those with last drink ≥ 10 days (HD1) before enrollment and 90 were those with last drink < 10 days before enrollment (HD2). We also compared the changes in the metabolic profiling with subjects with alcoholic liver disease (n=33).

Table 1: Summary of the study subjects

Group	Number of subjects
Controls	22
Heavy drinker 1 (HD1)	57
Heavy drinker 2 (HD2)	90
Alcoholic liver disease	33

Metabolite Summary and Significantly Altered Biochemicals

The present dataset comprises a total of 773 compounds of known identity (named biochemicals). Following normalization of the serum data to volume, log transformation and imputation of missing values, if any, with the minimum observed value for each compound, ANOVA contrasts and Welch's two-sample *t*-test were used to identify biochemicals that differed significantly between experimental groups. A summary of the numbers of biochemicals that

achieved statistical significance ($p \leq 0.05$), as well as those approaching significance ($0.05 < p < 0.10$), is shown below. Analysis by one-way ANOVA identified biochemicals exhibiting significant group effects. An estimate of the false discovery rate (q-value) is calculated to take into account the multiple comparisons that normally occur in metabolomic-based studies.

Statistical summary of the changes in serum metabolites among groups

Statistical Comparisons (Serum)			
ANOVA Contrasts	HD1 CTRL	HD2 CTRL	ALD CTRL
Total biochemicals $p \leq 0.05$	142	268	420
Biochemicals (↑↓)	89 | 53	195 | 73	282 | 138
Total biochemicals $0.05 < p < 0.10$	60	60	42
Biochemicals (↑↓)	41 | 19	41 | 19	27 | 15

Statistical Comparisons (Serum)			
ANOVA Contrasts	HD2 HD1	ALD HD1	ALD HD2
Total biochemicals $p \leq 0.05$	97	474	493
Biochemicals (↑↓)	66 | 31	305 | 169	301 | 192
Total biochemicals $0.05 < p < 0.10$	63	37	42
Biochemicals (↑↓)	44 | 19	29 | 8	23 | 19

Principle Component Analysis segregates ALD serums from healthy controls and heavy drinker serums.

Principal component analysis (PCA) is a mathematical procedure that allows differences across a large set of variables to be represented as a smaller set of variables. A description of the mathematical method is provided in the "Statistical Analysis" section of the report. PCA permits visualization of how individual samples, within a group, cluster with respect to their data-compressed "principle components". As such, this analysis tool aids in determining if serum samples can be segregated according to group, based on their global metabolite profiles. PCA (Fig1) was conducted using all serum samples, with two views of the 3D plot of results.

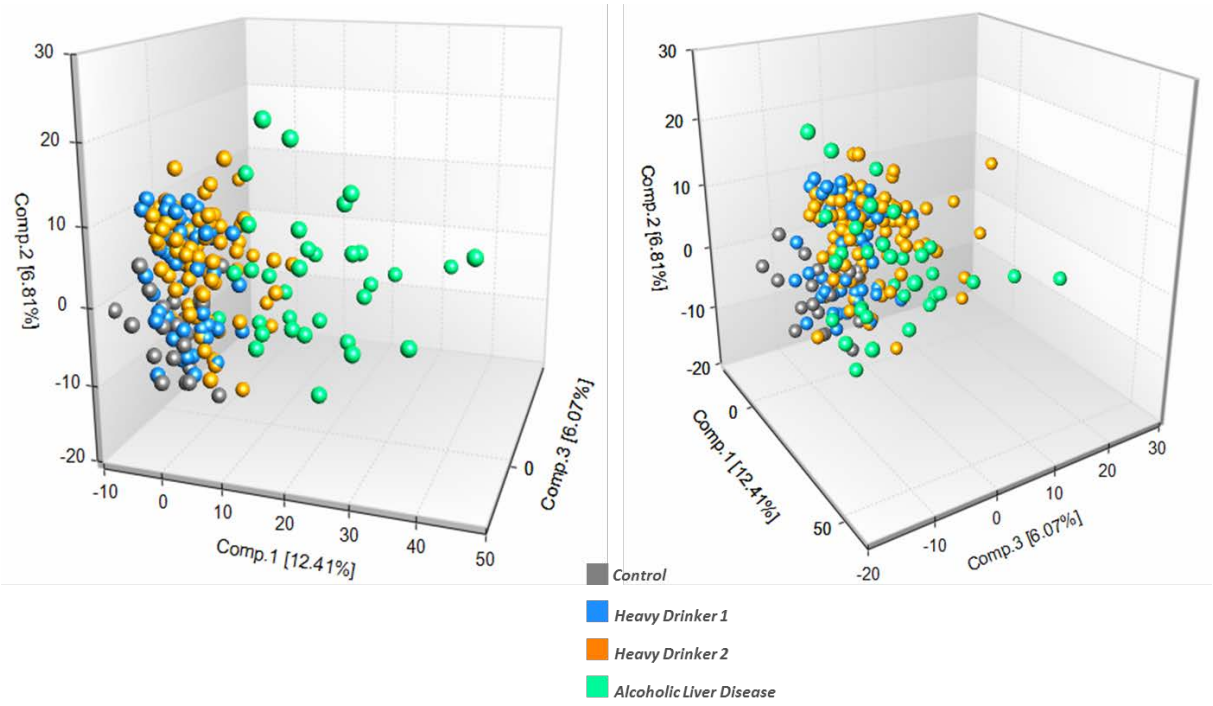

FIG 1: Principal component analyses

PCA differentiated the ALD sera from the controls and heavy drinker sera. There appeared to be some partial segregation of control sera from the heavy drinker-1 (HD1) and heavy drinker-2 (HD2) sera. Differentiation of HD1 from HD2 sera was not evident in the 3D plot. To further explore potential differences in the HD1 and HD2 samples, based on global metabolite profiles, a 2D PCA was conducted, using only the data for the HD1 and HD2 serum samples (**Figure 2**). While not impressive, there appeared to be some skewing of the HD2 samples, lower on the y-axis.

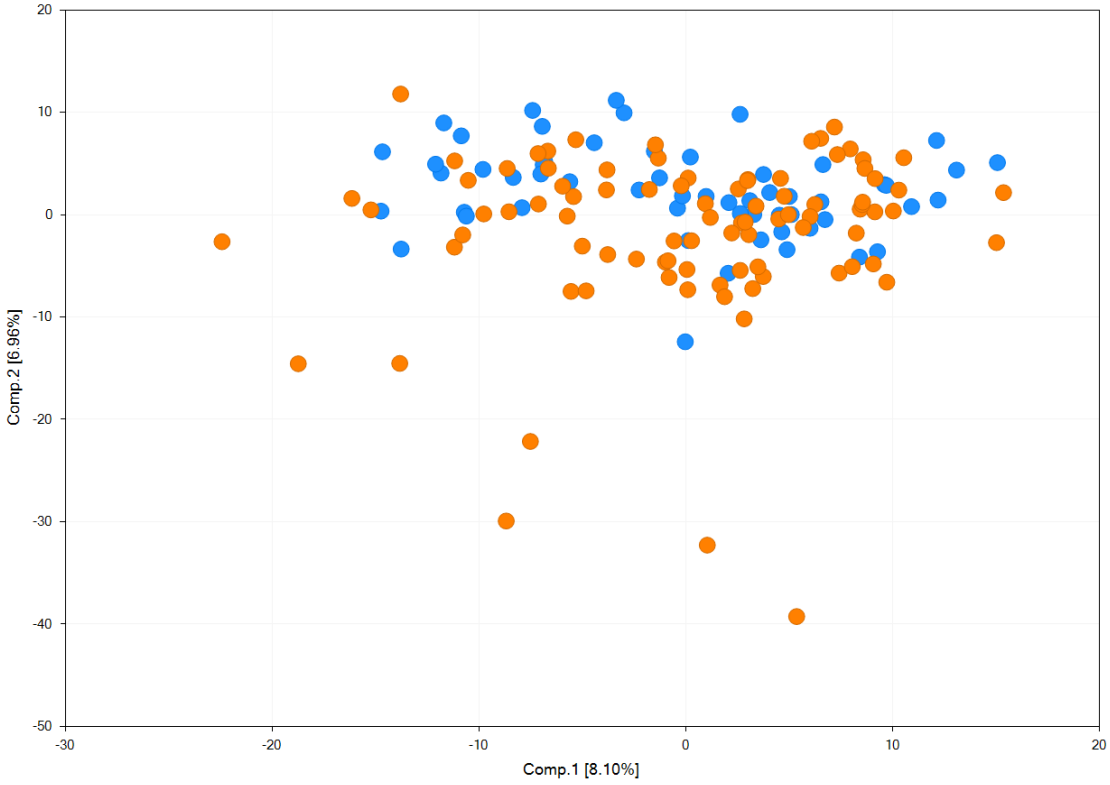

Fig 2: PCA analyses of HD1 and HD2

Hierarchical clustering displays some differentiation between serum groups.

Hierarchical clustering is another statistical tool which can be employed to explore global metabolite profile similarities among samples. A hierarchical clustering heatmap for all serum samples is presented in **Fig 3**. A majority of ALD samples cluster to the right of the plot. Control samples tend to cluster more to the left, in the plot, but are interspersed with HD1 and HD2 samples.

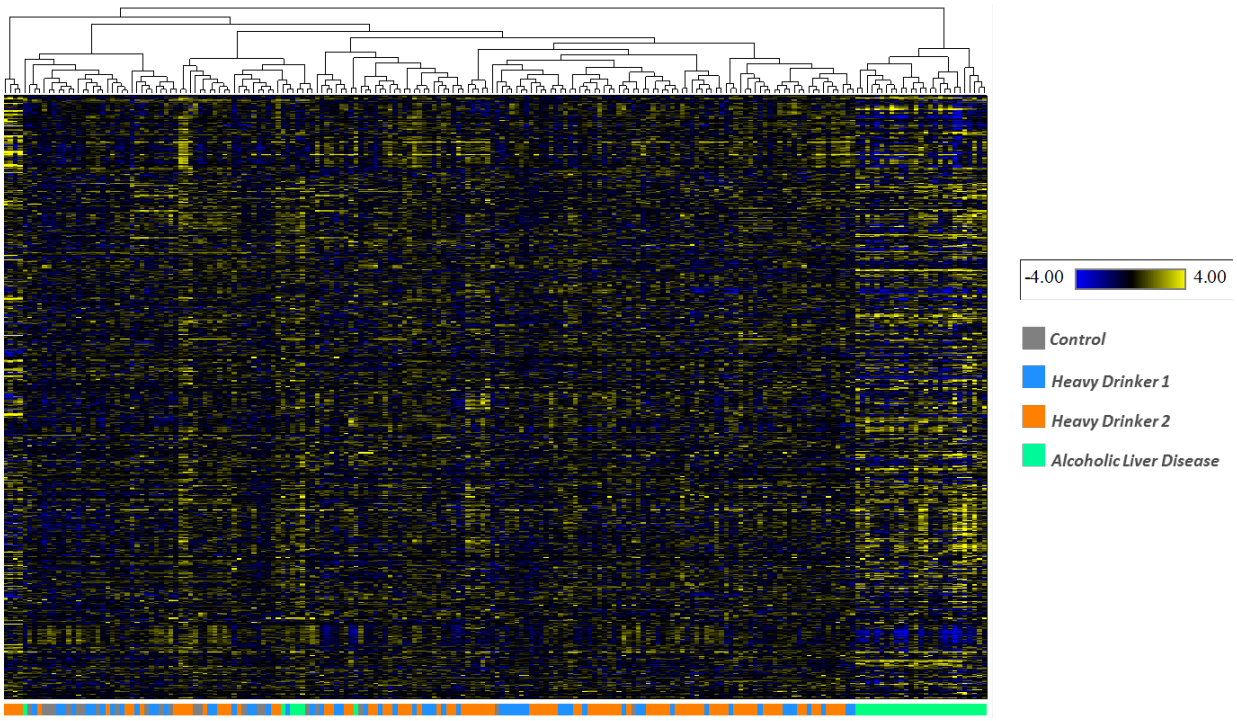

Fig 3: Hierarchical clustering – all serum samples

Random Forest analysis of serum samples.

Random Forest (RF) analysis attempts to bin individual samples into groups based on their metabolite similarities and differences. A description of the mathematical method is provided in the "Statistical Analysis" section of this report. Random Forest also defines which metabolites contribute most strongly to the group binning. RF analysis of serum samples is presented in **Fig 4.** RF analysis of the serum groups resulted in an overall predictive accuracy of 63%, significantly better than the 25% which would occur by chance alone. Control and ALD samples were binned with the greatest accuracy. HD1 and HD2 samples were frequently misseggregated to the alternate HD group or control, which may reflect a gradation of metabolite changes occurring with the development of liver disease. The biochemical importance plot displays the "Top 30" metabolites which most strongly contribute to the binning of individual samples into groups. Metabolites with higher "mean decrease accuracy" values contribute more strongly to group differentiation. The top three metabolites in mean-decrease-accuracy, were 1) 7-methylguanidine, a modified nucleobase, possibly derived from nucleotide degradation; 2) cystine an oxidized dimer of cysteine molecules and 3) 3-ureidopropionate, a pyrimidine nucleotide degradation product. Bile acids, such as glycocholate were disproportionately represented in the top 30.

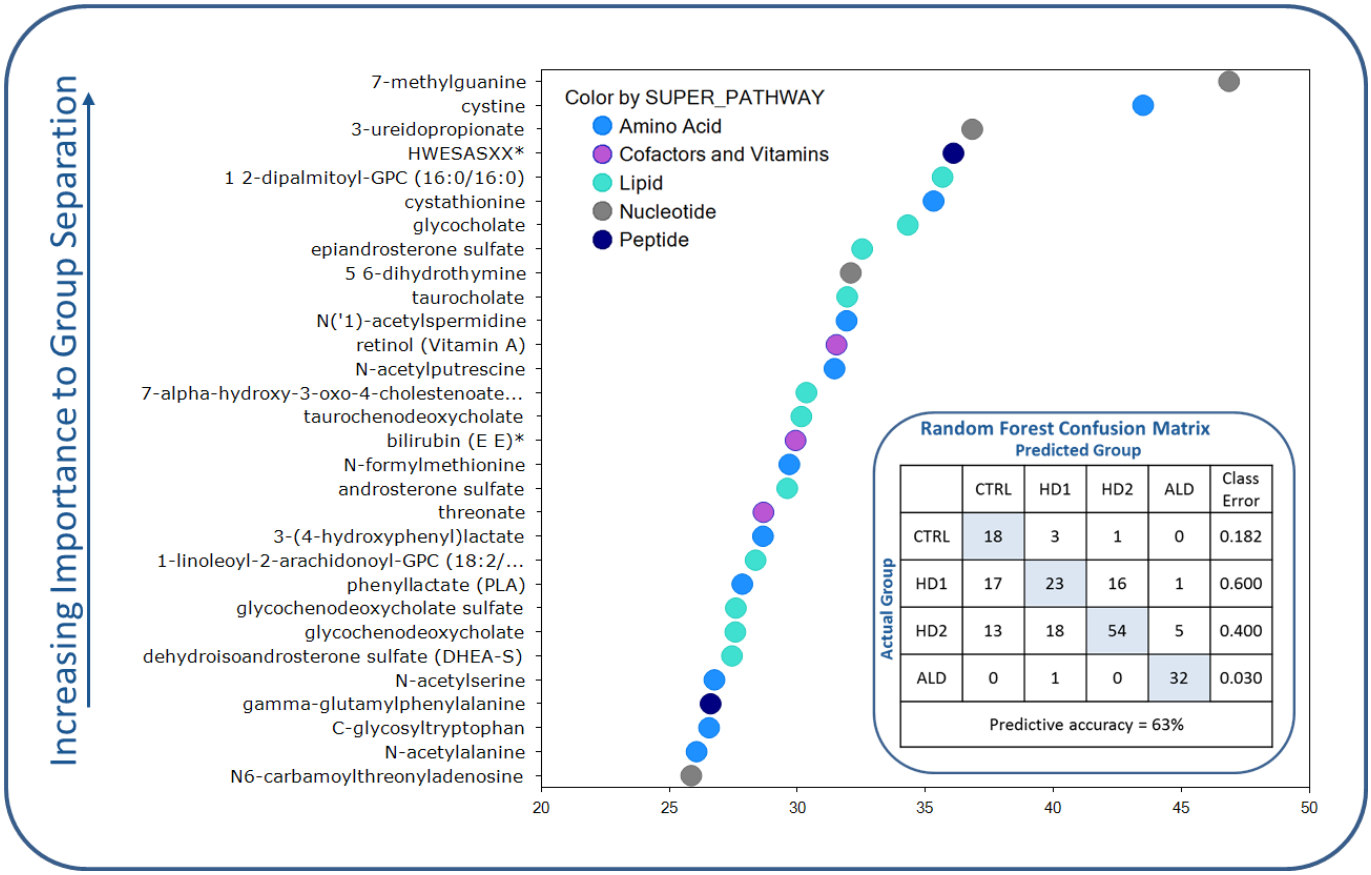

Fig 4: Random Forest classification all serum groups, using named metabolites

Sphingomyelin in ALD and HD serums, relative to control.

We performed the in depth analyses to determine the levels of metabolites in sphingolipid class and the results were shown in Table 2. We found that the following sphingomyelin levels were significantly higher in the serum of HD2 and ALD when compared to controls: myristoyl dihydrosphingomyelin (d18:0/14:0); palmitoyl dihydrosphingomyelin (d18:0/16:0); behenoyl dihydrosphingomyelin (d18:0/22:0).

Table 2: The levels of sphingomyelin in ALD and HD serums

	Fold changes					
	ANOVA Contrasts					
Sphingomyelin	**HD1 CTRL**	**HD2 CTRL**	**ALD CTRL**	**HD2 HD1**	**ALD HD1**	**ALD HD2**
myristoyl dihydrosphingomyelin (d18:0/14:0)*	1.13	**1.31**	**1.56**	**1.15**	**1.37**	**1.19**
palmitoyl dihydrosphingomyelin (d18:0/16:0)*	1.07	**1.19**	**1.57**	**1.11**	**1.46**	**1.32**
behenoyl dihydrosphingomyelin (d18:0/22:0)*	1.13	**1.43**	1.09	**1.27**	0.97	**0.76**
palmitoyl sphingomyelin (d18:1/16:0)	0.97	0.96	**1.09**	0.99	**1.12**	**1.13**
stearoyl sphingomyelin (d18:1/18:0)	**0.82**	**0.84**	**0.65**	1.03	**0.79**	**0.77**

behenoyl sphingomyelin (d18:1/22:0)*	0.99	0.96	**0.66**	0.97	**0.66**	**0.68**
tricosanoyl sphingomyelin (d18:1/23:0)*	**0.86**	**0.79**	**0.60**	0.92	**0.70**	**0.76**
lignoceroyl sphingomyelin (d18:1/24:0)	0.99	1.00	**0.69**	1.01	**0.70**	**0.69**
sphingomyelin (d18:1/14:0, d16:1/16:0)*	1.02	1.01	1.09	0.99	1.07	1.08
sphingomyelin (d18:2/14:0, d18:1/14:1)*	0.93	0.90	**0.77**	0.97	**0.84**	**0.86**
sphingomyelin (d17:1/16:0, d18:1/15:0, d16:1/17:0)*	**0.91**	**0.81**	**0.92**	**0.90**	1.01	1.13
sphingomyelin (d18:2/16:0, d18:1/16:1)*	**0.92**	**0.91**	**0.92**	0.99	1.00	1.01
sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0)	**0.82**	**0.74**	**0.66**	**0.91**	**0.81**	**0.89**
sphingomyelin (d18:1/18:1, d18:2/18:0)	**0.79**	**0.78**	**0.56**	0.98	**0.70**	**0.71**
sphingomyelin (d18:1/20:0, d16:1/22:0)*	0.96	0.91	**0.60**	0.94	**0.63**	**0.66**
sphingomyelin (d18:1/20:1, d18:2/20:0)*	**0.76**	**0.71**	**0.49**	0.94	**0.64**	**0.68**
sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)*	0.91	**0.78**	**0.54**	**0.86**	**0.59**	**0.69**
sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)*	**0.91**	**0.85**	**0.63**	**0.94**	**0.69**	**0.73**
sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1)*	**0.83**	**0.75**	**0.61**	**0.91**	**0.73**	**0.81**
sphingomyelin (d18:1/24:1, d18:2/24:0)*	0.96	1.01	1.02	**1.05**	1.07	1.01
sphingomyelin (d18:2/24:1, d18:1/24:2)*	**0.86**	**0.89**	0.93	1.04	**1.08**	1.04

Bile acids were elevated in ALD and HD serums, relative to control.

One of the strongest metabolite differences observed between ALD and control serums was an elevation in both primary (e.g. **taurochenodeoxycholate**, **taurocholate**) and secondary bile acids (e.g. **deoxycholate**) in the ALD sera **(Table 3)**. Many bile acids were also elevated in the HD1 and HD2 sera, relative to controls, though not all increases achieved statistical significance. As in the prior study, the bile acid synthesis intermediate **7-HOCA**, was sharply higher in the ALD sera, relative to all other groups. Bile acids are produced in the liver and emulsify dietary fats, eliminate cholesterol, and aid in the excretion of hepatic catabolites. Bile acid metabolism is also tied to microbial co-metabolism in the intestine. In the intestine, a significant portion of the bile acids are deconjugated by intestinal bacteria, and some of the deconjugated bile acids are structurally modified into secondary bile acids. A large percentage of the bile acids are actively recycled back to the liver, via the blood stream, through what is termed the enterohepatic recirculation system. Increases in serum bile acids may reflect, increased synthesis (consistent with **7-HOCA** elevation), increased reuptake from the gut, or a decrease in reabsorbtion by the liver, as part of enterohepatic recirculation.

Table 3: Bile acids were elevated in ALD and HD serums

Sub Pathway	Biochemical Name	Fold of Change ANOVA Contrasts HD1 CTRL	HD2 CTRL	ALD CTRL
Primary Bile Acid Metabolism	cholate	0.79	1.81	5.11
	glycocholate	2.16	3.93	21.77
	taurocholate	2.78	5.10	127.67
	chenodeoxycholate	1.66	2.57	7.99
	glycochenodeoxycholate	1.92	2.36	21.61
	taurochenodeoxycholate	2.55	3.29	131.02
	tauro-beta-murichолate	1.48	3.84	37.07
	glycochenodeoxycholate glucuronide	0.92	1.43	2.21
	glycochenodeoxycholate sulfate	1.68	2.47	15.84
	glycocholate sulfate	0.79	1.63	5.88
Secondary Bile Acid Metabolism	deoxycholate	1.03	1.05	0.83
	glycodeoxycholate	2.71	4.67	4.66
	taurodeoxycholate	1.84	3.50	2.94
	glycolithocholate	1.42	2.80	5.02
	glycolithocholate sulfate*	1.57	2.51	3.32
	taurolithocholate	1.02	1.05	3.97
	taurolithocholate 3-sulfate	1.27	1.65	6.07
	ursodeoxycholate	1.91	2.48	6.17
	isoursodeoxycholate	2.77	2.50	0.98
	glycoursodeoxycholate	2.27	2.08	11.28
	tauroursodeoxycholate	2.70	2.47	56.47
	hyocholate	0.78	1.19	3.14
	glycohyocholate	1.71	2.32	20.96
	glycocholenate sulfate*	0.86	0.87	1.67
	taurocholenate sulfate	1.26	1.02	6.08
	glycodeoxycholate sulfate	1.15	2.06	1.64
	ursocholate	1.06	1.38	10.15

Heme degradation metabolites were higher in ALD sera.

Heme degradation metabolites were elevated in ALD sera, relative to controls and the HD groups (**Table 4**). Unconjugated bilirubin, derived from heme degradation, circulates in the bloodstream and is removed from the blood, by the liver. In the liver, bilirubin is conjugated and then secreted in the bile. Serum **bilirubin** levels are known to be elevated with acute alcohol consumption. Elevation of serum **bilirubin** would be consistent with impaired ability of the liver to remove it remove bilirubin from the blood stream. **Biliverdin** and **urobilinogen** have also been found to be elevated in the blood of patients with liver diseases.

Table 4: Heme degradation metabolites were higher in ALD sera

Sub Pathway	Biochemical Name	Fold of Change ANOVA Contrasts HD1 CTRL	HD2 CTRL	ALD CTRL
Hemoglobin and Porphyrin Metabolism	heme	1.66	3.10	0.72
	bilirubin (Z,Z)	1.01	0.83	2.76
	bilirubin (E,E)*	0.59	0.84	4.10
	bilirubin (E,Z or Z,E)*	0.95	0.78	2.31
	biliverdin	0.90	1.21	8.54
	l-urobilinogen	0.92	1.21	51.62

Glycolysis intermediates were altered in HD and ALD sera.

Glucose levels were higher in ALD sera and in HD2 urines, although the urine results were strongly driven by two very high samples (**Fig 5**). Coincident with the higher **glucose** levels in ALD sera, levels of **1,5-anhydroglucitol (1,5-AG)** were lower in ALD sera, relative to the other groups. Declines in 1,5-AG were also noted in the previous study, though glucose was unchanged in that study. **1,5-AG** is a marker of average bloodstream **glucose** exposure, in that it's kidney re-uptake is inhibited by excess **glucose**. The reduced levels of **1,5-AG** in ALD sera would be consistent with the higher **glucose** levels in ALD samples. One of the consequences of heavy alcohol consumption, is a reduction in hepatic NAD^+ levels as a result of increased alcohol dehydrogenase and aldehyde dehydrogenase activity, which generates NADH from NAD^+. An increased NADH to NAD^+ ratio can inhibit fatty acid oxidation, glycolysis and gluconeogenesis. Two enzymatic steps in glycolysis utilize NAD^+ as a co-substrate (see **Slide 18** pathway schematic). Depletion of NAD^+ can inhibit the conversion of pyruvate to acetyl-CoA. NAD^+ can be regenerated from NADH through the conversion of **pyruvate** to **lactate**. **Pyruvate** levels were slightly increased in ALD sera (1.2X; not statistically significant) and **lactate** levels were elevated to a statistically significant levels in ALD and HD2 sera, relative to controls. Increases in **lactate** may be consistent with an alcohol-dependent increase in the NADH/NAD^+ ratio. An elevated NADH/NAD^+ level might also decrease the rate of glycolysis and consumption of **glucose**, as a result of decreased conversion of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate (pathway schematic).

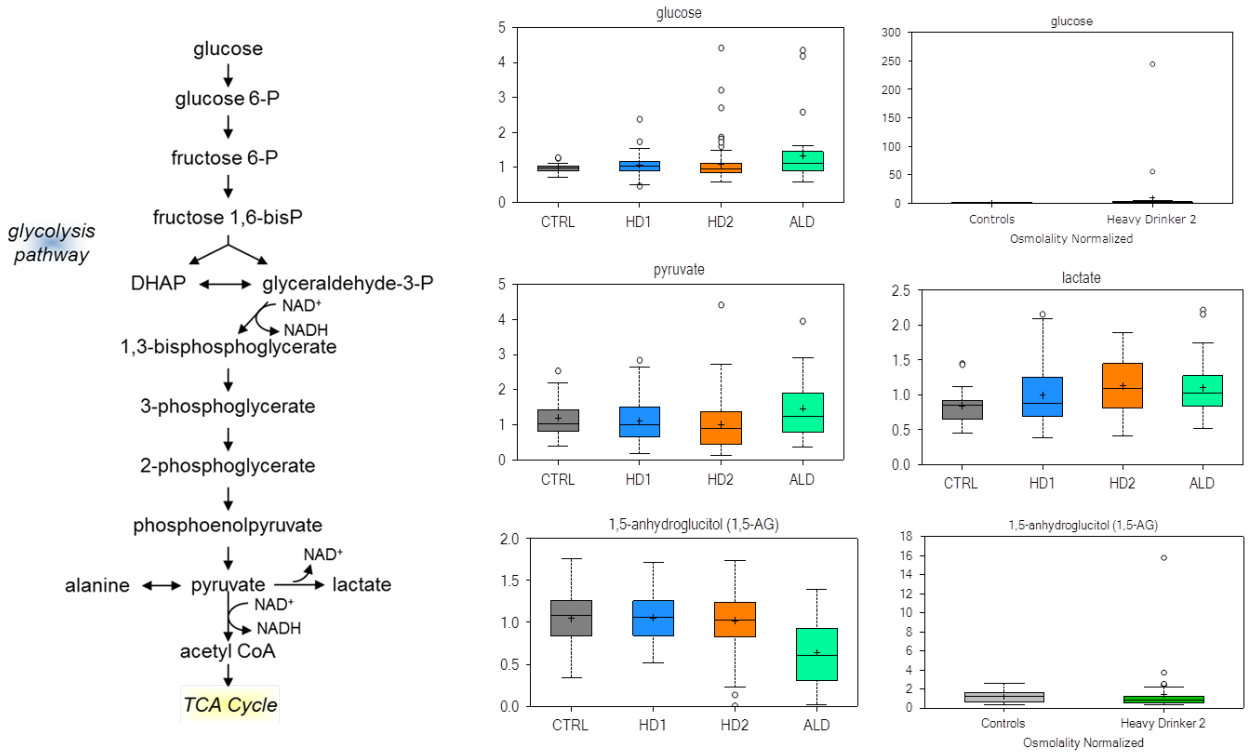

Fig 5: Alteration in glycolysis metabolites in HD and ALD sera

Conclusion:

Certain species of sphingolipid, bile acids, heme degradation products, metabolites in glycolysis pathway were significantly changed in ALD and HD vs. control sera. For some metabolite classes, such as bile acids, there may be evidence for progressively stronger metabolite changes going from HD1 to HD2 to ALD. Ongoing analyses as well as model constrctions are underway to determine the diagnostic performance of these non-invasive markers for HD or as markers predicting disease progression from HD to ALD.

8. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS
What individuals have worked on the project?

Name:	*Suthat Liangpunsakul, MD*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*36*
Contribution to Project:	*Dr. Liangpunsakul is the PI on the project. He oversees the subject's recruitment, preparing documents to the IRB, as well as quarterly and annual report to the DOD. He is also responsible for manuscript writing.*
Funding Support:	

Name:	*Rachel Bennett, BS*
Project Role:	*Research coordinator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*24*
Contribution to Project:	*She was the research coordinator for this project. She was responsible for subject recruitment as well as preparing the paper work for the IRB*
Funding Support:	

Name:	*Laura Heathers, BS*
Project Role:	*Research coordinator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*8*
Contribution to Project:	*She was the research coordinator for this project. She was responsible for subject recruitment as well as preparing the paper work for the IRB*
Funding Support:	

Name:	*Chi Westerhold*
Project Role:	*Research coordinator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*12*
Contribution to Project:	*She was the research coordinator for this project. She was responsible for subject recruitment as well as preparing the paper work for the IRB*
Funding Support:	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

Nothing to report

9. SPECIAL REPORTING REQUIREMENTS: Nothing to report