Ethanol-induced metabolomic differences in the Gut-Liver-Pancreas Axis

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1. Introduction

Excessive alcohol use is associated with neuropsychiatric disorders, cancers, cardiovascular disease, pancreatitis, and alcoholic liver disease. Although alcohol-induced disease is well characterized, the underlying pathology responsible for the development and progression of disease is poorly understood and few studies have considered the impact of ethanol-induced metabolomic changes in the gut-liver-pancreas axis. In this metabolomics study, untargeted high resolution mass spectrometry LC-MS/MS was used to measure changes in metabolite profiles in gut, liver and pancreas tissue samples following chronic exposure to ethanol in mice.

2. Methods

Animal experiment

- C57BL/6 Mice were subjected to chronic exposure to ethanol (8 un-dosed controls and 8 treated over an 8-week duration). Exposure to ethanol was achieved by feeding animals, ad libitum, with the Lieber-DeCarli ethanol diet, containing 5% extra pure ethanol. The study was carried out in accordance with EU and National ethical guidelines and was approved by the Aristotle University of Thessaloniki.

HRMS LC-MS/MS analysis

- Tissues were collected post-mortem and, following tissue lysis and extraction, high resolution mass spectrometry LC-MS/MS (LCMS-9030 Shimadzu Corporation) was used for untargeted metabolite analysis. MS and MS/MS data were acquired using data independent acquisition (DIA) and data dependent acquisition (DDA) methods with a mass range of m/z 100-1000 in MS and m/z 40-1000 in MS/MS.

Data processing

- Metabolic features were extracted from raw HRMS LC-MS data; precursor detection in the TOF MS mass scan used Analyze component detection algorithm (threshold set to low). LabSolutions Insight (Shimadzu Corporation) was used for data processing.

3. Results

3.1 Volcano plot analysis

Volcano plot analysis using data acquired in both positive and negative ionisation mode highlighted metabolite features in the gut, liver and pancreas that differed between control and ethanol treated mice with fold change >2 and p-value <0.05. FDR correction was applied to select the most significant features with log2 fold change >2 for annotation.

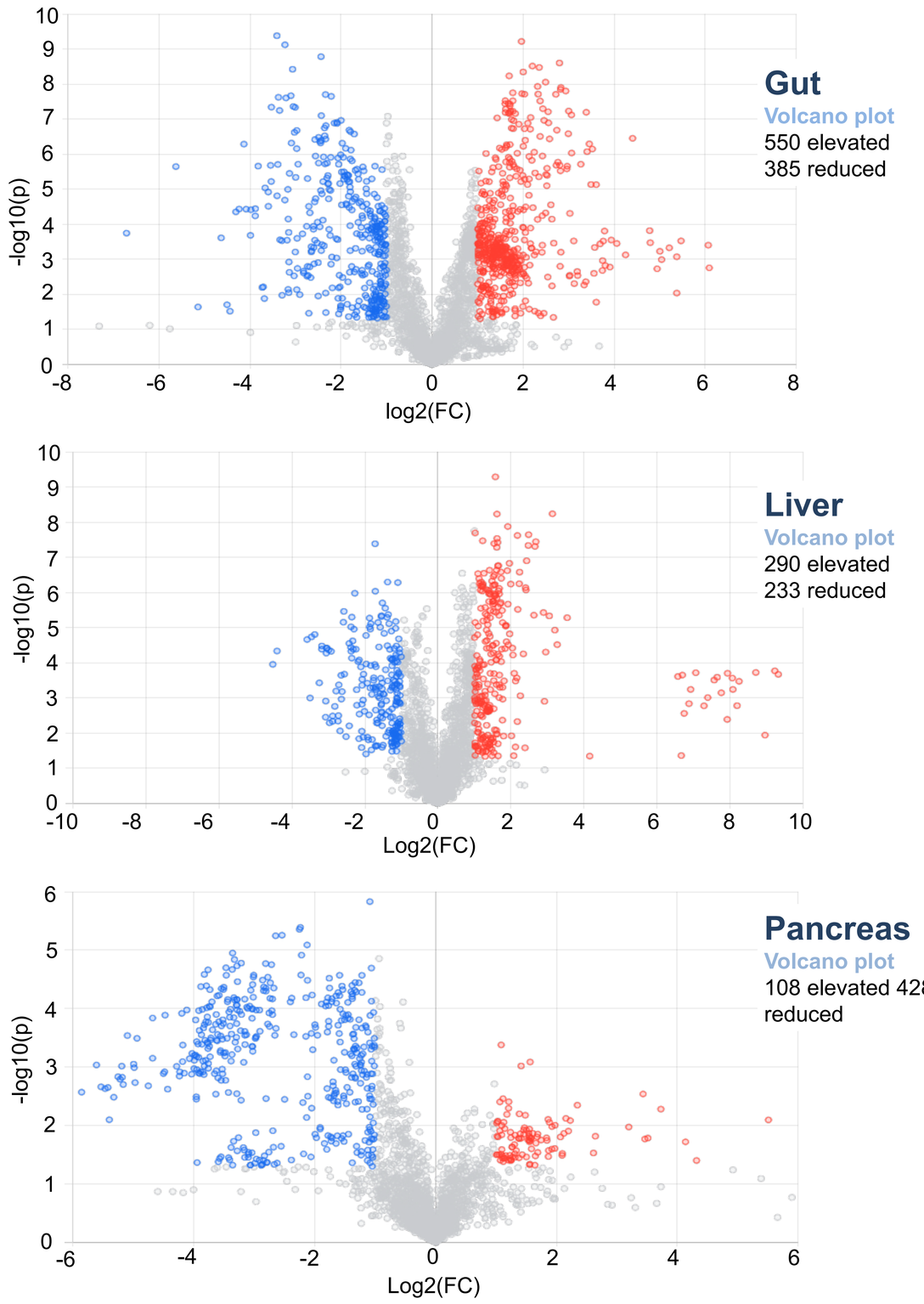

Figure 1. Volcano plots showing features extracted using HRMS LC-MS in positive and negative ion mode that were significantly increased or decreased in each tissue following ethanol administration (fold change >2, p<0.05).

Chronic exposure to ethanol resulted in the following changes:

- **Gut.** A higher number of metabolite features changed compared to the liver or pancreas tissue extracts. Notable increases included phospholipids and oleic acid ethyl ester. Lyso-phospholipids (particularly alkyl and alkenyl linked forms), glycerophosphocholine and glycerophosphoethanolamine were decreased.
- **Liver.** Fatty acid ethyl esters increased significantly with Log2 fold changes >6. Increases in N-acyl-taurines were also observed, likely as a protective mechanism response.
- **Pancreas.** The most significant features were decreases in monoacylglycerols with log2 fold decreases between 2 and 6.

3.2 PCA highlighting the effect of ethanol on each tissue

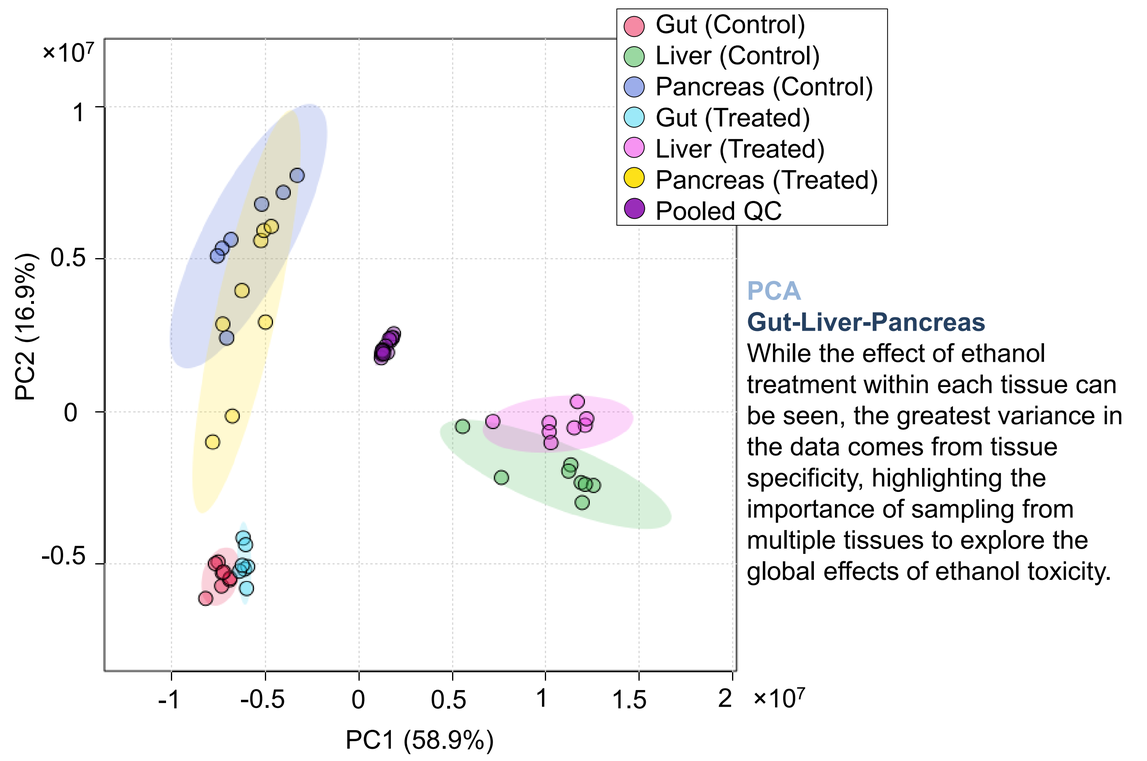

Figure 2. PCA scores plot for 2385 features extracted using HRMS LC-MS in positive ion mode with pooled QC presence >50% and RSD<30%. MetaboAnalyst software was used to generate PCA and volcano plot analysis.

3.3 Metabolites identified at the MSMS level (MSI level 2)

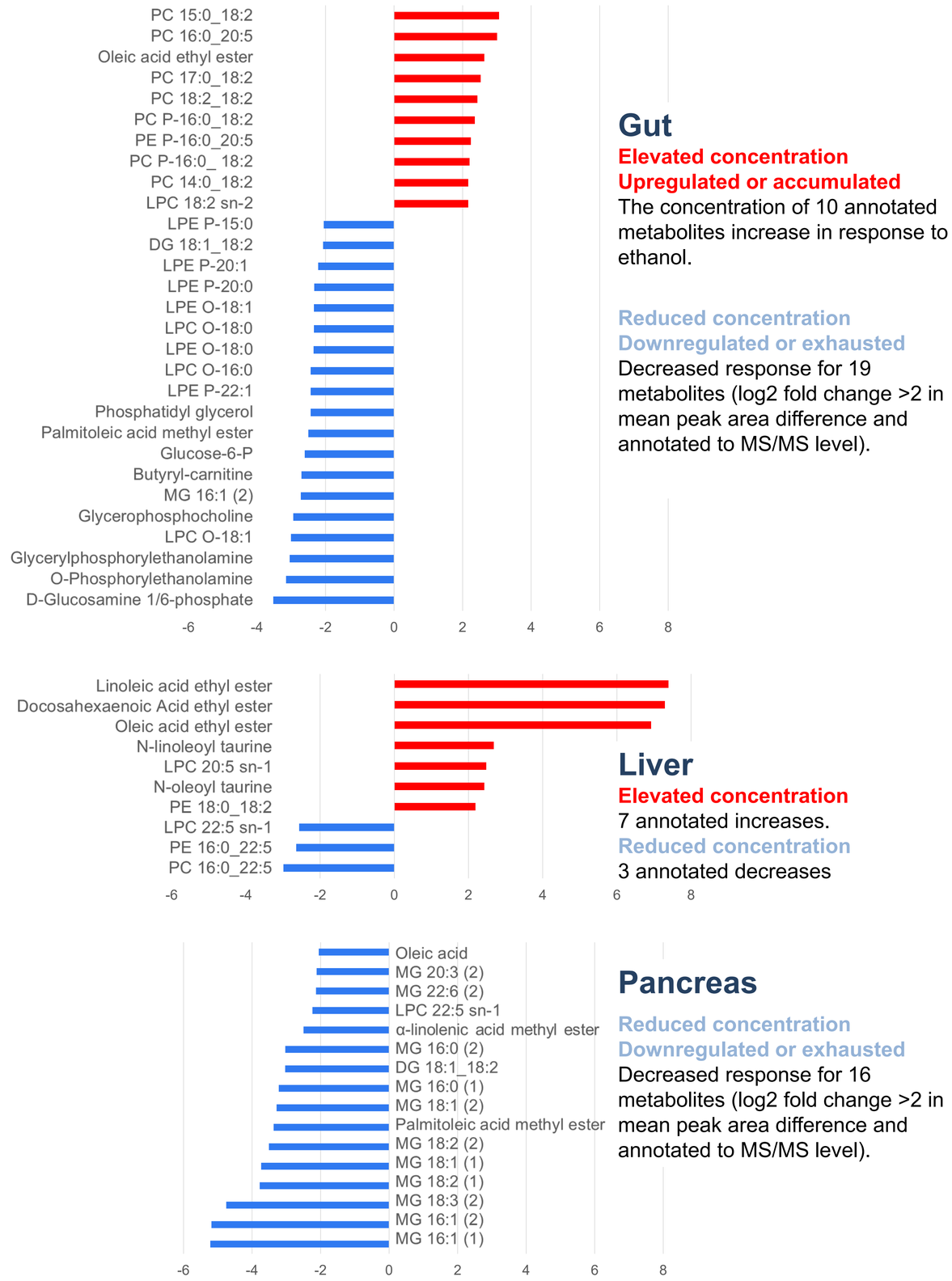

Figure 3. Bar-charts highlighting the most significant changes (log fold change >2) caused by ethanol administration in each tissue that could be annotated at the MSMS level (Metabolomics Standards Initiative level 2).

4. Conclusions

- A HRMS LC-MS/MS method was applied to study the ethanol-induced metabolomic differences in the gut-liver-pancreas axis. Significant changes in metabolite response were identified which were highly specific to each tissue.
- DIA and DDA HRMS LC-MS/MS data offered a rapid and reliable way to discover and annotate metabolite differences in untargeted metabolomics studies.
- The highest number of metabolite features changed in gut.
- The greatest log2 fold changes were observed in fatty acid ethyl esters in the liver.
- All significant changes in the pancreas were downregulated with ethanol treatment.
- Metabolites common to all tissue types (gut-liver-pancreas) included decreases in LPC 22:5 sn-1 and LPE 22:5 sn-1; LPC 20:4 sn-2 decreased in the pancreas and liver and increased in the gut.