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Lipid parameters associated with obesity identified using a high-throughput Shotgun Lipidomics technology

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ABSTRACT

Introduction

Lipid metabolism plays a central role in common lifestyle-associated diseases such as diabe-tes type 2 and cardiovascular disease. Hence, lipidomics, the quantitative analysis of lipid compositions of clinical and biological samples is becoming an increasingly popular tool in clinical research and biomarker studies. Mostly in order to achieve sufficient statistical power, there is a need for the analysis of hundreds to thousands of samples in a reasonable timeframe and with high analytical quality. Due to the absence of time-consuming LC-separation of individual lipid classes, high-resolution mass spectrometry-based Shotgun Lipidomics fulfills these requirements. Here, Shotgun Lipidomics was applied in order to screen for markers of obesity, a risk factor for diabetes type 2, in blood serum of 571 subjects from the Malmö Diet and Cancer Cardiovascular Cohort.

Methods

Quantitative lipid analysis was performed on a high throughput mass spectrometry-based shotgun lipidomics platform allowing for the measurement of 200 serum samples per day. Li-pids were extracted from 2 µl of blood serum using MTBE/MetOH 7:2 (v/v) with a Hamilton Starlet liquid handling station. Lipid class-specific internal standards were added prior to ex-traction. Mass spectra were acquired by direct infusion MS with a Triversa Nanomate nano-ESI source (Advion) coupled to a Q Exactive mass spectrometer (Thermo Fisher Scientific). Spectra were acquired for 5 min in positive and negative ion mode with polarity switch by data-independent acquisition. Lipids were identified based on precursor and fragment ions and normalized to internal standards with in-house developed software.

Preliminary data

Direct infusion mass spectrometry enabled by high resolution Q Exactive mass spectrometers (Thermo Fisher Scientific) allows for robust and efficient lipidomic screening of hundreds of samples without the need for time-consuming chromatographic separation of lipid classes. About 400 individual lipid molecules belonging to 22 lipid classes are quantified with sub-µM sensitivity, a precision of <10%, and an inter-site variation of <15% with a total measurement time of 5 min per sample.

In the example presented here, technical variation across the entire sample set was 9% (c.v.). Out of a total of 1151 lipidomic features, regression analysis revealed about 200 lipidomic features positively or negatively associated with BMI. For example, significant positive associations were found mainly for triglycerides while ether phospholipids were negatively associated with BMI. Furthermore, differential associations within lipid classes could be identified: triglycerides containing long and unsaturated fatty acid moieties were negatively associated with BMI. Categorization of subjects into low (BMI < 25 kg/m²) and high BMI (BMI > 30 kg/m²) groups and subsequent classification by a support vector machine based on serum lipidomes resulted in sensitivity and specificity of 82% and 86%, respectively. The area under the ROC curve was 89%. Thus, lipidomic screening enabled the identification of a robust lipid signature associated with BMI.

Novel aspect

High resolution MS-based Shotgun Lipidomics opens up exciting opportunities for efficient lipidomic screening in biomarker studies and clinical research.

WORKFLOW

Sample extraction ▸ Sample infusion ▸ MS analysis ▸ Lipid identification ▸ Data analysis

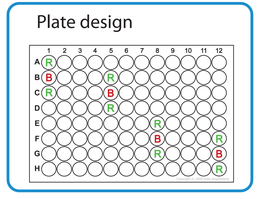
Sample preparation and infusion

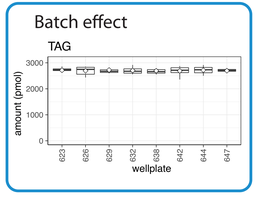
- Fully automated 1-step extraction
- Extraction and infusion in 96-wellformat
- Robotic nano-ESI source

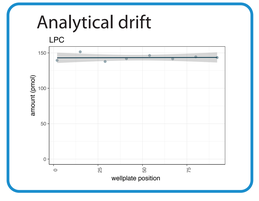
Mass spectrometry

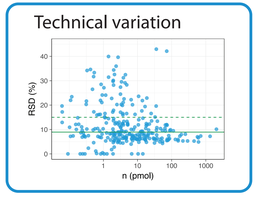
- ThermoFisher Q Exactive Plus
- Resolving power up to $R_{m/z=200}$ = 280,000
- Polarity switch - full lipidome coverage in 1 measurement
- Data-independent acquisistion (MSMS)

QUALITY ASSESSMENT







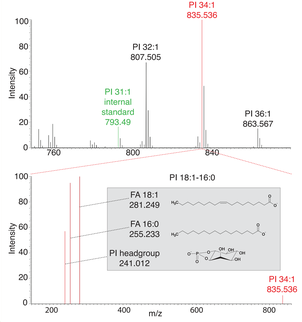


- 571 samples analysed in 8 batches
- 584 lipid molecules quantified
- 8 plasma reference samples per batch; evenly distributed across 96-well plate

= 58 reference samples for the entire screen

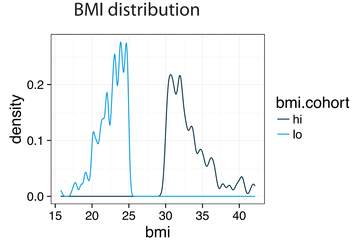
- Assessment of batch effects and analytical drift
- High throughput: 2 batches/day
- Technical variation = 9% (c.v.)
- ca. 85% of lipid molecules with c.v. <15%
- 2 µl of blood serum

METHOD FEATURES



- Full quantitation based on lipid class-specific internal standards
- Confirmation of identification by MSMS
- Detailed structural information: lipid species and molecular species
- 5 min per sample MS time
- High throughput: 200 samples/day
- Technical variation <10% (inter-site!)
- Coverage up to 2,300 individual lipid molecules belonging to >20 lipid classes

COHORT CHARACTERISTICS



Subjects with BMI < 25 (normal) and BMI >30 (obese) were selected in order to create high and low BMI cohorts. Subjects with intermediate BMI were excluded from the analysis.

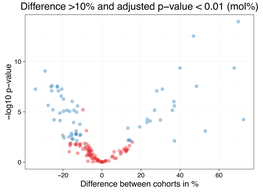
parameter	high	low	significance
subjects	117	205	
male (%)	37	33	
age (yr)	72	73.5	
HDL-chol (mM)	1.25	1.55	*
LDL-chol (mM)	3.18	3.14	
LDL/HDL	2.7	2.18	*
cholesterol (mM)	5.01	5.1	
TG (mM)	1.35	0.92	*

Cohorts differ significantly for HDL-cholesterol, LDL/HDL ratio, and Triglycerides (TG). Otherwise the characteristics of the subjects in the cohorts are similar.

RESULT

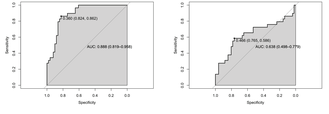
Data analysis approach

- T test to identify significantly different lipids
- Split data in test and training set for machine learning (support vector machine)
- 10-fold cross validation
- Classify samples into high and low BMI in test set
- Compare classification performance with clinical lipid parameters: triglycerides, HDL/LDL ratio, total cholesterol, HDL, LDL



60 out of 200 lipids differ significantly between high and low BMI.

Lipidomics Clinical parameters



ROC curves for the SVM classification algorithm.

parameter	lipidomics	clinics
AUC	89%	64%
Specificity (high)	86%	66%
Sensitivity (low)	82%	61%

CONCLUSION

- For 8-9 out of 10 subjects, lipidomics data allow for the correct prediction of BMI
- Higher information content in lipidomes than in clinical parameters

REFERENCES

"An Automated Shotgun Lipidomics Platform for High Throughput, Comprehensive, and Quantitative Analysis of Blood Plasma Intact Lipids.", Surma MA et al. 2015, EJLST (doi:10.1002/ejlt.201500145)

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