LIPIDOMIC PROFILING OF WILD TYPE AND FATTY ACID SYNTHASE (FASN) KNOCKOUT MICE WITH LC-cIMS-TOF MS AND MS/MS TECHNIQUES

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INTRODUCTION

Fatty acid synthesis in mammalians is catalyzed by a multienzyme complex named fatty acid synthase (FAS) which is encoded by FASN gene.[1,2] It catalyzes acetyl-CoA polymerization into short chain fatty acids (SCFA) that undergo further elongation to long chain FA (LCFA) through a different enzymatic system. Although this mitochondrial fatty acid synthesis pathway was proposed a few decades ago, its contribution to the overall fatty acid production in the body has not been evaluated.

Alternative uses for acetyl-CoA include cholesterol and or steroids synthesis, fatty acid elongation, other anabolic pathways, or it oxidation to CO_2 and H_2O for energy production. Thus, acetyl-CoA availability for these key pathways of structural and bioactive molecules production might depend upon the activity of FAS. However, FAS activity effect on these pathways has not been fully evaluated.

In the present study, we conditionally knocked out FASN in adult mice and performed lipidomics and ^{3}H labeling experiments to determine FASN KO effect on the major pathways for acetyl-CoA utilization.

METHODS

Animals

Homozygous $FASN^{flox/flox}$ mice (a generous gift from Dr. Semenkovich, Washington University) were crossed to UBC-Cre-ERT2 mice (Jackson Laboratory). Eight weeks old mice were treated with tamoxifen (i.p.) once a day for 6 days. Tissues were analyzed 5 days after last TAM injection. Livers and brains from FASN knockout mice (TG), as well as heterozygous (WT/TG) and wild type mice (WT) were extracted using the Folch method and diluted prior to chromatographic analysis.

Chromatography

Mobile Phase A: 60:40 ACN: 10 mM AmFormate 0.1% HFor

Mobile Phase B: 90:10 Isopropanol: ACN 10 mM AmFormate 0.1% HFor

Column: ACQUITY™ CSH C18 1.7 µm d_p 2.1x100 mm

Gradient:

Time	Flow (mL/min)	%A	Curve
0	0.4	50	6
0.5	0.4	47	6
4	0.4	45	6
7	0.4	35	6
7.5	0.4	20	1
10	0.4	1	6

Mass Spectrometry

Waters Select Series™ Cyclic™ IMS

ES+ and ES– data was acquired in single and multipass $HDMS^{E}$ mode.[3]

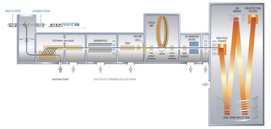

Figure 1. Schematic for Waters Select Series Cyclic IMS , Key components are the cyclic mobility separator and T-Wave Array used to change the direction of ion motion into and out of the mobility selector.

RESULTS

Figure 2. UNIFI™ Summary for Selected TAGs observed in Liver. Tabulated data includes mass accuracy, observed Collisional Cross Section (CCS), and detector counts. Arrival Time Distributions (ATD) and Mobility filtered low and high energy spectra for TAGS C54:5 and C54:6 are shown. Neutral loss is consistent with a C18:2 acyl chain.

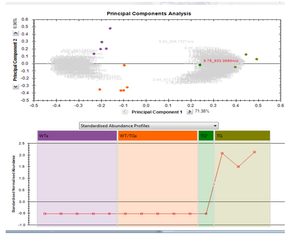

Figure 3. PCA Scores Bi-Plot for WT, WT/TG and TG animal groups, showing trend plot for selected species.

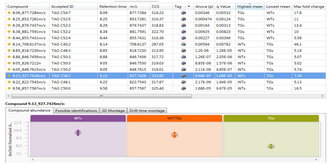

Figure 4. Selected TAGs identified in liver. Data was filtered on the basis of ANOVA <0.005. Combined Carbon chain and number of unsaturations are reported along with CCS, Highest/lowest group, and fold change. Bar plot is reported for TAG C56:7 showing standard deviations.

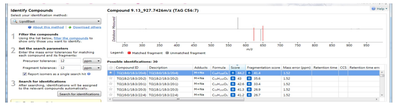

Figure 5. Results of LipidBlast database search for TAG 56:7. Based on the spectral match, the three acyl chains are C18:0, C18:3, and C20:4.

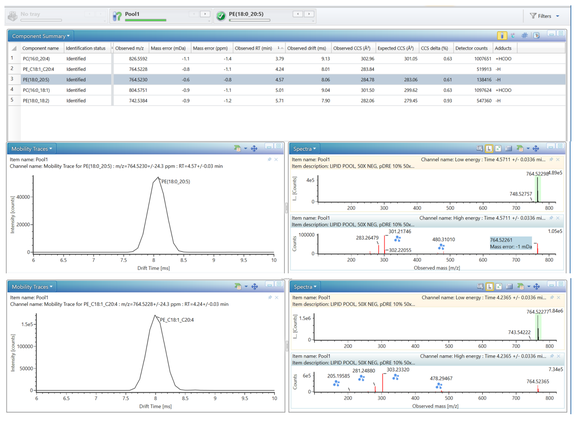

Figure 6. UNIFI Summary isomeric PEs observed in Liver. Tabulated data includes mass accuracy, observed CCS and difference from expected values, and detector counts. Arrival Time Distributions (ATD) and Mobility filtered low and high energy spectra for PE C18:0 C20:5 and C18:1 C20:4 with identified fragment ions are annotated.

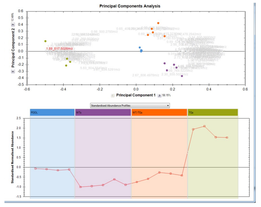

Figure 7. PCA Scores Bi-Plot for WT, WT/TG and TG animal groups, showing trend plot for selected species. Replicate assays of a pooled sample cluster tightly in the center of the plot.

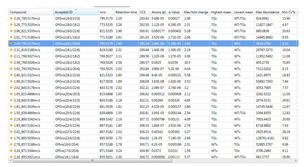

Figure 8. Selected Phosphatidylglycerols (GPGro) identified in liver. Data was filtered on the basis of ANOVA <0.005. CCS, Highest/lowest group, fold change, Maximum abundance and minimum CV % are reported.

Figure 9. Results of LipidBlast database search for GPGro C18:2 C22:6.

INCREASING PEAK CAPACITY WITH MULTIPASS METHODS

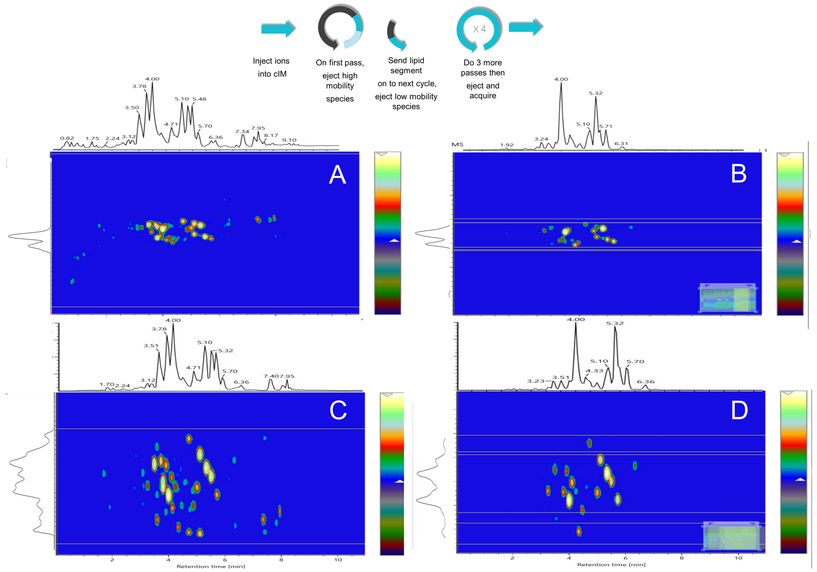

Figure 10A. ATD vs RT plot for single pass ES– analysis of liver phospholipids 10B. Extracted fragment ion single pass ATD vs RT for C18:0, C18:1 and C18:2 fatty acyl fragments. 10C. ATD vs RT plot for 4 total pass ES– analysis of liver phospholipids 10D. Extracted fragment ion ATD vs RT plot for 4 total pass for C18:0, C18:1 and C18:2 fatty acyl fragments.

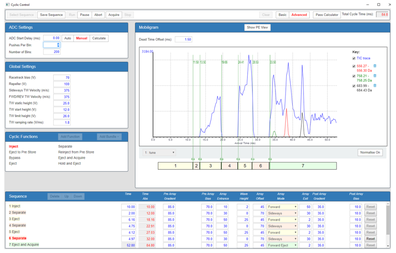

Figure 11. Cyclic Sequence Editor for 'Top and Tail' experiment. High and low mobility non-target ions are ejected in Steps 3 and 5, respectively. Target ions are further separated in Step 6.

CONCLUSIONS

- **Lipidomics experiments for characterization of differential metabolism for WT vs WT/TG and TG mice including identification of lipid species with altered metabolism**
- **Changes to Triacylglycerol and Phosphatidylglycerol lipid pools between wild type and transgenic groups suggest restructuring of liver metabolism**
- **Use of multipass IMS experiments to increase peak capacity for lipidomics experiments**

References

1. Smith, S. FASEB J. **8,** 1248-1259, 1994.
2. Bueno, M. and Quintela-Fandino, M. *Molecular & Cellular Oncology.* **7:2** 170389 2020.
3. Paglia, G and Astarita, G., *Nature Protocols.* **12,** 797-813, 2017.

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