Untargeted LC-MS/MS-based metabolic phenotyping applied to the CD248 knock out mouse model

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Overview

- Untargeted metabolomics utilizing HRAM LC-MS/MS analysis has been applied to study the impact of a C57Bl/6J CD248$^{-/-}$ (knock out) on mouse serum profiles following administration of a high fat diet, relative to chow diet controls.
- The response to diet has been compared to a C57Bl/6J CD248$^{+/+}$ wild type phenotype.

1. Introduction

Untargeted LC-MS/MS is a powerful tool by which to identify metabolic phenotypes. Here we applied metabolic phenotyping analysis to the CD248 knockout mouse model. CD248 is a transmembrane glycoprotein, expression of which is markedly upregulated in a considerable number of disease models including tumor growth, inflammation and injury-induced fibrosis. In human clinical studies, CD248 expression is upregulated in the fat cells of patients with diabetes, conversely, CD248 expression reverted to a normal range when obesity-associated diabetes was reversed through weight loss. In this work, an untargeted LC-MS/MS metabolic phenotyping analysis, using a reverse-phase LC separation and high resolution accurate mass (HRAM), was applied to a CD248$^{-/-}$ mouse model following high fat diet (HFD) feeding to study the effects of diet on serum metabolite profiles.

2. Methods

Serum samples were taken from C57Bl/6J CD248$^{-/-}$ knock out mice and C57Bl/6J CD248$^{+/+}$ wild type controls fed with high fat diet or a regular chow diet. Following protein precipitation with methanol and subsequent centrifugation, the precipitate was resuspended and re-extracted with water and methanol. Extracts were combined for each animal and analyzed, after randomization, using HRAM (high resolution accurate mass) mass spectrometry (LCMS-IT-TOF system and a QTOF LCMS-9030; Shimadzu Corporation, Japan). MS and MS/MS data were acquired in positive and negative ion mode on the Q-TOF with MS (m/z 100-1000; 100 msecs) and DDA-MS/MS (18 mass scans; 50 msec for each scan event with a collision energy spread 0-40V to acquire precursor and product ion data in each cycle). Cycle time of 1 second.

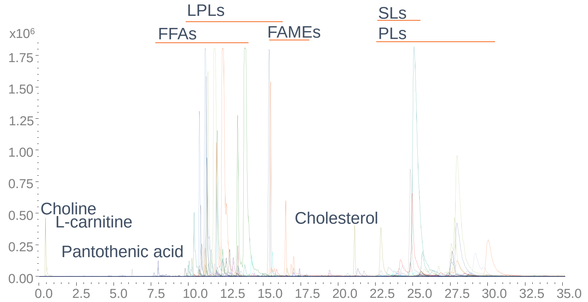


Figure 1. Precursor HRAM mass chromatograms of 95 annotated significant components detected in the pooled QC serum extract. LPLs (lyso-phospholipids), SLs (sphingolipids), FFAs (free fatty acids) and FAMEs (fatty acid methyl esters), in addition to cholesterol, L-carnitine, choline and pantothenic acid.

3. Results

Metabolic features were extracted from raw HRAM LC-MS data and filtered based on QC criteria (ion signals are present in at least 50 % of the QC samples and at RSD < 30%) as well as groups (ion signals present in at least 75 % of at least one of the 4 groups).

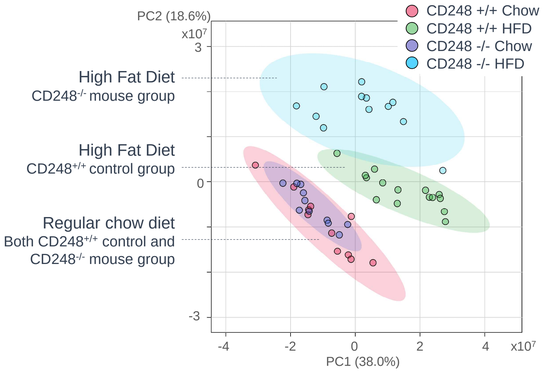


Figure 2. PCA scores plot for 5596 features extracted using HRAM LC-MS in positive ion mode. Metaboanalyst software was used to analyze the data using multivariate (PCA, heat map analysis, pattern hunter) and univariate (ANOVA) techniques.

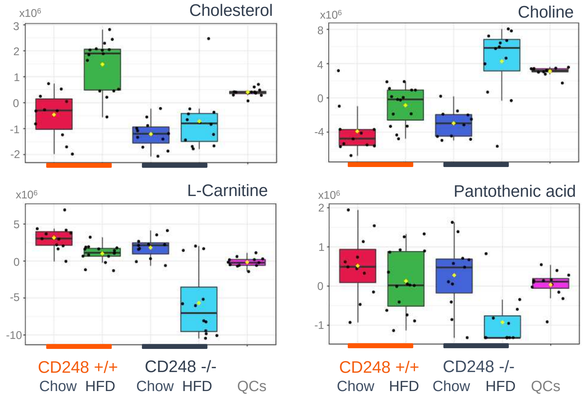
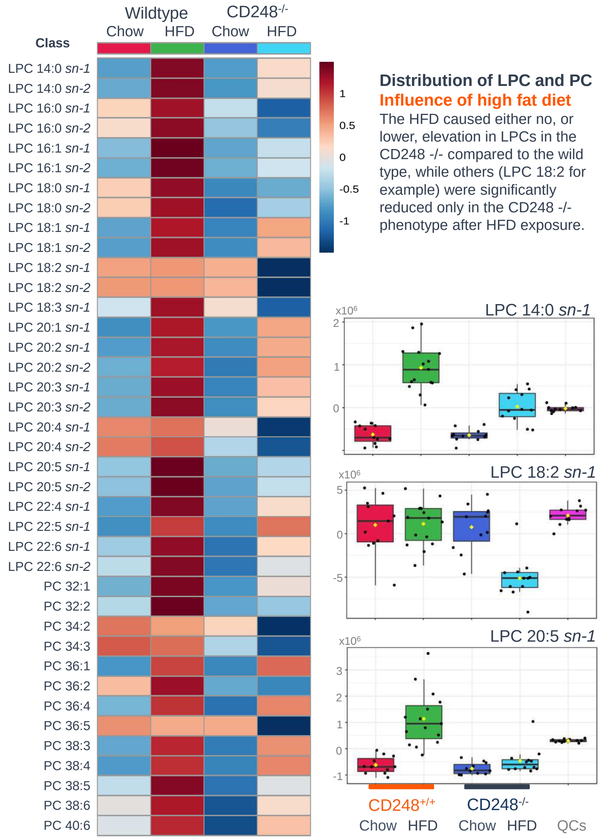


Figure 3. Boxplots generated using Metaboanalyst presenting 4 metabolites that significantly differed in response (peak area) to high fat diet that were specific to the CD248 knock out phenotype. When fed a high fat diet, the serum concentration of cholesterol was significantly increased in wildtype control mouse group whereas L-carnitine and pantothenic acid were reduced in the CD248 knock out mouse group.

3.1 Serum lipid profiling in the CD248+/+ knock out mouse model following a high fat diet

In the wild type control group different lyso-phospholipid species, mainly lysophosphatidylcholines (LPC) and phosphatidylcholine (PC) species were identified as being differentially changed following a HFD (Figure 4). Cholesterol was also differentially expressed. In this group the LPC and PC species were significantly elevated in the wild type control group C57Bl/6 CD248 +/+ following a HFD, with few exceptions.

In the C57Bl/6 CD248 knock out mice, LPC and PC species also differed from the wild type controls, but the magnitude of change was lower.



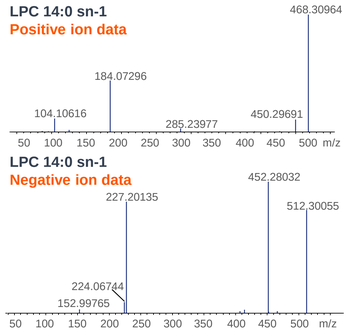
Distribution of LPC and PC Influence of high fat diet

The HFD caused either no, or lower, elevation in LPCs in the CD248 -/- compared to the wild type, while others (LPC 18:2 for example) were significantly reduced only in the CD248 -/- phenotype after HFD exposure.

Figure 4. Heatmap generated using Metaboanalyst highlighting the differential expression of LPC and PC species

3.2 High resolution accurate mass metabolite identification in metabolic phenotyping

- An untargeted LC-MS and MS/MS based metabolic phenotyping workflow was applied to the CD248 knock out mouse model following a high fat diet.
- For putatively annotating precursor ion metabolic features, data were acquired using a QTOF DDA-MS/MS method with a collision energy spread of 0-40V and 18 DDA-MS/MS scans with a mass scan time of 50 msecs.
- Metabolite identification was in agreement with published databases.
- Targeted DDA-MS/MS was further validated using a cross platform approach with different mobile phases to help enhance positive and negative ion data.



HRAM Metabolite Identification
Positive and negative ion data in lipid identification
Reported mass accuracy
Positive ion data
m/z 468.30964 2.498 ppm
m/z 450.29691 -2.199 ppm
m/z 184.07296 -1.956 ppm

Negative ion data
m/z 512.30055 2.264 ppm
m/z 452.28032 4.555 ppm
m/z 227.20135 -1.320 ppm

Figure 5. DDA-MS/MS spectra shown for the sn-1 isoform in positive ion mode and the [M+HCOOH-H]- adduct in negative ion mode (FWHM). As the complexity of the lipidome includes 8 major categories of lipids, over 80 major classes, 300 sub-classes and thousands of lipid species, acquiring targeted DDA-MS/MS in both ionization modes helped to provide high confidence in the identification of lipids.

4. Conclusions

- A HRAM LC-MS and DDA-MS/MS method was applied to study the metabolic effects of a mouse CD248 knock-out relative to a wild-type phenotype when a high fat diet was administered compared to a standard chow diet control. Significant differences in metabolite response were identified using multivariate and univariate statistics including PCA, ANOVA and heat-map analysis.
- Based upon their metabolite profiles, differential effects of the high fat diet were observed between the knock-out and wild type, suggesting CD248 is involved in an altered response to high fat diets. The most marked differences were observed in cholesterol, choline, carnitine, pantothenic acid, lyso-phosphatidylcholines, free fatty acids and fatty acid methyl esters.
- HRAM Q-TOF (LCMS-9030 Shimadzu Corporation) acquired MS and DDA-MS/MS data with a cycle time of 1 second over the MS/MS mass range of 40-1000 Da was performed to support metabolite and lipid identification.