Shotgun Lipidomics of prostate cancer cells using ESI-MS Shimadzu 8050 and simplified data analysis by SimLipid software

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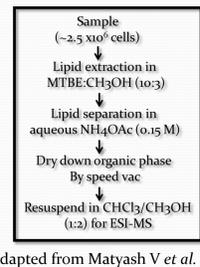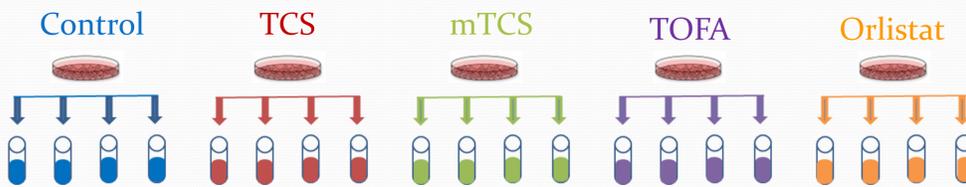
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Introduction

Lipidomics is a relatively recent omics field of research which includes complex lipidome analysis. It is an emerging field in biomedical research as lipids play an important role in cell, tissue and organ physiology and have potential as biomarkers of disease or treatment success. Shotgun lipidomics[1] involves identification and quantification of lipids by direct infusion of complex lipid samples into the mass spectrometer without any chromatographic separation. In 2017 prostate cancer is the most commonly diagnosed cancer in Australia among males. It is estimated that the risk of a male being diagnosed with prostate cancer by his 85th birthday will be 1 in 7.

Method

### LNCaP cells with different treatments

Control   TCS   mTCS   TOFA   Orlistat

Sample (~2.5 x10^6 cells) → Lipid extraction in MTBE:CH$_3$OH (10:3) → Lipid separation in aqueous NH$_4$OAc (0.15 M) → Dry down organic phase By speed vac → Resuspend in CHCl$_3$/CH$_3$OH (1:2) for ESI-MS

Adapted from Matyash V *et al.* 2008

Shimadzu QqQ 8050 (LC-ESI-MS)

Lipid class	Ion mode	Scan type	S/N > 3
Head group scan			
PC/SM	+Ve	PI 184.1	√
PE	+Ve	NL 141	√
PS	+Ve	NL 185	√
PS	-Ve	NL 87	√
PG	+Ve	NL 172	√
PA	+Ve	NL 98	X
PI	-Ve	PI 241	√
Ceramides	+Ve	PI 364.3	X
Cholesterol Esters	+Ve	PI 369.4	X
Triacylglycerides			
TAG (14:0)	+Ve	NL 245	√
TAG (16:1)	+Ve	NL 271	√
TAG (16:0)	+Ve	NL 273	√
TAG (18:3)	+Ve	NL 295	√
TAG (18:2)	+Ve	NL 297	√
TAG (18:1)	+Ve	NL 299	√
TAG (18:0)	+Ve	NL 301	√

Lipid class	Ion mode	Scan type	S/N > 3
Triacylglycerides contd.			
TAG (20:5)	+Ve	NL319	√
TAG (20:4)	+Ve	NL 321	√
TAG (20:3)	+Ve	NL 323	√
TAG (20:2)	+Ve	NL325	√
TAG (20:1)	+Ve	NL 327	√
TAG (20:0)	+Ve	NL 329	√
TAG (22:6)	+Ve	NL 345	√
TAG (22:5)	+Ve	NL 347	√
TAG (22:4)	+Ve	NL 349	√
TAG (22:3)	+Ve	NL 351	√
TAG (22:2)	+Ve	NL 353	√
TAG (22:1)	+Ve	NL 355	√
TAG (22:0)	+Ve	NL 357	√
TAG (18:3)	+Ve	NL 295	√
TAG (18:2)	+Ve	NL 297	√
TAG (18:1)	+Ve	NL 299	√
TAG (18:0)	+Ve	NL 301	√

Example Phospholipid scans

PC

PE

PS

PI
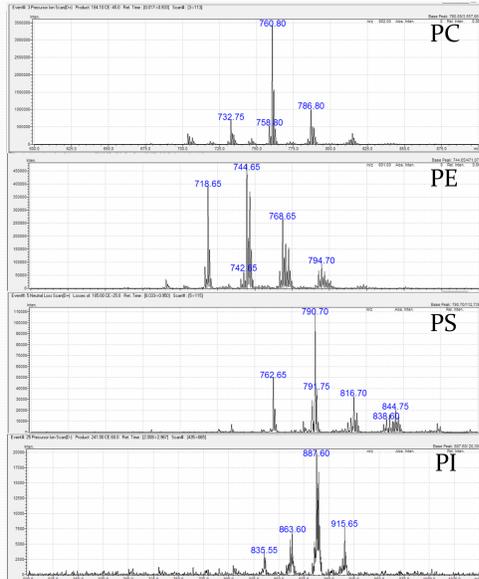
Example Triacylglyceride scans

TAG 16:1

TAG 16:0

TAG 18:1
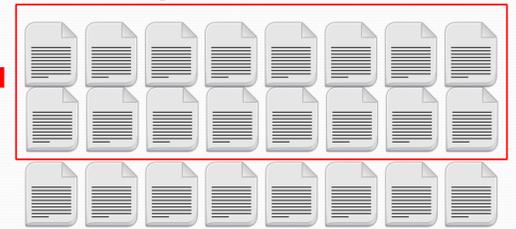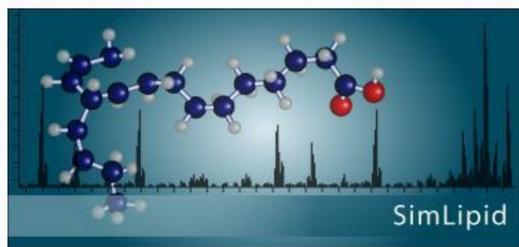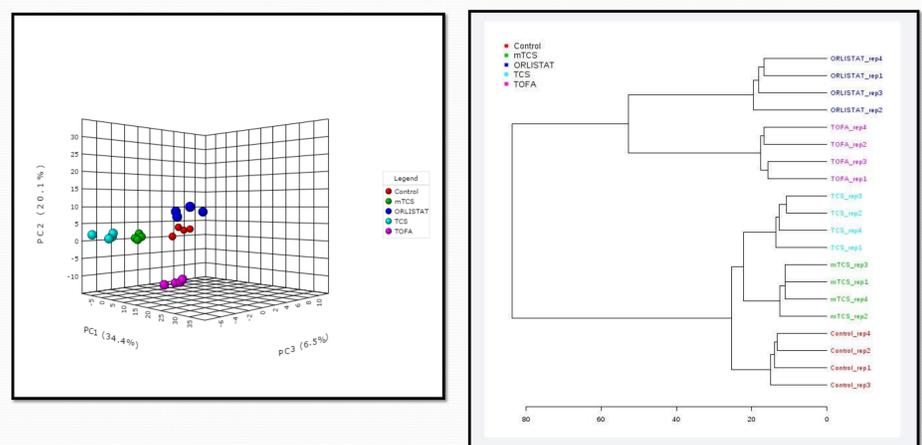
TAG 18:0
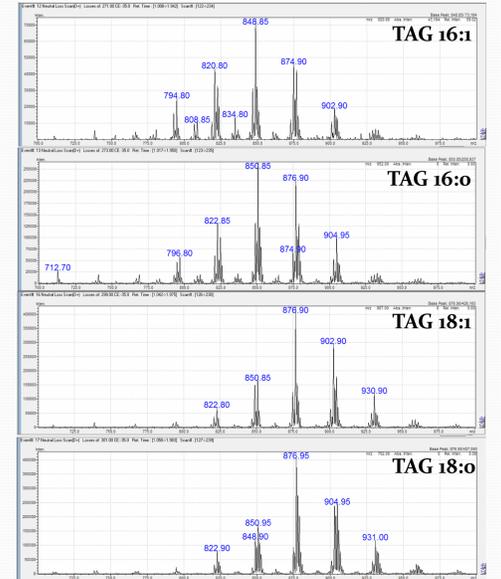
Good quality mass spectra obtained from different classes of lipids

High throughput data analysis

Import raw data from native file format (.lcd) into SimLipid software in batch mode

MetaboAnalyst software

Export SimLipid results into CSV file, and perform Statistical analysis using MetaboAnalyst software

Summary

High throughput data analysis was performed by directly importing raw Shimadzu data files (.lcd format) into SimLipid. Preliminary data analysis with Simlipid has shown some promising functionalities for both qualitative and quantitative analysis. The statistical analysis revealed very good correlation by PCA analysis and good stratification of different treatments .

Future work will include addition of class specific internal standards for absolute quantitation.

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References:
1. Han X and Gross R. W. The journal of Lipid Research, (2003) 44: 1071-1079.
2. Matyash V *et al.* The journal of Lipid Research, (2008) 49: 1137-46.