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Metabolic Alterations Observed in Plasma of Mice Fed High-Fat Diet

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ABSTRACT

Purpose: Monitor known metabolic differentiators and discover novel biomarkers of diet-induced obesity.

Methods: Plasma samples from 20 mice fed normal diet and 20 mice fed high-fat diet for 4 weeks were analyzed with a Thermo Scientific™ Vanquish™ UHPLC system and a Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer and data were processed using Thermo Scientific™ Compound Discoverer™ software.

Results: A semi-targeted workflow was developed for the detection of known obesity markers, such as branched-chain amino acids, while at the same time, enabling comprehensive metabolic phenotyping of mouse plasma samples. Over 4,000 metabolites were detected. Both diet and sex contributed to modified metabolite levels as detected by differential analysis.

INTRODUCTION

Obesity has reached pandemic proportions in the US and has been implicated in the development of cardiovascular disease, diabetes and cancer. Obesity has been associated with changes in blood markers, such as glucose, insulin and triglycerides. Metabolomics analysis of plasma can provide further insight into metabolic pathways involved in obesity induced by high-fat intake. Here, a semi-targeted workflow was designed to confidently measure known metabolic differentiators, such as branched-chain amino acids, while allowing for the discovery of previously unidentified metabolites that are altered during diet-induced obesity. This approach combines high resolution accurate mass Orbitrap™ technology for maximum detection of known and unknown metabolites in plasma samples, with intelligence-driven fragmentation for the identification of knowns and structural elucidation of unknown biomarkers.

MATERIALS AND METHODS

Sample Preparation

Plasma samples of 20 mice fed normal diet and 20 mice fed high-fat diet for 4 weeks were obtained from BioIVT. Each group consisted of 10 male and 10 female mice. A pooled sample was created from all samples and was used for quality control and identification of unknowns. Metabolites were extracted with an excess of cold methanol (3x) containing internal standards. After evaporation of the supernatant, metabolites were resuspended in solvent A.

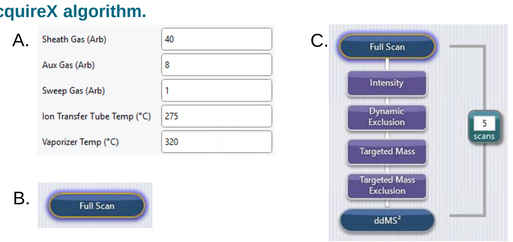
LC/MS Methods

Two microliters of the resuspended metabolites were injected on a Thermo Scientific™ Hypersil GOLD™ column (15cm ×2.1mm ID, 1.9µm particle size). The mobile phase consisted of solvent A (water with 0.1% formic acid) and solvent B (methanol with 0.1% formic acid). The flow rate was 0.3mL/min and the gradient is described in Table 1. Instrumentation included a Vanquish UHPLC system equipped with a binary pump H, in-line with an Orbitrap Exploris 240 mass spectrometer. Samples were randomized and pooled QC samples were analyzed at the beginning and the end of the sequence, and every 10 samples. Authentic standards were analyzed with the same experimental conditions. MS methods are illustrated in Figure 1.

Table 1. LC gradient.

Time(min)	%B
-3	0
0	0
6	50
8	98
13	98
13.1	0
15	0

Figure 1. MS methods. A. Source settings. B. Full scan method used for study samples and pooled QC samples. C. MS/MS identification method for the pooled sample utilizing the AcquireX algorithm.



Data Analysis

Data were processed using the Compound Discoverer software for unknown identification, differential analysis and pathway mapping. Authentic standard data were analyzed with Compound Discoverer software to create a custom spectral library. Thermo Scientific™ TraceFinder™ Software was used to monitor internal standards for quality control.

RESULTS

Confirming Data Quality

The internal standards were used to monitor instrument performance, such as retention time drift, mass accuracy and signal response. The reproducibility of the measurements was evaluated by monitoring the retention time, mass error, and peak area of D_8-valine and D_8-phenylalanine (Figures 2,3, and 4, respectively).

Figure 2. The retention times of internal standards remain constant during the study (47 injections, 15 h).

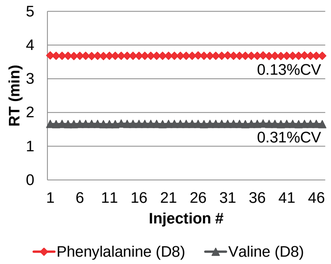


Figure 3. Internal standards are measured with sub-ppm mass accuracy during the study (47 injections, 15 h).

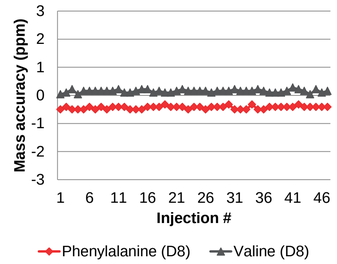


Figure 4. The peak areas of internal standards remain constant during the study (47 injections, 15 h).

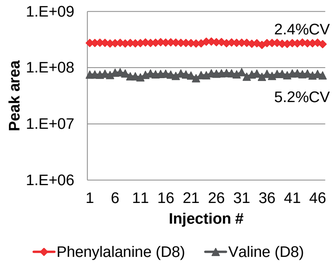


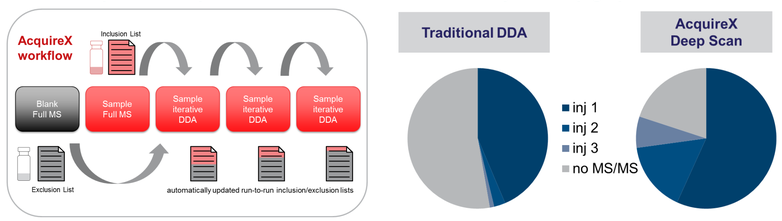
Figure 5. The Orbitrap Exploris 240 MS provides robust performance and confident identifications with AcquireX intelligent acquisition.

IMPROVED METABOLOME ANNOTATION

AcquireX Provides Fragmentation for More Compounds than Traditional DDA

Small molecules form different types of adducts and cluster ions during electrospray ionization. Highly abundant compounds, in the form of a parent ion or any of its accompanying features, such as isotopes and adducts, may prevent the fragmentation of metabolites of lower abundance. By populating the inclusion list with the preferred ion for each metabolite, more compounds can be sampled by MS/MS in a single run. Additionally, by automatically updating inclusion and exclusion lists after each injection during analysis, we can ensure that compounds not selected for MS/MS will be prioritized during a subsequent injection (Figure 6).

Figure 6. The AcquireX workflow (depicted on the left) delivers improved MS/MS sampling by automatically excluding background ions and focusing acquisition on true sample components. AcquireX significantly increased the number of compounds with fragmentation data in the pooled sample over traditional DDA after three injections (right).



DETECTION OF KNOWN AND UNKNOWN OBESITY MARKERS

Differential Analysis Reveals Metabolic Changes in the Two Groups

To quickly visualize the results of differential analysis, a principal component analysis (PCA) plot and a volcano plot were used. Both plots revealed significant metabolic changes for the group fed a high-fat diet versus the one fed a normal diet. (Figure 7)

Figure 7. A PCA plot (left) and a volcano plot (right) illustrate significant metabolic changes due to high-fat diet.

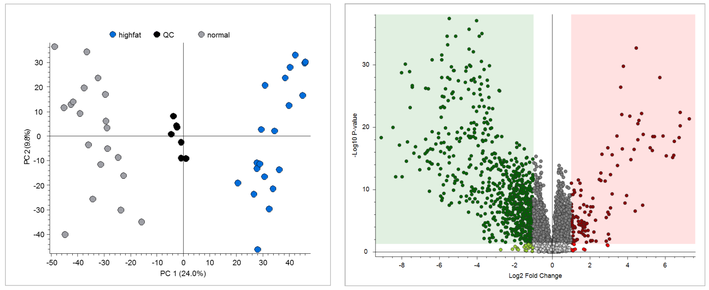


Figure 8. Known obesity markers, such as acetylcarnitine, are confidently identified against authentic standards (left) and changes in abundance can be accurately measured (right).

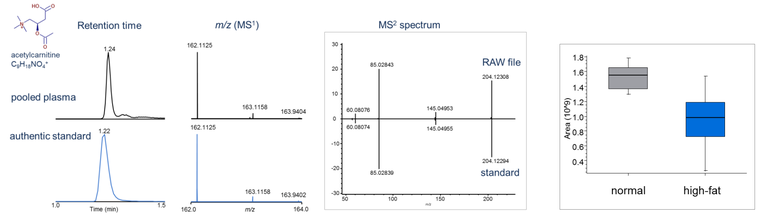
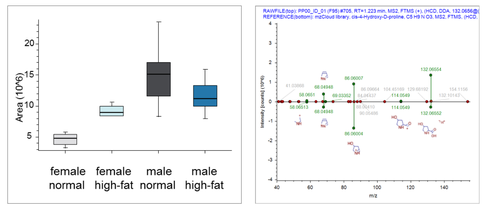


Figure 9. Previously unknown discriminants of diet-induced obesity are confidently measured, clearly indicating sex-specific changes due to diet (left). This compound was confidently annotated as 4-hydroxyproline, by searching against mzCloud (right).



CONCLUSIONS

The development of a semi-targeted workflow enabled simultaneous detection of known metabolic differentiators and discovery of previously unidentified obesity markers, shedding light to the biochemical pathways involved in diet-induced obesity and related diseases.

- The accurate *m/z* measurements afforded by Orbitrap Exploris 240 MS allowed molecular formula determination and putative annotation of thousands of metabolites by searching against structure databases, such as ChemSpider.
- AcquireX intelligent acquisition software maximized the number of unique metabolites interrogated by MS/MS, by annotating non-biological and redundant features on-the-fly.
- Differential analysis detected altered metabolite levels due to high-fat diet that could be mapped to dysregulation of lipid and energy metabolism.

TRADEMARKS/LICENSING

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