IMS-DIA-MS CHARACTERISATION AND IMS-MRM QCONCAT QUANTITATION OF THE LIPIDOME AND APOLIPOPROTEIN COMPLIMENTS OF OBESITY AND DIABETES COHORTS

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INTRODUCTION

Risk factors associated with an 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 and is known to initiate inflammation, in turn leading to type 2 diabetes. The exact mechanism of this inflammation process is still not well understood. Here, we describe a quantitative lipidomic and targeted proteomic 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. Apolipoprotein alleles were also targeted using an IM-MRM method with QconCAT equivalents. Both datasets were interrogated using pathway analysis tools, suggesting pathways such as HDL-mediated lipid transport are implicated with chronic metabolic syndrome disorders such as obesity and diabetes.

METHODS

Sample preparation

Lipids were extracted from human plasma, which originated from 6 control, 6 obese and 6 diabetic patients. Extractions were performed as previously described by Sarafin *et al*[2]. 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 centrifuged at 14,000g for 20 min. The resulting supernatant was collected for LC-MS analysis. Plasma samples were also spiked with a QconCAT equivalent of Apolipoprotein B (apoB) prior to overnight tryptic digestion (Figure 1).

LC-MS conditions

Lipids were chromatographically separated using a BEH 1.7 µm C18 RP 2.1 x 100 mm LC column, whilst peptides utilised a Symmetry C18, 5 µm, 180 µm x 20 mm (trap column) and HSS T3 1.8 µm C18 RP 75 µm x 150 mm (analytical column).

Analyte	Mobile Phase	Gradient	Flow Rate
Lipids	IPA:acetonitrile (9:1)/ 10mM ammonium formate Acetonitrile:Water (6:4)/ 10mM ammonium formate	20 min (3–40% B)	0.5 mL/min
Peptides	0.1% formic acid/water 0.1% formic acid/acetonitrile	90 min (1–40% B)	300 nL/min

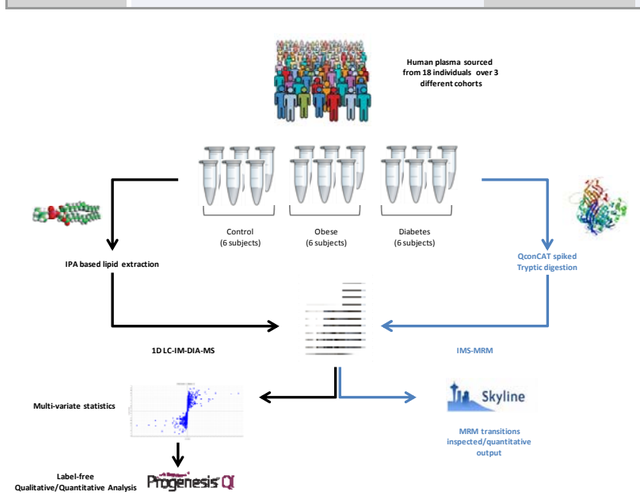

Figure 1. Lipidomic and proteomic experimental design study from human plasma extracts.

Lipid and peptide measurements were conducted using a Vion IMS QTof mass spectrometer operating in positive and negative (lipids) ESI mode (Figure 2). A data independent acquisition workflow combined with ion mobility (IM-DIA) was used in conjunction with the acquisition schema. Peptide data were acquired using a targeted MRM method incorporating ion mobility (IM-MRM).

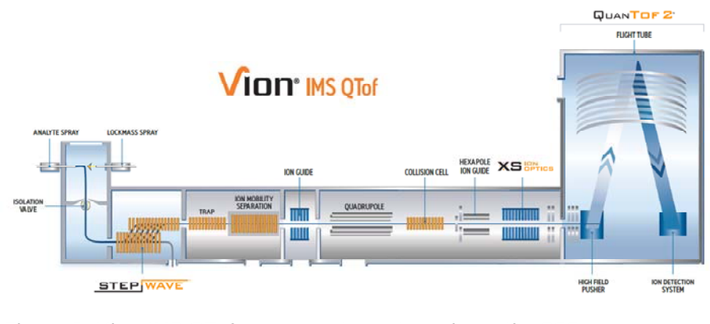

Figure 2. Vion IMS QTof mass spectrometer schematic. CCS measurements were derived using a IM-DIA-MS workflow.

Bioinformatics

The LC-MS lipid data were processed and searched with UNIFI and Progenesis QI. Normalized label-free quantification and CCS values were achieved from Progenesis QI with additional statistical analysis conducted using 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 were also interrogated further using MetaboAnalyst[3].

Skyline (University of Washington) was used for peptide quantitative analysis using libraries specific to apoB. Both data sets were combined and submitted for pathway and network analysis using a variety of pathway tools including MetaboAnalyst and IMPaLA[4].

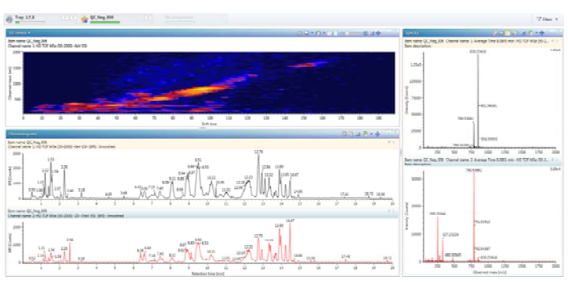

Figure 3. Negative ion data is represented showing chromatograms (lower traces), spectra (right-hand traces) and mobility viewer (upper trace).

RESULTS

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

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. Representative identifications correlating with high fold changes and statistical significance are plotted as box and whisker plots.

Analysis of the IM-MRM data (Figure 6), shows a representative apoB peptide. The inclusion of ion mobility highlights increased specificity with drift time aligned transitions. Comparison of the three cohorts indicates apoB mean quantitative differences of approximately 6 and 11-fold for diabetic and obese cohorts respectively when compared with controls.

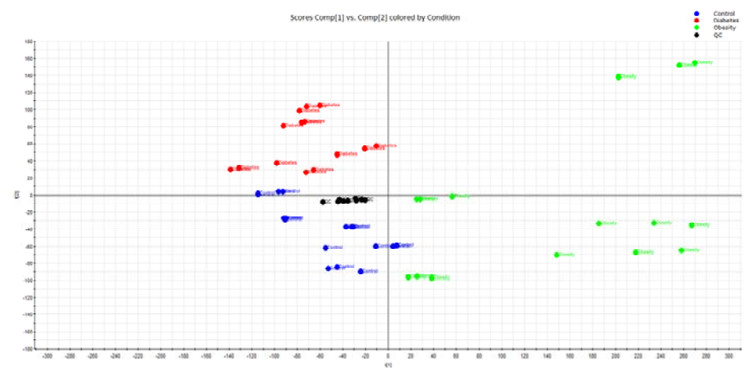

Figure 4. Unsupervised PCA scores analysis. Control (blue), diabetic (red), obese (green) and pooled QC's (black) show clear separation between groups in addition to good technical reproducibility.

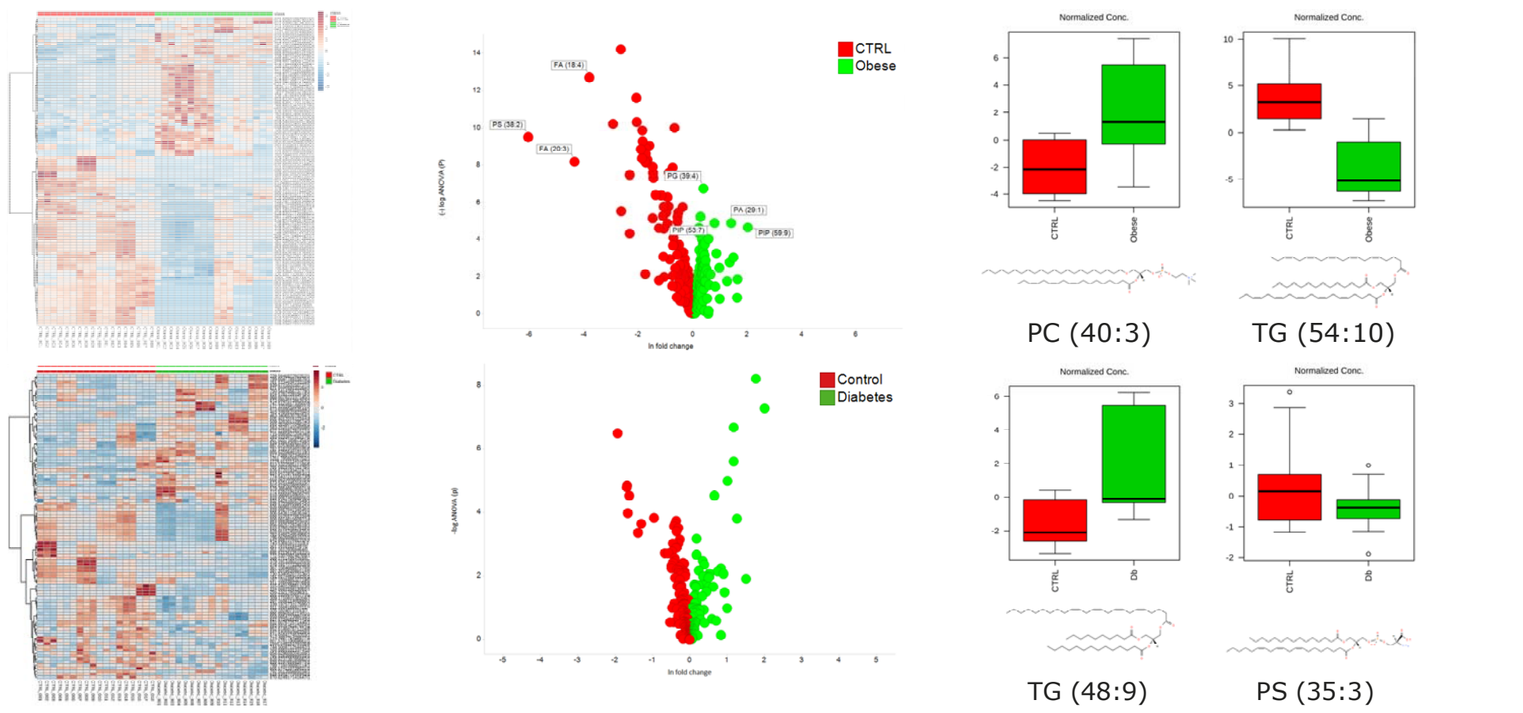

Figure 5. Comparative analysis of controls/obese (upper row) and controls/diabetic (lower row) cohorts. Heatmaps for each of the comparisons show significant differences in regulation profiles for a variety of lipid species (column A). Lipids demonstrating over-expression are shaded red, whilst those under-expressed are shaded blue. Statistically relevant identifications with significant fold change can be assessed using volcano plots (column B). Example lipids corresponding to fold changes > 2 and high statistical significance (log ANOVA (p) > 2) are presented in column C.

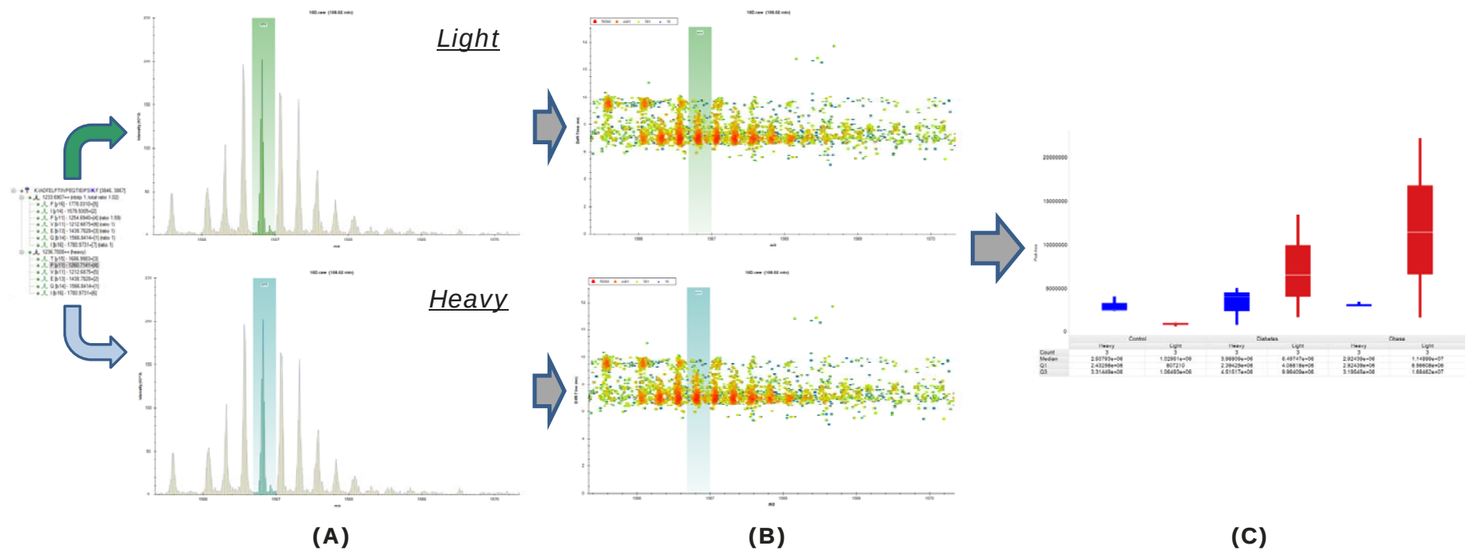

Figure 6. Skyline interrogation of an example apolipoprotein B peptide (IADFELPTIIVPEQTIEIPSIK) and its QconCAT equivalent (A). Monitoring of the b14 transition (m/z 1566.8) for example shows that in addition to retention time, implementation of drift time provides increased quantitative precision and accuracy (B). Comparisons of the three cohorts show a significant increase in apoB levels for diabetic (5.7 x10^5 ng/mL) and obese (13.65 x10^5 ng/mL) subjects when compared with controls (1.45 x10^5 ng/mL). Light and QconCAT versions are represented in red and blue respectively (C).

Lipid identifications were curated (fold change ≥2, CV ≤30% and ANOVA (p) ≤0.001) and pathway analysed with the associated protein data using IMPaLA. A number of pathways/networks implicated with metabolic syndrome, including phospholipid metabolism[5] and lipid digestion/mobilization are highlighted (Figure 7).

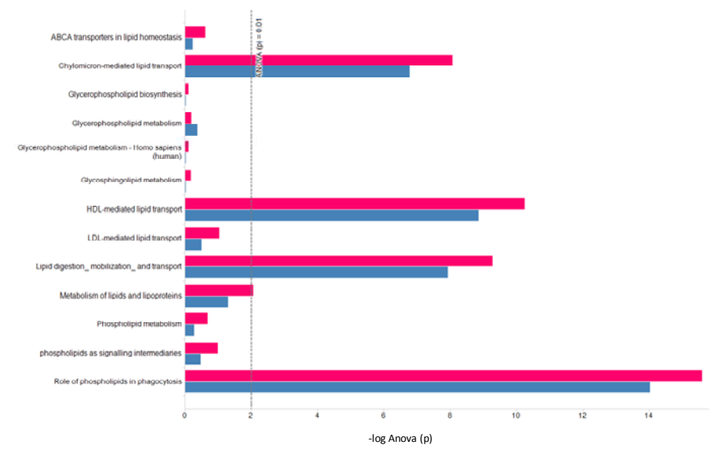

Figure 7. Combined pathway analysis of lipid and protein datasets using IMPaLA. Statistically relevant lipids (blue) and proteins (pink) above a threshold of 0.01 ANOVA (p) are considered (e.g. HDL mediated transport[6] and chylomicron-mediated transport)

CONCLUSIONS

- **A multi-omic 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 phosphocholines, triglycerides and sphingomyelins have been identified to be 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**
- **IM-MRM provides greated specificity for peptide quantitation. ApoB related data shows elevated levels between 6 and 11-fold for diabetic and obese patients, respectively**
- **Pathway analysis identified a number of significant pathways such as HDL mediated transport as being involved in metabolic syndrome disorders.**

References

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