Analysis of minor lipids in subcutaneous and epicardial fat tissue

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Introduction

Over the last years fat tissue has come to the fore as an important endocrine organ secreting adipokines. The adipokines participate on metabolism, satiety control, fat distribution, insulin managment, energy expenditure or inflammation processes. Risk of metabolic diseases significantly depends on adipose tissue distribution and activity. Fat depots are anatomically divided into two classes as the subcutaneous (SAT) and visceral white adipose tissue (VAT).

Adipose tissue is composed primarily from triacylglycerides (TAGs). Jové et al. described lipid profile of human omental and SAT. As expected most of identified lipids (99%) were the glycerolipids [1]. However, for better understanding of development of metabolic diseases it is inevitable to measure not only lipids involved in energy storage, but also minor lipids, which can play role in signaling pathways and membrane composition. However, to the best of our knowledge, there is no study focused on analysis of nonpolar metabolites, with special accent on minority compounds.

[1] M. Jové, J.M. Moreno-Navarrete, R. Pamplona, W. Ricart, M. Portero-Otín, J.M. Fernández-Real, Human omental and subcutaneous adipose tissue exhibit specific lipidomic signatures, FASEB J. 28 (2014) 1071–1081. doi:10.1096/fj.13-234419.

The aim of the study in the first step was to optimize and validate LC/MS method for analysis of both major and minor bioactive lipids in adipose tissue.
The optimized method was then used for analysis of real patients samples of subcutaneous and epicardial adipose tissue.

Methods

Method validation: Mixture of subcutaneous tissues sample was divided into 5 samples and underwent extraction process (Fig. 1). Accuracy, reproducibility, optimal way of storage were tested (with or without RNAlater) .

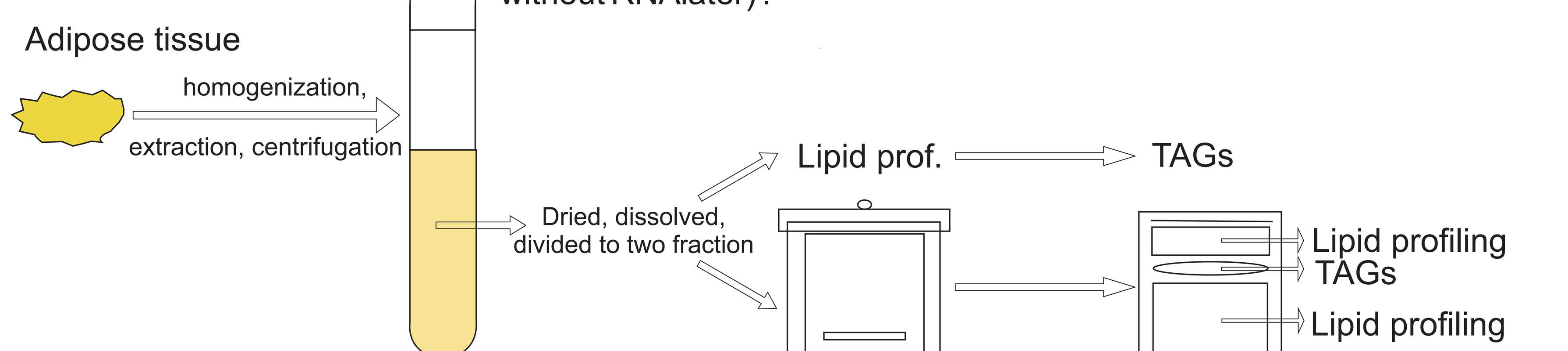

Fig. 1 Experiment workflow

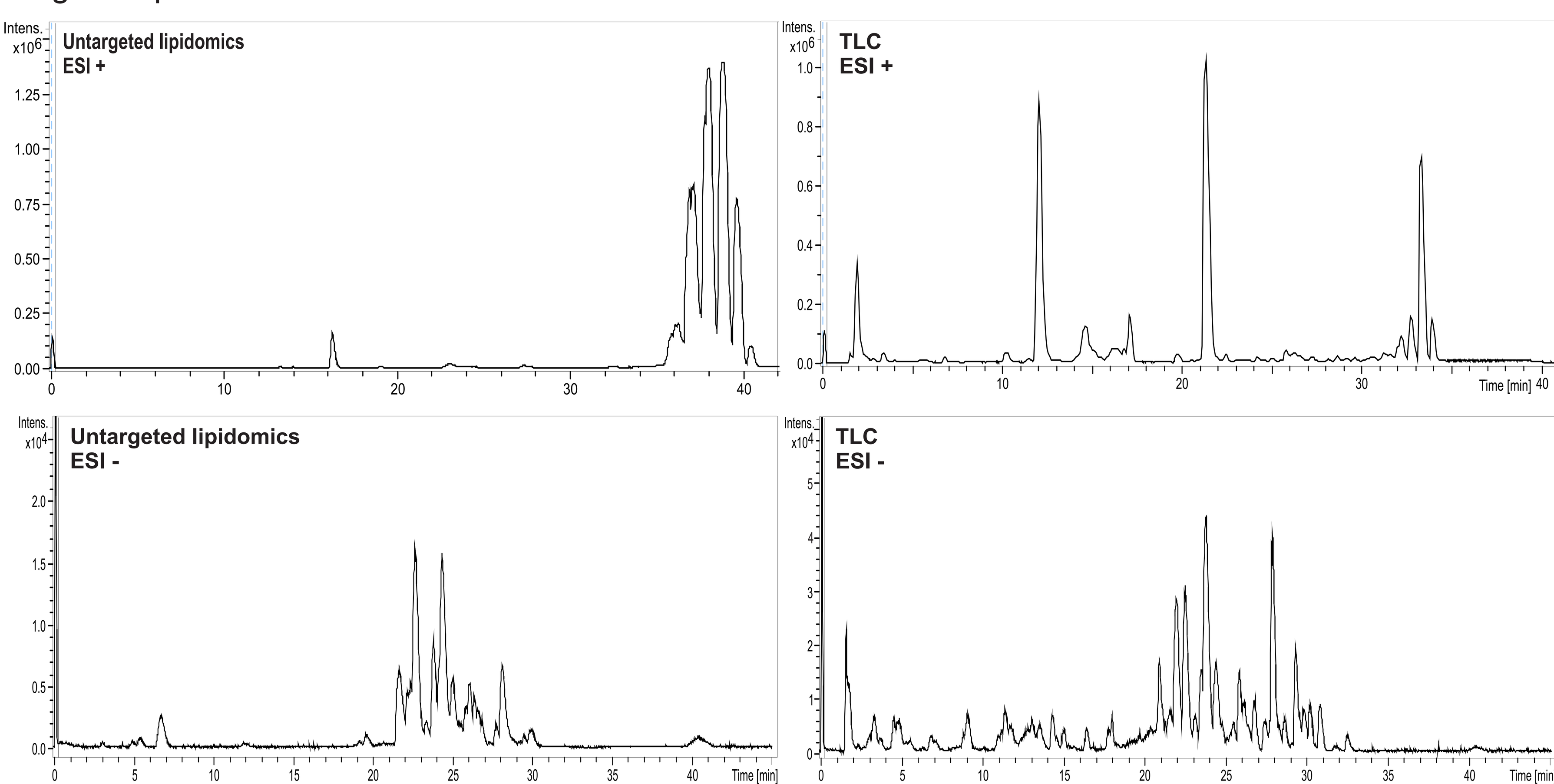

Fig. 2 LC/MS chromatograms of untargeted lipidomics and lipid profile after removel of TAG by TLC in negative and positive mode

LC/MS: LC- HPLC Agilent 1200 LC system (Agilent Technologies, California, USA), MS- micrOTOF-Q III (Bruker Daltonics, USA), ESI

Lipidomics: column- Accucore C30 (Thermo Fisher Scientific, Waltham, USA), flow rate 0.22 ml/min, temperature 40°C, A mobile phase:10 mM ammonium formate:acetonitrile 4:6 (v:v), 0.1% FA, B mobile phase:10 mM ammonium formate in acetonitrile:isopropanol 1:9 (v:v) 0.1% FA
The elution gradient was: 30% B for 5 min, 30% - 43% B for 2 min, 43% - 50% B for 1.3 min, 50% – 70% B for 11.3 min, 100% % B for 7 min, 18% B for 10 min. The injection volume was 1 μl.

Data analysis: The MS was calibrated by clusters of natrium formate before sequences started and also during sample analysis in death time of analysis, software: DataAnalysis 4.2 (BrukerDaltonics, USA), MZmine 2.23, S/N ratio: 5. mass range of 100 - 1500 Da, time tolerance 0.15 min, mass tolerance 10 ppm, minimal peak duration 0.2, maximal peak duration 1.2 min, intensities of peaks were normalized on sum of peaks.

Database: LIPID MAPS website (http://www.lipidmaps.org), Human Metabolome Database (http://www.hmdb.ca)

Real patients samples: Samples of subcutaneous and epicardial fat tissue were obtained from patients (n=10) who underwent cardiovascular surgery. Samples were processed by previously validated method.

Conclusions

Using our method we separated major TAGs from less abundant metabolites and thus we identified broad range of metabolites such as di- and mono-acylglycerides, phosphocholines, phosphoserines, phosphoinositols, phosphatidic acids, sphingomyelins, ceramides, cholesteryl esters, phosphoethanolamines, free fatty acids, and other metabolites in adipose tissue.
Method was used for analysis of real patients samples of epicardial and subcutaneous fat tissue. We observed 18 lipids significantly changed between epicardial and subcutaneous adipose tissue.

Acknowledgements

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Results and discussion

Method validation:

Repeatability was evaluated by coefficient of variation (CV) for both fraction and all picked peaks. Meadian of CV for untargeted lipidomics was 5 %, however this fraction contained mostly triacylglycerols (TAGs). Study was extended by analysation of minor bioactive lipids (phosphocholines, phosphoserines, phosphoinositols, phosphatidic acids, sphingomyelins, ceramides, cholesteryl esters, phosphoethanolamines and also other metabolites). As we expected, measurement of minor metabolites and TLC separation was characteristic by higher CV, especially for phosphatidic acids.Median CV was 12.5%.

Accuracy was tested through the comparison of TAGs obtained from TLC and from untargeted lipidomics. Most of metabolites (148) did not differ significantly (p-value > 0.05). 12 metabolites differ significantly (p-value < 0.05). Despite antioxidizing measures (adding BHT to sample, darkness during sample preparation) some molecules with residuals of unsaturated fatty acids was degraded on TLC. In case of real patients samples it is necessary to compare data acquired by the same method.

The impact of **samples collection** in RNAlater was verified for minor lipids. 11 out of 163 metabolites, which were affected, were not identified by exact mass or were included in derivatives of phosphatidic acid, which were also known for greater scattering of CV.

Real patients samples

Tab 1. Lipids significantly changed between subcutanous an epicardial adipose tissue

	m/z	Adducts	R.T.	FC	p-value
SM(16:1)	703.5700	$[M+H]^+$	21.2	1.50	0.021
Cer(18:1)	610.5413	$[M+HCOO^-]^-$	26.0	0.56	0.017
PI(38:5)	883.5386	$[M-H]^-$	20.9	0.36	0.010
PE(36:4)	766.5366	$[M-H]^-$	24.4	0.55	0.014
PC(32:0)	734.5693	$[M+H]^+$	23.5	13.30	0.001
PC(32:1)	732.5529	$[M+H]^+$	21.3	6.40	0.045
PC(34:1)	760.5821	$[M+H]^+$	24.1	1.55	0.014
PC(34:2)	758.5664	$[M+H]^+$	21.2	1.86	0.006
PC(36:1)	788.6130	$[M+H]^+$	26.4	1.56	0.009
PC(36:2)	786.6001	$[M+H]^+$	24.7	1.83	0.002
PC(36:4)	782.5657	$[M+H]^+$	21.4	8.55	0.001
PC(36:5)	780.5495	$[M+H]^+$	21.8	7.12	0.009
PC(38:4)	810.5982	$[M+H]^+$	23.8	7.80	0.002
DG(18:1/18:0)	640.5840	$[M+NH_4]^+$	28.3	1.90	0.004
TAG(16:0/18:1/18:2)	874.7848	$[M+NH_4]^+$	33.7	0.67	0.006
TAG(18:1/18:2/18:1)	900.8008	$[M+NH_4]^+$	33.8	0.64	0.026
TAG(16:0/18:1/18:1)	876.8012	$[M+NH_4]^+$	34.3	0.65	0.021
TAG(18:3/18:1/20:0)	928.8256	$[M+NH_4]^+$	33.0	0.77	0.026

FC- fold change (relative intensity of epicardial adipose tissue to subcutaneous adipose tissue), R.T.- retention time, SM- sphingomyelin, Cer- ceramide, PI-glycerophosphatidylinositol, PE- gycerophosphatidylethanolamine, PC- glycerophpatidylcholine, DG-diacylglycerol, TAG- triacylglycerol

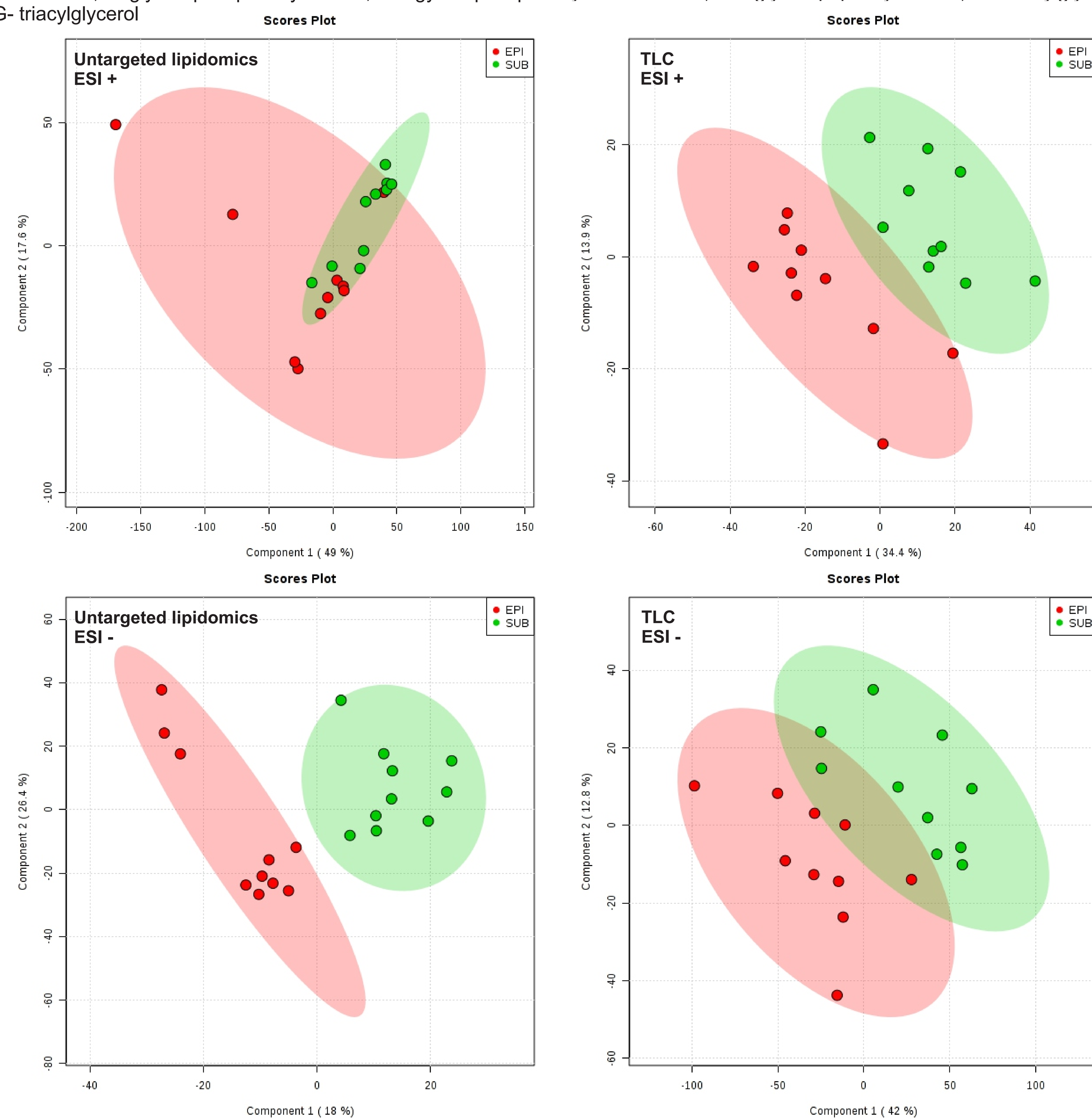

Fig. 3 PLS of untargeted lipidomics and lipid profile after removel of TAG by TLC in negative and positive mode