High-throughput targeted lipidomics analysis of dihydroceramide desaturase-1 (DES1) knockout mice

Targeted lipidomics on the QTRAP® 6500+ System

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Targeted lipidomics approach using liquid chromatography (LC) coupled with triple quadrupole-based instrumentation in the multiple reaction monitoring (MRM) mode provides high sensitivity and specificity. Amide column separation is an attractive chromatographic strategy that effectively resolves both polar and non-polar lipids into classes and subclasses, which is critical for reliable lipid identification and quantification.

A targeted global lipid profiling strategy has been developed, enabling a broad array of different lipids to be quantified at the molecular species level (~1150 molecular species). The target list of lipids is comprehensive, covering most major lipid classes and categories, and MRMs were selected to cover lipids containing fatty acids with 14-22 carbons and 0-6 double bonds. The optimized chromatography method was developed to minimize isobaric interference through the chromatographic separation of lipid class. The method is customizable, so new lipid categories, classes, and molecular species can be added.

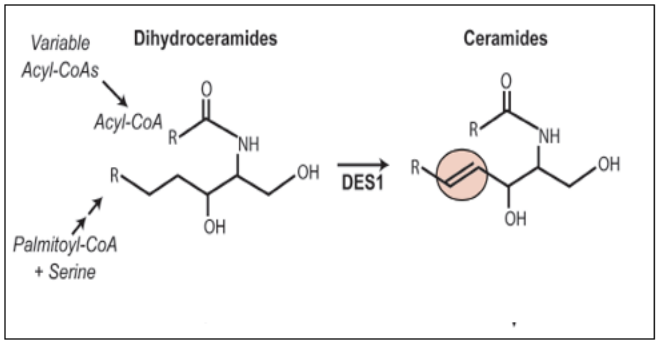

Figure 1. Deletion of Dihydroceramide Desaturase 1 (DES1) in knockout mouse model. DES1 is the enzyme involved in the conversion of dihydroceramide into ceramide By inserting the 4,5-trans-double bond to the sphingolipid backbone of dihydroceramide. DDase requires the O2 and the NAD(P)H as a cofactor. Impact of knocking down this enzyme on the lipid profiles in liver and epididymal white adipose tissue was studied.

To validate this method, which allows for a relatively rapid and specific lipid screening technique, a DES1 knockout mice model was selected. A small feasibility study was performed to measure lipid changes in liver and adipose tissues to determine if expected changes could be measured.

Key features of targeted lipid profiling using the QTRAP 6500+ System

- A fully developed method that is easily adopted for broad lipid profiling in a wide range of sample matrices[1]
- Optimized chromatography separates lipids by class, which reduces inter-class isobaric interferences (24 mins per sample)
- Scheduled MRM™ Algorithm enables large numbers of lipids to be profiled in a single acquisition (1150 lipids used here)
- Offers wide dynamic range, fast polarity switching scan (< 5msec), as well as high sensitivity even at high acquisition rates (2-5 msec dwell times)
- This method utilizes internal standards for developing time scheduled MRM methods as well as for downstream relative quantitative analysis

Methods

Sample preparation: Liver and epididymal white adipose tissue (eWAT) was homogenized in cold methanol and extracted using the Bligh-Dyer method[2], n=8 and 7 for wildtype and knockout, respectively. The extracts were diluted 100-fold in ethanol before injection. Avanti SPLASH standard mixture was spiked at 1:10 ratio. The volume of Lipidyzer™ Platform standards were calculated using the Lipidomics Workflow Manager. Accurate quantitation (less than 10% quantitative bias) for most classes of lipids analyzed can be achieved by using the Lipidyzer Platform standards. Classes not included were relatively quantified with the addition of SPLASH standards.

LC-MS analysis: LC separation was performed using an ExionLC™ System consisting of a binary high pressure mixing gradient pump with degasser, a thermostated autosampler, and a column oven (35 ºC). The QTRAP 6500+ System, equipped with an IonDrive™ Turbo V source, was operated in low mass mode with polarity switching. Retention times are first roughly determined using either the BHE with spiked internal standards or a representative matrix sample and the unscheduled MRM method. Analysis of this preliminary data enables the development of a time-scheduled MRM method leveraging the enhanced features in the Scheduled MRM Algorithm Pro. Details on the chromatographic and MS methods can be obtained from the Comprehensive Targeted Method for Global Lipidomics Screening. [2]

Data processing: All data was processed using MultiQuant™ Software 3.0.2. Automated computation of the time scheduled final MRM methods was performed using the sMRM Pro Builder 1.1. [3]

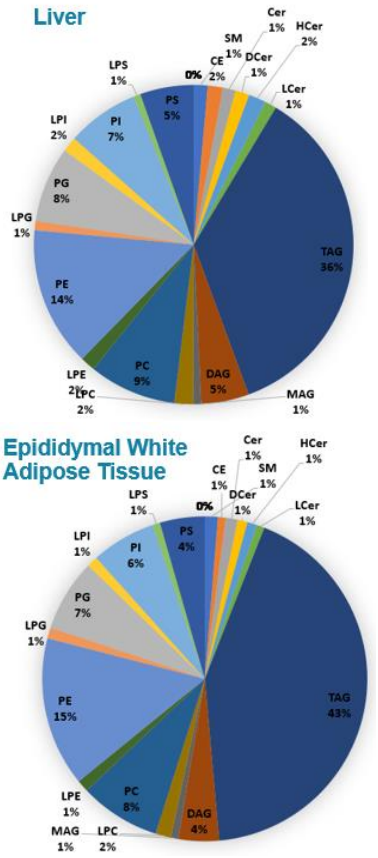

Figure 2. Distribution of detected lipid by tissue. 1150 lipids were profiled in liver and eWAT tissue using the targeted LC-MRM method. After data analysis, the number of detected lipids by class were counted and plotted here. Distribution of lipids was fairly similar except for a noticeable increase in the number of TAGs found in the eWAT tissue.

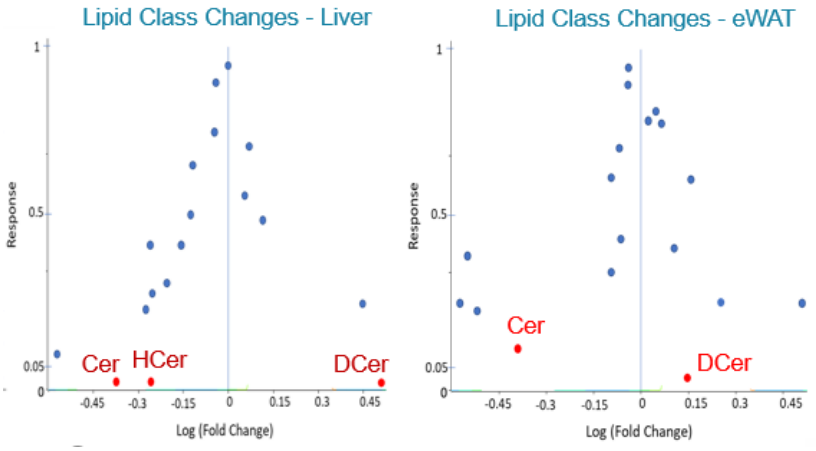

Figure 3. Lipids with significant changes in DES1 knockout mice vs control. Concentrations for each class were calculated by summing each analyte in their respective class and is expressed in µM. The volcano plot shows p-value versus log fold change. In both tissues types, the DCer show increased amounts relative to control while the Cer and HCer show decreases as expected.

Changes in lipid abundance

Lipid profiling using the targeted LC-MRM method was performed on both liver and epididymal white adipose tissue obtained from the control and knockout mice. Many lipids were measured across a broad range of lipid classes (Figure 2). Many were found to have minimal changes in abundance (Figure 3). Of interest in this model system were the changes to the ceramides and dihydroceramides (Figure 4).[4] They are expressed in very low levels in adipose tissue but were easily measured with this sensitive, targeted approach.

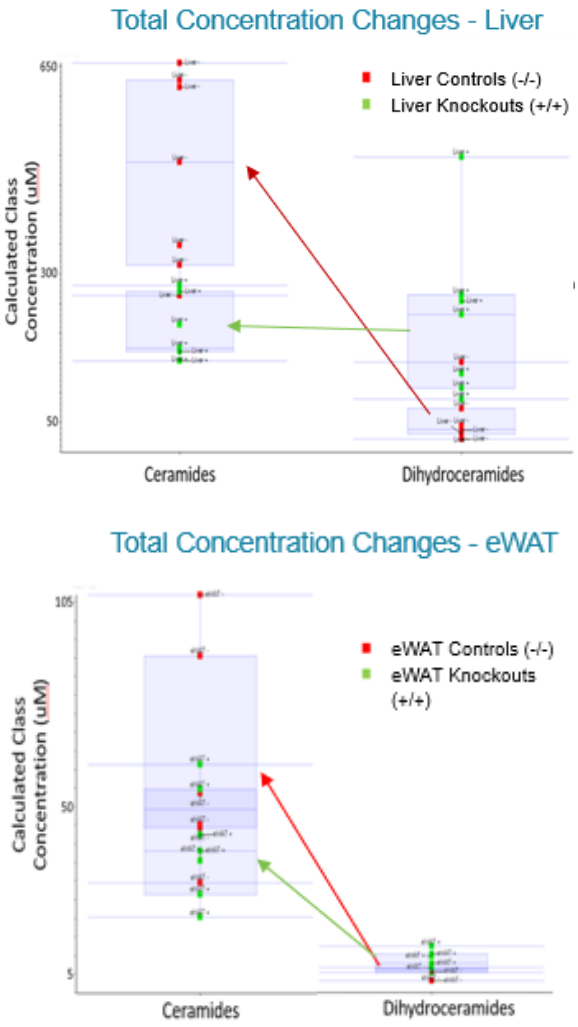

Figure 4. Reduced abundance of ceramides in knockout mice. When comparing the levels of the dihydroceramides relative to the ceramide levels between the control and the knock out mouse, it was found that there was less accumulation of ceramides as expected due to the reduction in the DES1 enzyme responsible for the conversion.

Conclusions

Here a short feasibility study was performed to test the global lipid profiling methodology using LC-MRM on a QTRAP 6500+ System using two tissue types in mouse models. A DES1 knockout model was chosen to validate the method as changes were expected in the CER and DCER species.

- Ceramides and dihydroceramides were found to have significant differences between the wildtype and knock out mice
- In the DES1 knockout mice, many lipid classes were not found to be changing (17 classes) relative to the control
- While CER and DCER are lower abundance lipids, the targeted method provided enough sensitivity for quantitation
- Expected changes in ceramides and dihydroceramides were observed in this characterized knockout mouse model to confirm the methodology

References

1. Achieve Broad Lipid Quantitation using a High-Throughput Targeted Lipidomics Method. SCIEX technical note RUO-MKT-02-8482-B.
2. Download method information.
3. Download the sMRM Pro Builder Template.
4. Chaurasi B, *et al.,* (2019) Targeting a ceramide double bond improves insulin resistance and hepatic steatosis. *Science* 365 (6451), 386-392.

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