# Hepatic Lipid Analysis in an Experimental Model of Chronic Alcohol Exposure using MALDI-TOF and MALDI-FT-ICR Mass Spectrometry

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## Overview

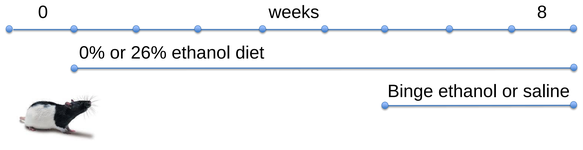
**Aim**:
- Determine the effects of chronic plus binge ethanol exposures on hepatic lipid composition, abundance, and alterations in biochemical lipid profiles using mass spectrometry.

**Methods**:
- Long Evans rats were fed with 26% ethanol containing diet for 8 weeks and binged with 2 g/kg of ethanol 3 times/week during the last two weeks.
- Lipids were extracted from frozen livers by the Folch method.
- The samples were analyzed in the negative ion mode by MALDI-TOF (Ultraflextreme) and FT-ICR (solariX XR) mass spectrometers.

**Results**:
- Both MALDI-TOF and FT-ICR analysis revealed altered intensities of hepatic phospholipids in chronic ethanol exposed livers relative to controls.
- With the high mass accuracy of FT-ICR, we were able to confidently identify various phospholipid species including phosphatidylinositols, phosphatidylserines, and phosphatidylethanolamines.

## Methods

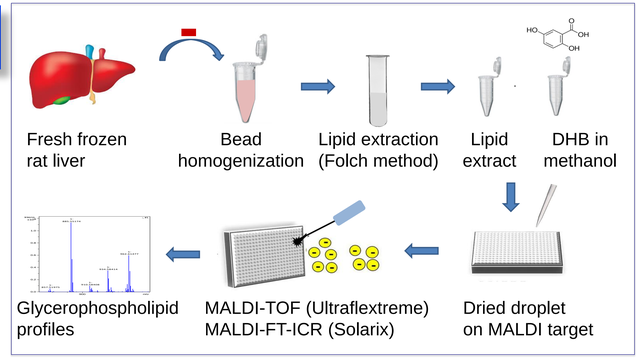
### Experimental Model Overview




Adult male Long Evans rats were pair-fed with isocaloric liquid diets containing 0% or 26% ethanol for 8 weeks and binged with 2 g/kg of ethanol 3 times/week during the last two weeks. After sacrifice, livers were snap frozen on dry ice and stored in -80°C for MS analysis or formalin-fixed for histology.

### Liver Analysis

Lipid extracts were prepared from fresh frozen livers (50 ± 5 mg) by the Folch method after homogenization in sterile deionized water using a TissueLyser (Qiagen, Valencia, CA). The organic phase was evaporated to dryness in a SpeedVac vacuum centrifuge and the pellets were dissolved in 100 µL HPLC grade methanol. 2,5-dihydroxybenzoic acid (DHB, 75 mg/mL) prepared in methanol was used as matrix and mixed 1:1 (v/v) with lipid extract. The samples were analyzed in the negative ion mode by MALDI-TOF (Ultraflextreme) and FT-ICR (solariX XR) mass spectrometers.




## Results

### Liver Histology

- H&E staining of mouse liver sections demonstrate histology common with excessive liver exposure, as shown in **Figure 1**.

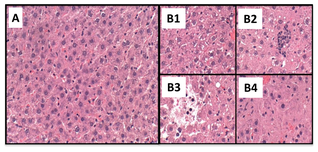



**Figure 1.** Formalin fixed paraffin embedded liver sections were stained with Hematoxylin & Eosin. (A) Control liver with normal chord-like architecture. (B) Ethanol exposed liver with (B1) loss of hepatic chord architecture and microsteatosis, (B2) lobular inflammation and microsteatosis, (B3) focal necrosis, and (B4) apoptosis.

### Lipid Mass Spectrometry

- Mass spectrometry of lipid extracts by FT-ICR produced abundant lipid signal, as shown in **Figure 2A.**
- Analysis of these peaks produced confident assignments, with experimental data matching theoretical isotope distributions as shown in **Figures 2B and 2C**.
- Suggested assignment of the experimental *m/z* are shown in **Table 1**. These assignments were made based only on mass accuracy.
- Similar lipid mass spectra were produced with MALDI-TOF, as shown in **Figure 3**. A zoom of the suggested assignment of *m/z* 885 as PI(38:4) is shown in **Figure 3B**.
- Comparing the TOF and FT-ICR data sets, TOF and FT-ICR measured 116 unique lipids, and TOF measured an additional 20 unique lipids, as shown in **Figure 4**. These 20 extra lipids were found in low abundance.

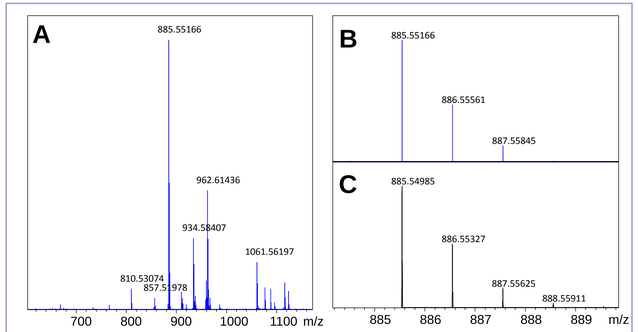



**Figure 2. (A)** Representative MALDI-FT-ICR mass spectrum of lipid extract from control liver from *m/z* 600-1200. **(B)** Expanded FT-ICR spectrum showing the isotope profile near *m/z* 885. **(C)** The theoretical isotope profile for the PI(38:4).

| Lipid ID | Experimental *m/z* | Theoretical *m/z* | Error (ppm) |
|---|---|---|---|
| PS(40:6) | 834.527961 | 834.52931 | 1.2 |
| PS(38:4) | 810.527961 | 810.53074 | 3.7 |
| PI(36:4) | 857.517456 | 857.51978 | 2.3 |
| PI(38:5) | 883.533106 | 883.53570 | 3.4 |
| PI(38:4) | 885.548756 | 885.55166 | 3.4 |
| PS(36:1) | 788.543611 | 788.54732 | 5.0 |

**Table 1.** Exact masses of 6 lipids detected directly from rat liver lipid extracts by MALDI-FT-ICR.

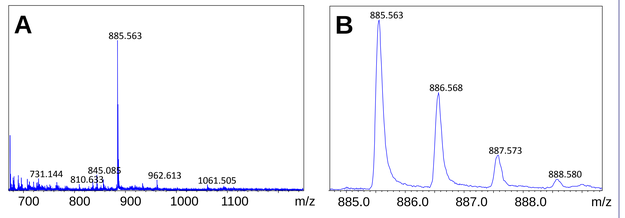



**Figure 3. (A)** Representative MALDI-TOF mass spectrum of lipid extract from control liver from *m/z* 600-1200. **(B)** Expanded spectrum of liver lipid extract in the region near *m/z* 885 Da.

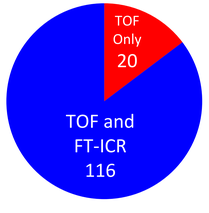



**Figure 4.** Chart of **unique lipids** observed in TOF and FT-ICR, or only TOF data. 116 unique lipids were observed in both TOF and FT-ICR data, while an additional 20 lipids were observed only in TOF data.

### Phospholipid Abundance Analysis

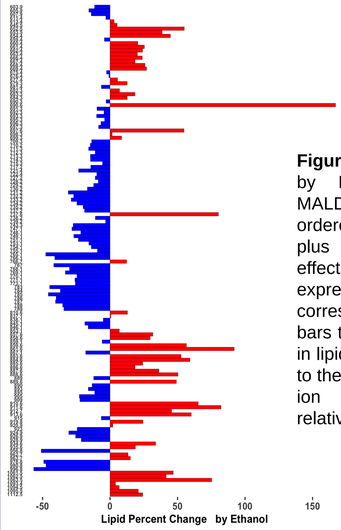



**Figure 5.** Lipid Percent Change by Ethanol Exposure from MALDI TOF data. Data bar plot ordered by *m/z* showing chronic plus binge ethanol exposure effects on hepatic phospholipid expression compared to corresponding controls. Blue bars to the left depict reductions in lipid abundance, and red bars to the right reflect increased lipid ion abundance in ethanol relative to control livers.

- Relative abundances of lipids were compared between the control and ethanol exposure data, as shown in **Figure 5**.
- Many lipids show significant increase or decrease in relative abundance.

## Conclusions

- Chronic plus binge alcohol exposures cause striking alterations in phospholipid profiles in the liver. These biochemical signatures may help refine diagnostic criteria and disease stage.
- MALDI-TOF and MALDI-FT-ICR distinguished alcohol induced alterations in glycerophospholipid expression and profiles of ethanol exposures.
- The high mass resolution and mass accuracy achieved by FT-ICR provided more confident lipid assignment. However, more lipids were tentatively observed with TOF.
- Further work is needed to validate lipid assignments and determine significance of lipid abundance changes.

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