UTILIZATION OF SIMLIPID FOR THE CHARACTERIZATION OF METABOLIC SYNDROME RELATED LIPIDS ACQUIRED USING A NOVEL SCANNING QUADRUPOLE DIA ACQUISITION METHOD

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INTRODUCTION

Lipidomics has become a rapidly increasing area of research over recent years with a focus on its use and application for disease processes including metabolic syndrome disorders, cancer and cardiovascular disease for example. Obesity, a metabolic disorder risk factor, is known to initiate inflammation, which in turn can lead to type 2 diabetes. The exact mechanism as to how this occurs is not understood.

In the method, a low-resolution quadrupole mass filter is scanned repetitively and both precursor and MS/MS data acquired at spectral rates approaching 2000 spectra/s. The method produces a high duty-cycle, highly specific and unbiased two-dimensional data that can be viewed and processed using readily available informatics.

Here, we describe an LC-MS based lipidomic approach to reveal molecular factors that may be involved in obesity and diabetes. Data acquired using a novel scanning quadrupole DIA method and processed through SimLipid provided a list of curated lipids that can be used to identify multi-factorial disease associated components and pathways.

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.[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 (Figure 1).

LC-MS parameters

Lipids were chromatographically separated using a CSH 1.7 µm C18 reversed phase (RP) 2.1 x 100 mm LC column. A gradient of 20 min from 3 to 40% isopropanol:methanol (10 mM ammonium formate) at 400 µL/min was conducted using an ACQUITY I-class system.

A Xevo G2-XS QToF (Waters Corporation), Figure 2, was operated in SONAR™ mode. The optimized quadrupole window and the other parameters employed for the analyses are described in Figure 3.

Bioinformatics

The data were processed and searched using SimLipid software (version 6.0) with compound databases, providing comprehensive qualitative lipid characterisation. EZinfo and MetaboAnalyst[2] were also used for statistical and data analysis respectively.

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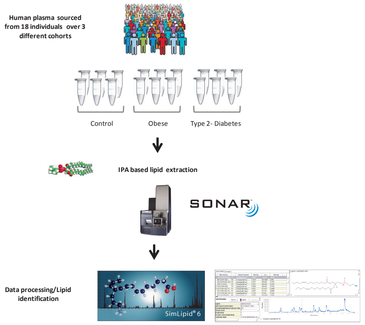


Figure 1. Lipidomic experimental design study for human plasma.

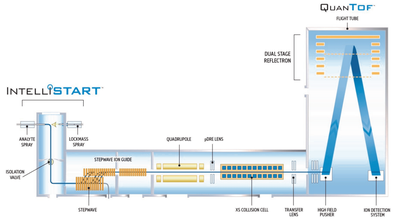
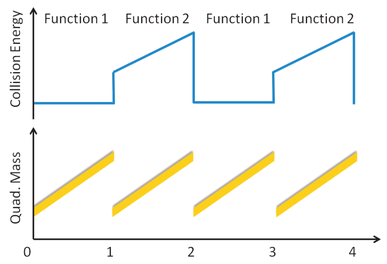


Figure 2. Schematic of the Xevo G2-XS mass spectrometer used for SONAR™ data acquisition

RESULTS

Data from all patients were acquired using SONAR and processed using SimLipid. Data were peak picked and database searched against the SimLipid library (Figure 4). Example results for a typical plasma sample are presented in Figure 5 with a wide range of lipid classes included as part of the search. Lipidomic analysis is challenged with issues such as co-elution and the presence of isobarics.

The SONAR/SimLipid workflow provides additional selectivity (both precursor and fragment ions), which is provided by the scanning quadrupole to reduce interference effects and thereby provide cleaner fragmentation spectra.



Ionisation Mode	Quad Scan (Da)	Quad Window (Da)	ToF Scan (Da)	Function Integration Time (s)	Function CE (V)	Function 2 CE (V)
+	400-1000	10	50-1200	0.2	6	20-50
-	400-1000	10	50-1200	0.2	6	25-55

Figure 3. SONAR™ acquisition method and DIA acquisition parameters used in the different experiments.

The isobaric nature of lipids can also make identification challenging, however searching against the SimLipid library allows for isobarics to be differentiated. Figure 6 provides an example which consists of three co-eluting phospholipids, were the combined SONAR/SimLipid workflow is shown to be of benefit in cases of co-eluting and isobaric species.

Resulting identifications were combined with previous quantitative studies and interrogated further using EZInfo and MetaboAnalyst for statistical analysis and data visualisation (Figure 7). Example discriminative markers between diabetic and control patients included PC, SM, TAG and Cer classes.

Figure 4. Example SimLipid processing parameters. Typical peak picking settings are shown for RT Peaks (upper), Precursor Ions (middle) and Product Ions (lower).

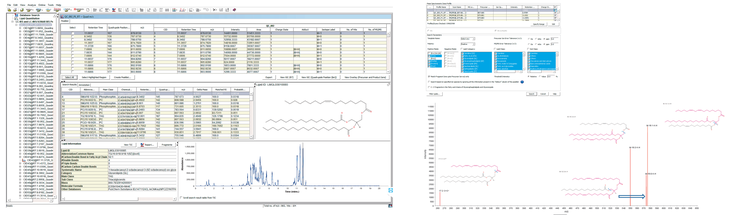


Figure 5. SimLipid processed data for a representative lipid extract. Upper screenshot shows SONAR data for a TG (50:3) which has been peak picked and database searched against the SimLipid library. The corresponding fragment ions for the same TG species are shown in the lower trace with full sequence information being provided (pink segments of the lipid structures indicate fragments of interest).

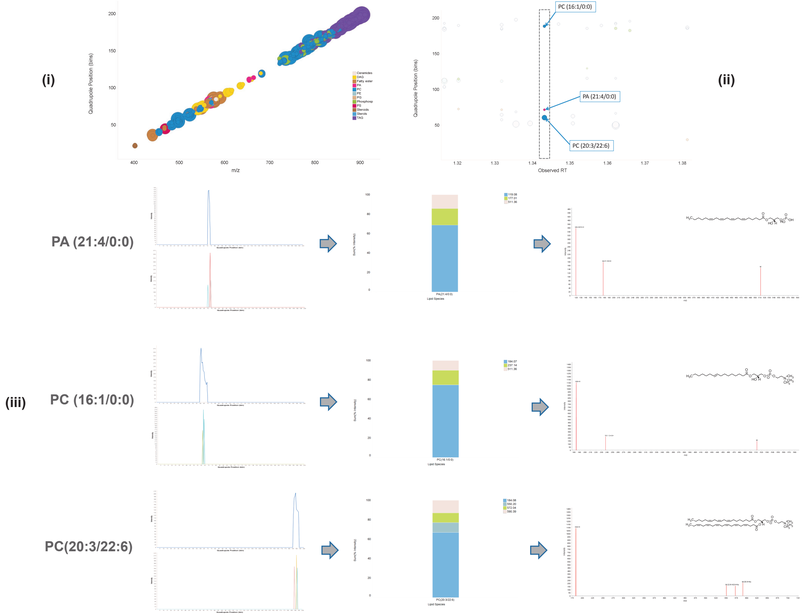


Figure 6. (i) SONAR based identifications using SimLipid with a distribution profile (m/z vs Quadrupole Position) showing the various lipid classes identified; (ii) Implementing SONAR demonstrates high specificity provided by the technique for co-eluting components; (iii) Quadrupole profiles for each co-eluting lipid further highlights the specificity of the workflow, showing the quadrupole position for precursor (low energy scan) and associated fragments (high energy scan). Fragment ion intensities are presented with accompanying bar charts and representative fragment ion spectra shown for each lipid. Isobaric species (m/z) are differentiated on the basis of the SimLipid assigned fragment ions.

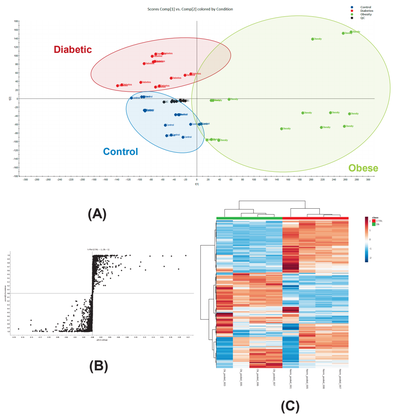


Figure 7. Multivariate statistical analysis of SONAR data acquired for the metabolic syndrome disorder study. Unsupervised PCA (A) shows group differentiation between diabetic, obese and control groups. Clustering of the pooled QC's indicate high technical reproducibility. S-plots resulting from OPLS-DA analysis (B) show statistically probable features with high fold change, whilst heatmaps (C) show expression trends within the data between control/diabetic cohort.

CONCLUSION

- **A lipidomic workflow comprising of SONAR acquisition with SimLipid processing has been demonstrated using plasma extracts originating from a metabolic syndrome cohort.**
- **SONAR provides additional specificity in regions of co-elution, as shown with TAG based examples.**
- **High quality data is shown to be rapidly acquired with UPLC-based chromatography.**
- **Lipid characterisation using SimLipid provides high scoring identifications and can distinguish isobaric lipid species.**
- **Multi-variate statistical analysis of data resulting from a metabolic syndrome cohort show clear distinction between control, diabetic and obese subjects.**

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; 86:5766-74.
2. Xia et al. Using MetaboAnalyst 3.0 for Comprehensive Metabolomics Data Analysis. Current Protocols in Bioinformatics, 55:14.10.1-14.10.91.