Discovering Potential Diabetic Lipid Biomarkers Using HRAM LC-MS-MS Approach on A Hybrid Quadrupole – High Field Orbitrap Mass Spectrometer

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Overview

Purpose: Develop a high throughput untargeted lipidomics workflow for rapid biomarker discovery using HRAM LC-MS-MS on a Thermo Scientific™ Q Exactive™ HF hybrid quadrupole-Orbitrap mass spectrometer

Methods: Rat plasma were recovered from whole blood lean (3 animals) and Zucker fatty (3 animals) from Bioreclamation-IVT. The lipid profiling for the rat plasma is performed by UHPLC-MS-MS using a C30 column (2.1x150mm, 3μm) and a Q Exactive mass spectrometer. A top 15 data dependent MS-MS experiment was used for collecting data, in which each high resolution MS scan (120K at m/z 200) was followed by 15 high resolution MS/MS scans (30K at m/z 200). Thermo Scientific™ LipidSearch™ 4.1 SP1 software was used to process all raw data for lipid identification and lipidomics profiling.

Results: The Q Exactive HF MS system delivers high mass accuracy and resolution combined with faster scan speed, enabling faster and deeper lipidome coverage. More than 700 lipid species were simultaneously identified and quantified with high confidence and great analytical precision from the two types of rat plasma. Significant concentration increases of many different triglycerides and phospholipid species were observed from ZDF rat plasma.

Introduction

Lipids play a key role in cell, tissue and organ physiology with diseases such as diabetes which involve disruption of their metabolic enzymes and pathways. Identification of unique lipid biomarkers to distinguish healthy humans compared to those with a disease can have an impact on the early detection of diseases and personalized medicine. Here we demonstrate that HRAM LC MS-MS approach on a hybrid quadrupole high field Orbitrap mass spectrometer enables rapid putative biomarker discovery through lipidomics profiling experiments. In these experiments phenotypic ZDF rat plasma (fatty vs. lean wild type) were used to demonstrate the capabilities of this method.

Methods

Sample Preparation

The rat plasma were purchased from Bioreclamationivt (Westbury, NY). Rat plasma were recovered from whole blood of Zucker Lean (3 lots) and Zucker Diabetic Fatty (ZDF, 3 lots) using EDTA as anti-coagulant by Bioreclamationivt. The solvents of Chloroform, Methanol and water were used for the lipid extraction and Table 1 shows the extraction procedure used.

Table 1. Plasma Lipid Extraction Procedure

1. Take 80 µl of the sample aliquot into 4 ml glass tube.
2. Add 10 ul of internal standard purchased from Avanti (LM: 1004, 17:0-14:1 PE)
3. Add 600 µl of methanol, vortex.
4. Add 1000 µl of chloroform, vortex.
5. Add 500 µl of water, vortex.
6. Centrifuge at 3000 R for 10 min.
7. Collect the lower (chloroform) phase.
8. Evaporate the combined organic phases to dryness in a vacuum centrifuge.
9. Reconstitute extracted lipids in 100 µl of IPA/methanol (50:50) for storage.

Liquid Chromatography

A Thermo Scientific™ Dionex™ UltiMate™ 3000 Rapid Separation LC (RSLC) system performed HPLC separations using the gradient conditions as shown in Table 2. Mobile phase A was 60:40 Acetonitrile / Water and mobile phase B was 90:10 IPA / Acetonitrile; both A and B contained 10mM ammonium formate and 0.1% formic acid. The column was a C30 column (2.1x150mm, 2.6μm) operated at 45°C, flow rate of 260 μL/min and the injection volume was 2 μL.

Table 2. HPLC Gradient

Time	% A	%B
0	70	30
2	57	43
2.1	45	55
12	35	65
18	15	85
20	0	100
25	0	100
25.1	70	30
30	70	30

Table 3. MS Set Up

ESI Probe	Q Exactive HF
Sheath gas 45	Pos. Ion (250-1200 amu)
	Neg. Ion (200-1200 amu)
Aux gas 8	MS Resolution, R = 120K FWHM at m/z 200
Spray Voltage 3.5 kV	Top15 dd-MS^2, R = 30K
	FWHM at m/z 200
S-Lens 50	MS^2 Isolation Width 1 Da
Cap. Temp.	Stepped NCE
320°C	Pos. Ion: 25, 30
	Neg. Ion: 20, 24, 28
	AGC target
Heater Temp. 350°C	1E+6 MS, 50 msec max.
	1E+5 MS^2, 80 msec max.

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Mass Spectrometry

A Q Exactive HF mass spectrometer equipped with HESI-II ion source was employed. The Q Exactive HF mass spectrometer features an ultra-high field Orbitrap analyzer which doubles its speed and resolution compared to the first generation of Orbitrap analyzer (Figure 1). The ultra-high resolution (up to 240,000, FWHM at m/z 200) of the Q Exactive HF MS allows accurate mass measurement with less than 3 ppm accuracy with external calibration, and the faster scan speed (up to 18 Hz) of the Q Exactive HF MS result in higher number of precursor ions selected for MS/MS experiments. As a result, more lipid identifications in a single HPLC-MS-MS run can be achieved with improved sensitivity, accuracy, and productivity. The instrument was operated via a data dependent LC MS/MS method (top15 MS^2) in both positive mode and negative modes, respectively. The instrument method and operating conditions are shown in Table 3.

Data Analysis

LipidSearch 4.1 SP1 software was used for lipid identification and quantitation with following data processing workflow (Figure 2).

1) Peak Detection. Read raw files, MS^n and precursor ion accurate masses.

2) Identification. Candidate molecular species are identified by searching a large database > 10E+7 entries of accurate masses (lipid precursor and fragment ions) predicted from potential lipid and positive/negative ion adducts.

3) Alignment. The search results for each individual sample are aligned within a time window and the results are merged into a single report. Positive and negative ion adducts are grouped for lipids eluting at the same retention time.

4) Quantification. Accurate-mass extracted ion chromatograms are integrated for each identified lipid precursor and peak areas are obtained. Analyte concentration for each lipid class was estimated relative to the concentration of internal standard.

5) Statistical Analysis. t-Tests determine which molecular species are significantly different between sample and control groups, and the results are displayed in whisker plots.

FIGURE 1. Layout of Q Exactive HF Mass Spectrometer

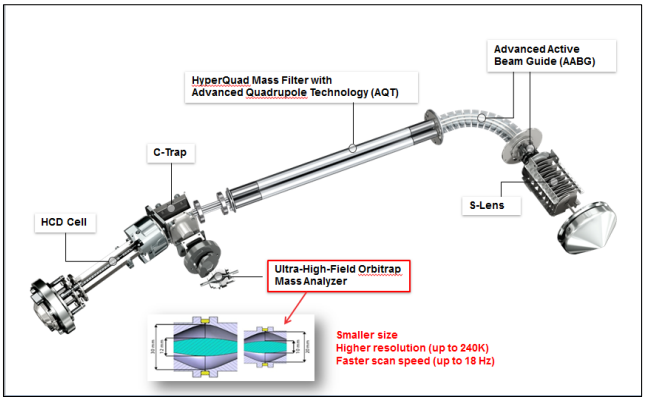
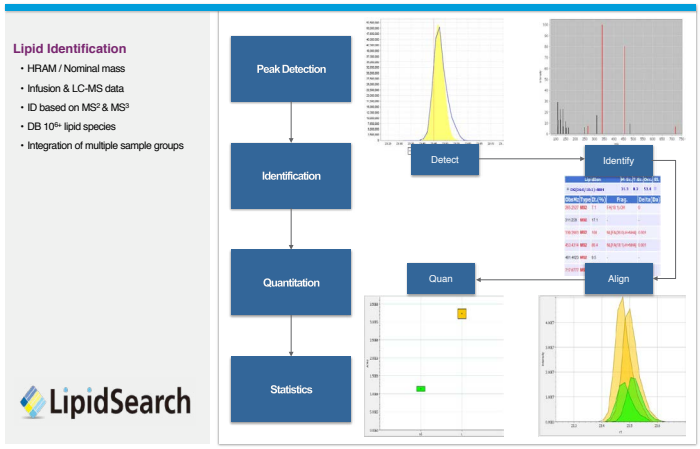


FIGURE 2. Simultaneous Lipid Identification and Quantification Using LipidSearch 4.1 SP1 Software



Results

Each rat plasma was analyzed in triplicate using positive and negative ionization modes, respectively. Figure 3 shows both positive and negative base peak chromatograms observed from one ZDF rat plasma extract. Ultra high resolution and faster MS/MS scan speed enabled high mass accuracy and good quality of MS/MS over wide dynamic range. The C30 column coupled with the Q Exactive HF mass spectrometer successfully identified and quantified large range of lipid molecule from the ZDF plasma and lean control plasma samples using Lipid Search software. 750 lipid species were identified with high confidence and quantified with great analytical precision from the rat plasma samples (Table 4). More than 300 identified lipid species, especially PC and TG showed significant fold changes between the ZDF and lean groups. Figure 4 shows that the fatty acids were confidently assigned for two closely eluting two PC isomer species using the MS-MS data. The fold changes of two identified PC species were calculated using the integrated full MS peak areas using 3 ppm mass extraction window. While PC 18:3_18:2 did not show any significant change between the ZDF and Lean rat group PC 16:1_20:4 was significantly higher in the ZDF rat group and could be a potential biomarker candidate.

FIGURE 3. Basepeak Chromatogram of ZDF Plasma

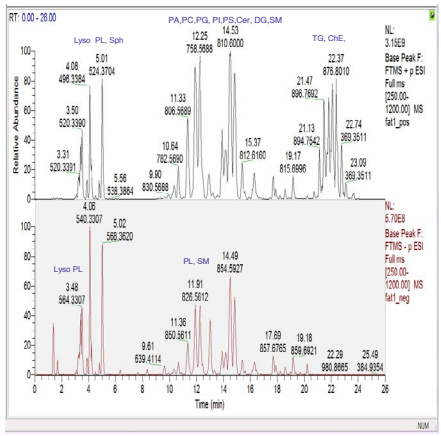
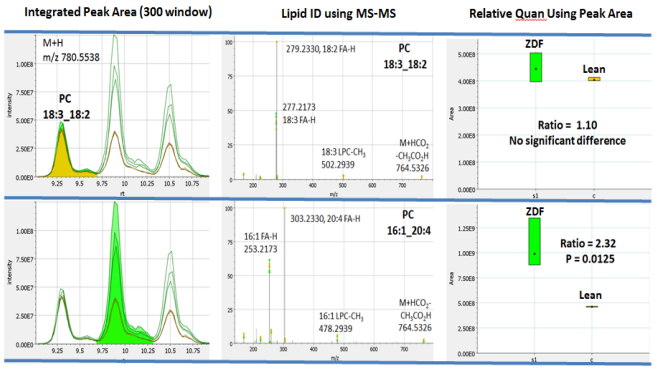


Table 4. Summary of Lipid IDs and Quantification

Class	Filtered*	p < 0.05
PA	1	0
PC	135	93
LPC	110	77
PE	38	0
LPE	4	3
PG	4	2
PI	42	30
PS	3	3
Cer	21	13
$CerG_1$	13	10
SM	28	15
AcCa	19	14
ChE	11	5
SiE	5	3
StE	3	0
DG	30	29
TG	283	52
Total	**750**	**349**

FIGURE 4. Simultaneous Lipid ID and Relative Quantification of Two Different PC Species in Rat Plasma Using LipidSearch Software



Conclusion

- A HRAM LC-MS-MS approach was successfully applied to lipidomics profiling of total lipid extracts from Zucker Diabetic Fatty (ZDF) and Zucker Lean rat plasma using the Q Exactive HF mass spectrometer coupled with a C30 column.
- Orbitrap data combined with LipidSearch software allows the simultaneous lipid identification and quantitation in the same experiment.
- 750 lipid species were identified with high confidence and quantified with very good analytical precision from rat plasma extracts. More than 300 different molecular lipid species had significant fold changes between the ZDF and lean groups and provide valuable information for discovering potential diabetic biomarkers.

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