UTILISING AN IMS-DIA-MS WORKFLOW TO CHARACTERISE AND QUANTIFY THE OBESITY OR DIABETES LIPIDOME IN HUMAN PLASMA

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INTRODUCTION

Risk factors associated with increased possibility of developing diseases are commonly referred to as metabolic syndrome. Obesity is one such risk factor causing excess body fat to be accumulated to the extent that it adversely affects health and life expectancy. Obesity is known to initiate inflammation, which in turn can lead to type 2 diabetes. The exact mechanism as to how diabetes results from inflammation is still not well understood. Here, we describe a quantitative lipidomic approach based on human plasma from obese and diabetic patients to reveal molecular factors that may be involved in these biomolecular processes. Lipid analyses have been conducted using a label-free LC-IM-DIA-MS approach, providing qualitative and quantitative information from a single experiment. Ion-mobility and associated collision cross section (CCS) databases, obtained with a novel geometry travelling wave IMS-QTof MS platform were used to increase peak capacity and specificity of compound identifications. The curated datasets were then combined with data from a previous study which investigated obesity effects within a mouse model. Both were interrogated using pathway analysis tools, suggesting glycerophospholipid and arginine/ proline metabolism pathways are implicated with chronic metabolic syndrome disorders such as obesity and diabetes.

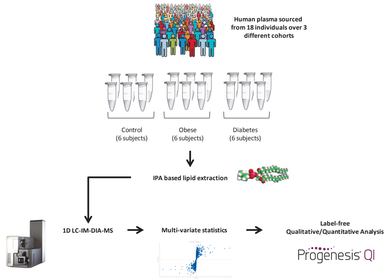

Figure 1. Experimental design study for lipids extracted from human plasma.

METHODS

Sample preparation

Lipids were extracted from human plasma (Innovative Research Inc), which originated from 6 control, 6 obese and 6 diabetic patients. Extractions were performed as previously described by Sarafin et al.[1] Briefly, plasma (200 µL) was treated with isopropanol which had previously been stored at –20°C (3:1, v/v). Samples were then vortexed and left at room temperature for 10 min before incubation at –20°C overnight. Samples were then centrifuged at 14,000g for 20 min. The resulting supernatant was collected for LC-MS analysis. An overview of the experimental and analytical workflow is provided in Figure 1.

LC-MS conditions

For lipid identification, the LC-MS experiments consisted of a 20 min gradient from 3 to 40% isopropanol:methanol (10 mM ammonium formate) at 500 µL/min using a ACQUITY I-Class UPLC system, configured with a BEH 1.7 µm C18 reversed phase 2.1 x 100 mm LC column.

Lipidomic measurements were conducted using a Vion IMS QTof mass spectrometer operating in positive and negative ESI mode (Figure 2). A data independent acquisition workflow combined with ion mobility (IM-DIA) was used in conjunction with the acquisition schema.

Poly-DL-alanine (10 mg/L) was used for ion mobility calibration, extending across a CCS range of 151 to 306 Å (positive) and 150 to 308 Å (negative).

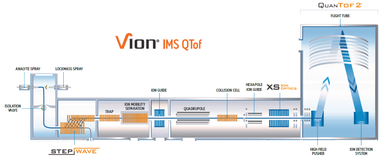

Figure 2. Vion IMS QTof mass spectrometer schematic. CCS measurements derived using a IM-DIA-MS workflow.

Bioinformatics

The LC-MS lipid data were processed and searched with both UNIFI (Figure 3) and Progenesis QI. Normalized label-free quantification and CCS values were achieved from the Progenesis QI software and additional statistical analysis conducted with EZInfo. Compound searches were conducted using a combination of LipidMaps and a customized version comprising CCS values derived from a series of standard lipids. The data was further interrogated using MetaboAnalyst.[2]

Pathway and network analysis was also performed using a variety of pathway tools including MetaboAnalyst and KEGG.

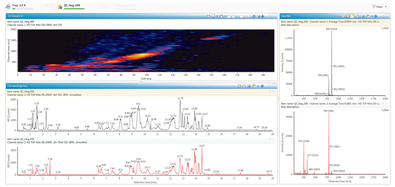

Figure 3. UNIFI data analysis. Negative ion data is represented showing chromatograms (lower traces), spectra (right-hand traces) and mobility viewer (upper trace).

RESULTS

Small amounts of the purified plasma extracts were analyzed to identify, quantify and investigate the lipidomic variance between control, diabetic and obese cohorts. Principal component analysis (PCA) was used to identify significant changes between the three cohorts, of which an example is shown in Figure 4.

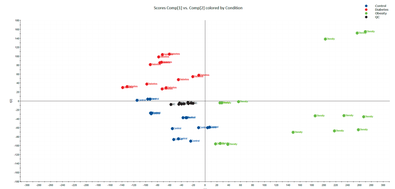

Figure 4. Unsupervised PCA scores analysis. Control (blue), diabetic (red), obese (green) and pooled QC's (black) show clear separation between groups in addition to good technical reproducibility.

A comparative analysis between controls/obese, controls/ diabetic and diabetic/obese are presented in Figure 5. Representative heatmaps highlight regions of differential regulation for specific classes of lipids in each of the three cases. Contrasting volcano plots allow lipids consisting of high fold change with high statistical significance to be extrapolated. The dynamic range of the plasma lipidome in all cases is shown to be in the region of 3 orders. Representative identifications correlating with high fold changes and statistical significance are plotted as box and whisker plots.

Identifications from an obese mouse model (previous work) have been curated (fold change ≥2, CV ≤30% and ANOVA (p) ≤0.001) and cross-referenced with identifications from this study, highlighting similarities between classes of lipids and the biological pathways perturbed (Figure 6).

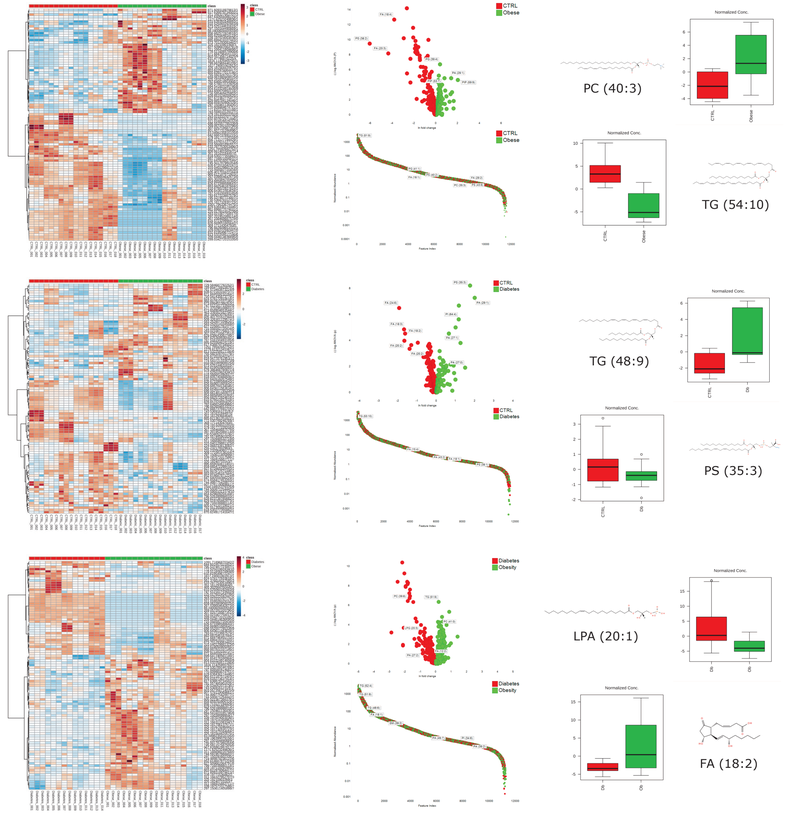

Figure 5. Comparative analysis of controls/obese (upper row), controls/diabetic (middle row) and diabetic/obese (lower row) cohorts; (A) Heat maps constructed using Euclidean distance and Ward clustering highlight significant differences in regulation profiles for a variety of lipid species. Lipids demonstrating over-expression are shaded red, whilst those under-expressed are shaded blue; (B) Volcano plots display features which are both statistically relevant (ANOVA) and significant fold change. The normalised abundance of individual features demonstrates the dynamic range achieved during this analysis. In both plots, tentative identifications have been appended as examples; (C) Example lipid identifications corresponding to fold changes >2 and high statistical significance (ANOVA >0.05) are presented as box and whisker plots.

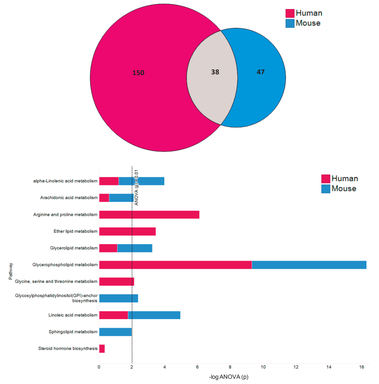

Figure 6. KEGG pathway analysis of curated lipids from human (pink) and mouse (blue) datasets. Combining both species resulted in 235 individual lipids with 38 common between both. These were mapped to 11 pathways with 8 meeting the statistical ANOVA (p) threshold of 0.01. Of these, the most prevalent is the glycerophospholipid metabolism pathway, which is shown to participate in a variety of metabolic syndromes including obesity.[3,4]

CONCLUSIONS

- **A lipidomic study utilizing a label-free IM-DIA-MS approach has been applied to human plasma samples from patients diagnosed as obese or diabetic.**
- **A variety of lipids including fatty acids, triglycerides and sphingomyelins have been identified to be amongst the most contrasting classes between obese and diabetic cohorts.**
- **Ion mobility derived CCS measurements have provided increased specificity as part of the database searching to aid with identification.**
- **Cross-referencing human lipidome results with those from mice, show complimentary lipids contributing significant to pathways involved in metabolic syndrome disorders (e.g. glycerophospholipid metabolism).**

References

1. Sarafian et al. Objective Set of Criteria for Optimization of Sample Preparation Procedures for Ultra-High Throughput Untargeted Blood Plasma Lipid Profiling by Ultra Performance Liquid Chromatography-Mass Spectrometry. Anal. Chem. 2014;86;5766-5774.
2. Xia et al. MetaboAnalyst 3.0—making metabolomics more meaningful. Nucl. Acids Red. (DOI: 10.1093/nar/gkv380).
3. El-Najjar et al. Increased levels of sphingosylphosphorycholine (SPC) in plasma of metabolic syndrome patients. PLoS ONE 10 (10), doi: 10.1371/journal.pone.0140683.
4. Jove et al. Plasma lipidomics discloses metabolic syndrome with a specific HDL phenotype. FASEB J. 2014; 28: 5163-5171.

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