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Ethanol-induced metabolomic differences in mice using HRAM Q-TOF analysis

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Overview

- Metabolomics has been used to study the impact of ethanol on liver and plasma but few metabolomics studies have reported the effect of acute or chronic exposure on the brain.
- Untargeted metabolomics utilizing HRAM Q-TOF analysis with MS and DIA-MS/MS acquisition at 50 Hz has been applied to compare brain tissue from mouse collected post-mortem after chronic exposure (8 weeks) and acute exposure (11 days) to ethanol.

Introduction

Alcohol consumption continues to be a major risk factor for disease development and is associated with neuropsychiatric disorders, cardiovascular diseases, cirrhosis of the liver, various cancers and fetal alcohol syndrome. Alcohol-specific metabolic responses and the underlying pathophysiology and effects on the brain are poorly understood. This study considered the effect of ethanol-induced metabolite changes in brain tissue using a mouse model.

Materials and Methods

The most widely used model to study alcohol liver injury in mouse is the ad libitum feeding with the Lieber-DeCarli liquid diet containing ethanol for 4-6 weeks which induces mild steatosis and minimal inflammation. Modifying the model to include a chronic-plus-single-binge ethanol feeding mimics acute-on-chronic alcoholic liver injury in patients. HRAM Q-TOF (LCMS-9030 Shimadzu Corporation) untargeted analysis was used to measure the effects of ethanol on metabolite profiles of mouse brain tissue following acute exposure (11 days) and chronic exposure (8 weeks) to ethanol with respective untreated controls. A total of 42 samples were analyzed in random order. A pooled Quality Control (QC) sample was injected several times at the start and systematically throughout the batch in addition to 4 group specific QC pool samples analyzed at the start, middle and end. Group specific QC samples were used for component detection using the Find algorithm within Insight Explore software (Shimadzu Corporation) and the generated compound table was applied to process data from all samples.

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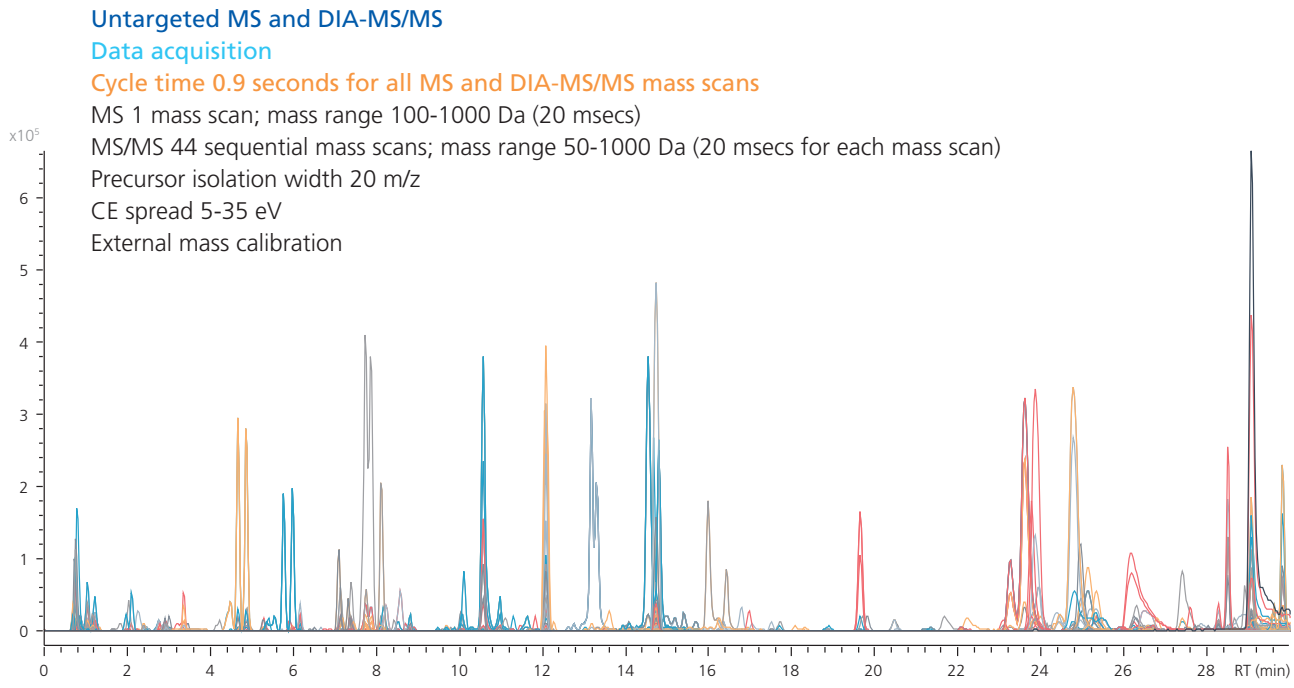

Figure 1. HRAM mass chromatograms of 627 components detected in the QC (5ppm extraction window).

Results

To negate the effects of idiosyncratic ion behavior a pooled QC was used throughout the batch analysis to identify components that resulted in peak area variance <30%. A total of 627 components were considered in the statistical data analysis.

Brain metabolic response to acute ethanol exposure

Acute exposure to ethanol resulted in the down regulation of 17 endogenous metabolites relative to respective untreated controls. In this screening study, components were putatively identified using DIA-MS/MS spectra compared to METLIN.

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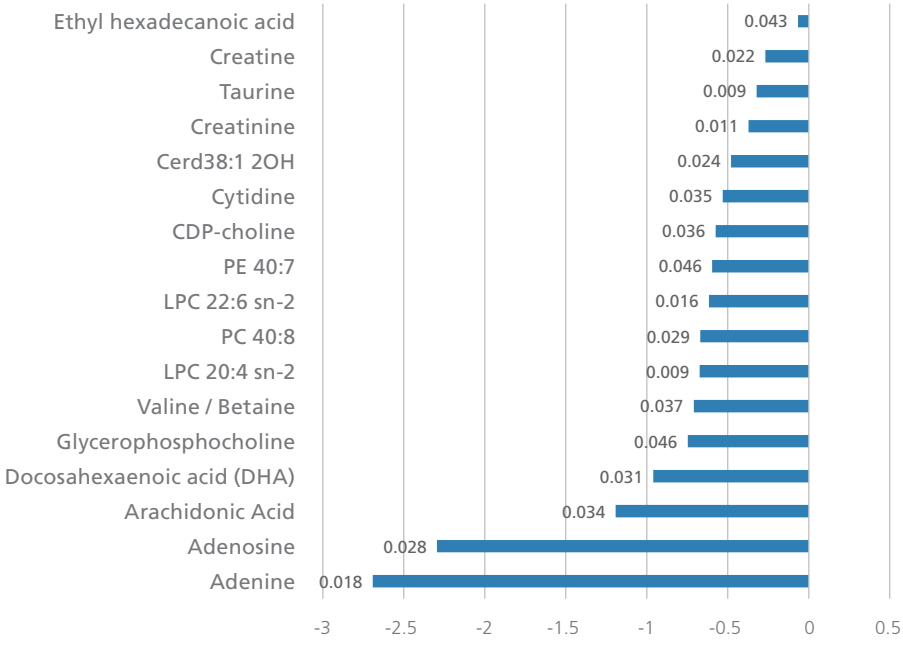

Metabolites that were found to differentiate between groups in response to acute exposure to ethanol

Log 2 fold change

17 metabolites show a reduced response following ethanol administration compared to untreated controls.

The fold change is also annotated with *p*-values from t-test.

Figure 2. Following the acute administration of ethanol to mice a number of putatively identified metabolites are lower compared to control group.

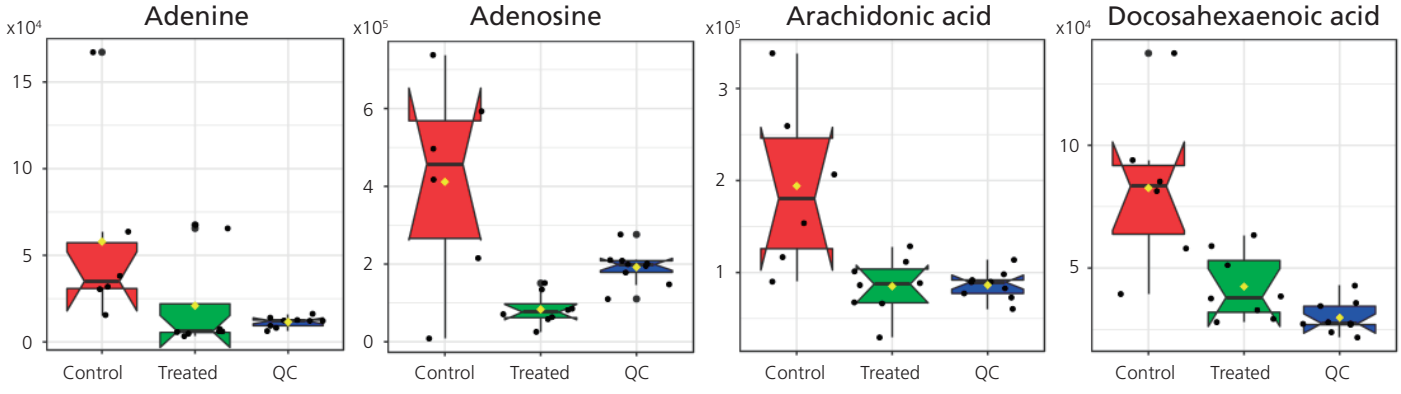

Figure 3. Notch box plots generated using MetaboAnalyst presenting 4 of the metabolites that significantly differ in response (peak area) between the study groups following acute exposure. Notches indicate the 95% confidence interval (CI) around the median (±1.58*IQR/√n). Individual sample responses (peak areas) are marked by the black dots; the yellow symbol shows the mean and inverted edges depict where the 1st / 3rd quartile is out of range of the 95% CI.

Observed trends are consistent with previous literature. Ethanol is reported to enhance adenine degradation, it is also associated with a reduction in xanthine and hypoxanthine (Yamamoto *et. al*. 2005). Thalmic levels of taurine and creatine diminish in ethanol fed mice (Xu *et. al*. 2018). Ethanol targets a conversion from arachidonic acid (ARA) to prostaglandin E_2 in brain, where the main source of ARA is derived by release from *sn-2* LysoPC containing ARA – LPC 20:4 (Luo *et.al*. 2001). Similarly, brain concentration of DHA has been shown to diminish under ethanol exposure as part of a neuroinflammatory and neuropathological response (Collins 2015).

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Brain metabolic response to chronic ethanol exposure

Metabolite profiles differed markedly between the study groups, in chronic ethanol exposure analyte concentrations both increased and decreased (Figure 4).

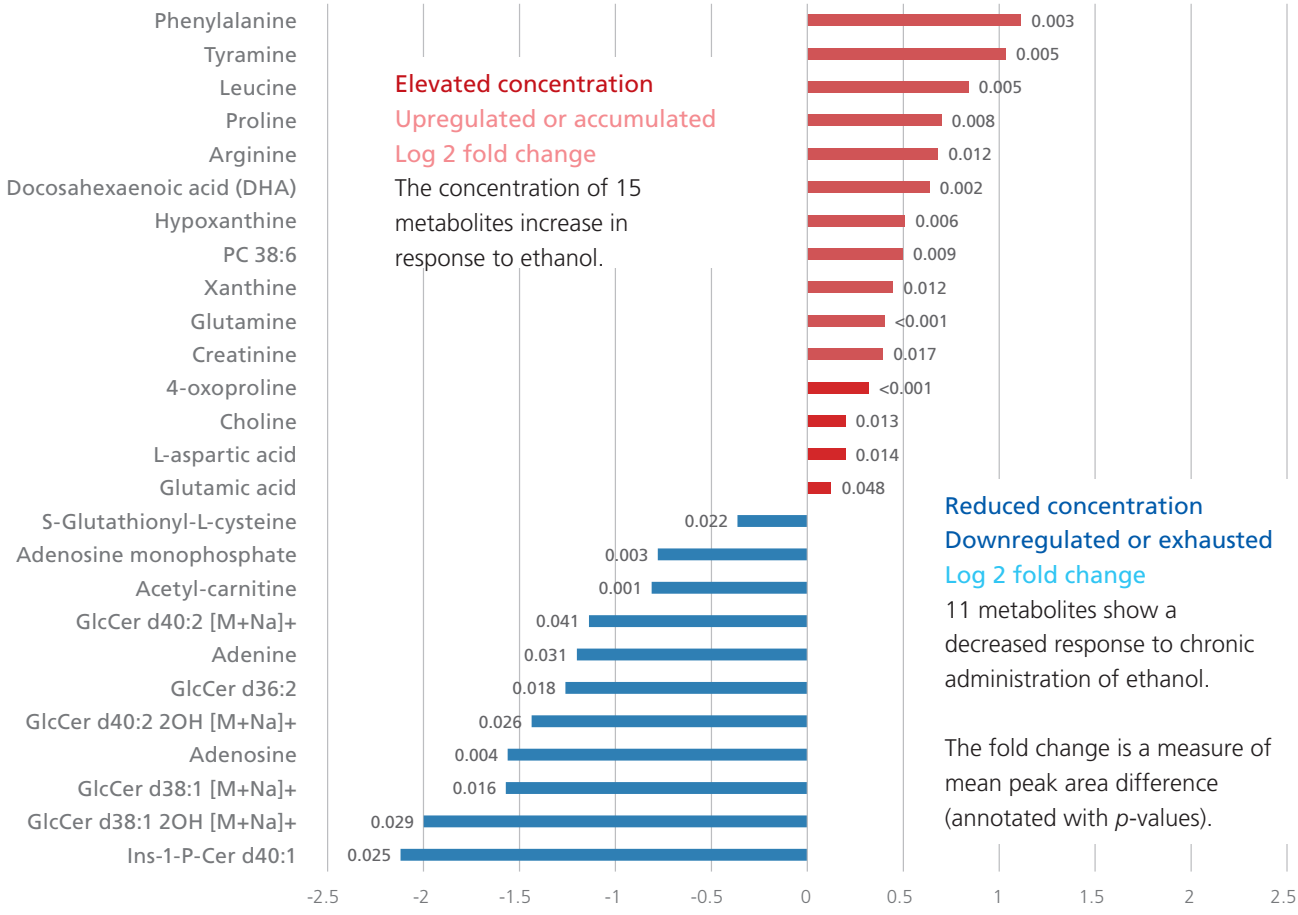

Figure 4. Chronic administration of ethanol to mice resulted in a number of metabolites that differ significantly from the control group.

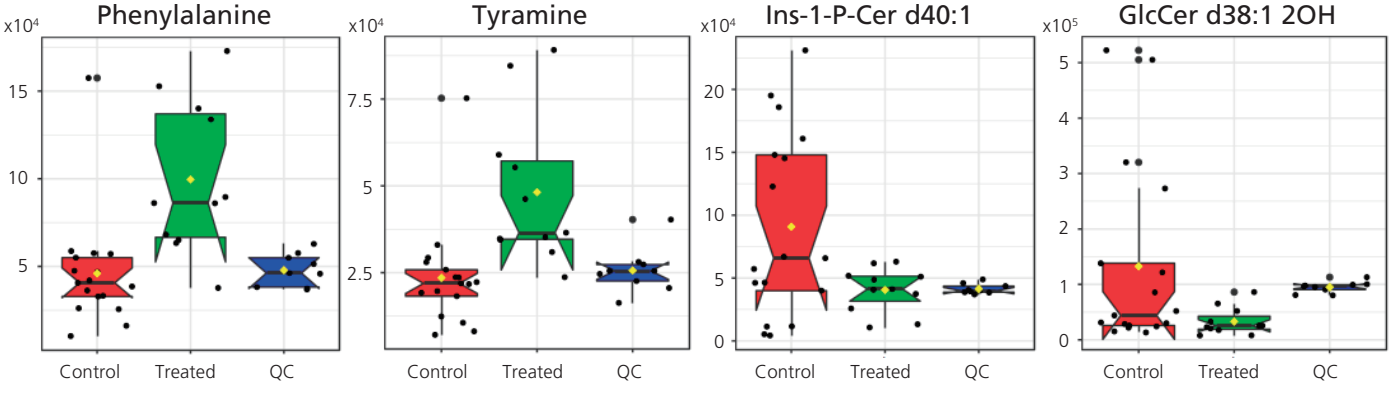

Figure 5. Following chronic exposure to ethanol, the analysis showed increased response (peak area) for phenylalanine and tyramine and decreased response for Ins-1-P-Cer d40:1 and GlcCer d38:1 2OH $[M+Na]^+$. Notch boxplots generated using MetaboAnalyst as described in figure 3.

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Consistent with our data, ethanol induced increases in brain amino acids (Griffiths and Littleton 1977). Diminishment of glucosylceramides has also been reported in neurons following ethanol exposure (Saito et. al. 2006). Though DHA was decreased following acute ethanol exposure, the apparent increase in DHA following chronic ethanol exposure observed is consistent with human studies reporting elevated incorporation in the brains of chronic alcoholics (Umhau et.al. 2013). In that study authors indicate increased avidity of the brain for circulating unesterified DHA; incorporation from plasma into brain phospholipid is augmented, replacing the DHA that has been metabolically lost through alcohol exposure previously described.

Metabolite identification with DIA-MS/MS

In this work, the HRAM LC-MS/MS data was acquired using a MS and DIA-MS/MS method with a cycle time of 0.9 seconds for 45 mass scans (MS/MS mass range 120-1000 Da; each MS/MS mass scan had a precursor isolation width of m/z 20 and a collision energy spread to acquire precursor and product ion data in the same scan). The MS and DIA-MS/MS method showed highly reproducible peak area response and constant mass accuracy (within 5ppm for all QC ion signals throughout the analysis). As one example, MS and DIA-MS/MS was used in the analysis of *sn-1* and *sn-2* forms of Lysophosphatidylcholine 16:0. As the LC-MS/MS method acquired 45 events in less than one second the sampling rate was 14 MS and DIA-MS/MS data points across a chromatographic peak width of approximately 12 seconds resulting in robust peak detection and high quality MS/MS spectra.

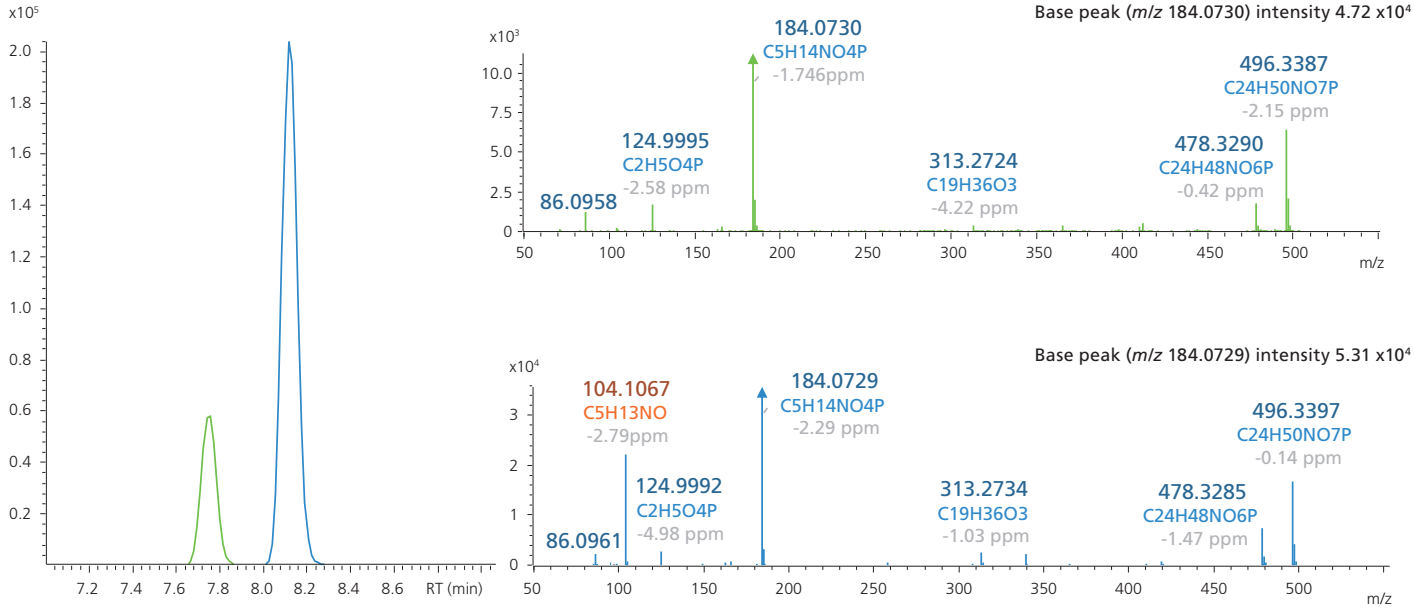

Figure 6. MS chromatogram for *m/z* 496.3398 +/-5ppm corresponding to LPC 16:0 *sn-1* and *sn-2* isoforms. Average MS/MS spectra shown for each peak (FWHM). The fragment at *m/z* 104 is a characterized fragment of the *sn-1* isoform known to exceed a 30 fold difference in intensity relative to the same ion in the *sn-2* isoform (Han and Gross 1996). Neutral formulae for each fragment are shown.

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Conclusions

- An LC-MS/MS method has been developed for the untargeted metabolomics analysis of brain extracts.
- HRAM Q-TOF (LCMS-9030 Shimadzu Corporation) acquired MS and DIA-MS/MS data with a cycle time of 0.9 seconds over the MS/MS mass range of 50-1000 Da.
- The method was applied to study the metabolic effects of ethanol in mouse brain extracts following acute (11 days) or chronic (8 weeks) exposure. Significant differences in metabolite response were identified using t-test and percentage change analysis and MS/MS spectra were used in putative identification.
- Based upon their metabolite profiles, both acute and chronic exposure to ethanol could be differentiated from the untreated control group. Acute ethanol exposure was found to cause significant decreases in 17 identified metabolites while chronic exposure resulted in the coordinated decrease in response to 11 and increase in response to 15 identified metabolites.

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