Lipidomics on a High Fat Diet mouse model indicate alterations in lipid metabolism upon aerobic exercise and calories restriction

Thomai Mouskeftara[1, 2]; Christina Virgiliou[2, 3]; Ioannis I. Moustakas[4]; Antonios Chatzigeorgiou[4]; Eric Halls[5]; Carsten Baessmann[6]; Helen Gika[1]

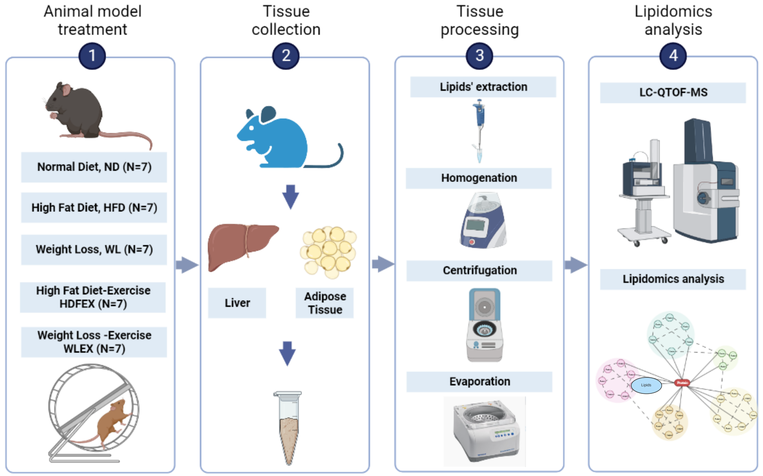
1. Department of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece
2. Biomic AUTh, Center for Interdisciplinary Research and Innovation, Thessaloniki, Greece
3. School of Chemical Engineering, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
4. Medical School, National and Kapodistrian University of Athens, Athens, Greece
5. Bruker Daltonics, Billerica, MA, USA
6. Bruker Daltonics GmbH & Co. KG, Bremen, Germany

Introduction

Liver is a major metabolic organ in the human body and the dysregulation of hepatic lipid metabolism is associated with obesity, metabolic syndrome, T2D and NAFLD.
Adipocytes are cells that form and store lipids in adipose tissue, they play a major role in energy homeostasis. Detailed investigation of the lipidome remodeling upon high fat diet (HFD) or weight loss (WL; caloric restriction) as well as the influence of physical activities in those conditions can help to understand the mechanisms underlying dyslipidemia in metabolic conditions.
To this end, lipidomics signatures of HFD fed or mice undergoing weight loss (WL; calories restricted) were compared with animals with and without exercise (EX) exposure. The aim was to address liver and adipose tissue specific metabolic processes involved in weight loss upon exercise activities.

Methods

C57Bl6 male mice (7 animals per group) were fed calorie restricted or HFD for 20 weeks, followed by aerobic exercise or caloric restriction or both interventions for the last 8 weeks.
Mouse liver tissues were extracted with MTBE: MeOH 3:1 (v/v), while for adipose tissue a mixture of $CHCl_3$: MeOH 2:1 (v/v) was used. Homogenization was followed, using a Bead mill Homogenizer. Lipids were separated on Acquity UPLC CSH C18, 2.1×100 mm, 1.7 μm column (Waters Ltd., Elstree, U.K.) with H_2O-ACN-IPA (containing 10 mM ammonium format and 0.1% formic acid) gradient with a flow rate of 0.4 mL/min and column temperature of 55 °C.
LC-MS/MS data were acquired on a timsTOF mass spectrometer (Bruker Daltonics, Bremen, Germany) in both polarities for liver and only in positive mode for adipose tissue, using data depended acquisition (DDA). Data were acquired in a mass range 50-1200 m/z with fixed spectra acquisition rate for MS/MS data at 20 Hz and 0.3 sec cycle time. Collision energy was set to 30 volts for all molecules. The timsTOF was calibrated prior each analysis with 10 mM sodium formate. Data was processed using the MetaboScape software (Bruker Daltonics).



Results

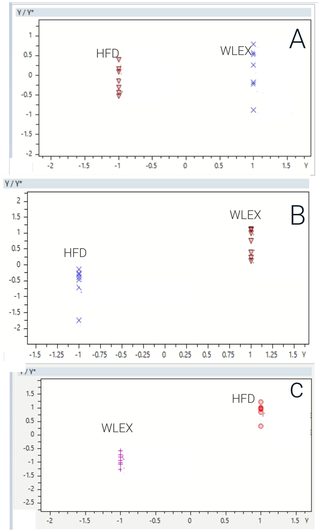


Figure 1. PLS models showing the classification of HFD –WLEX (+ESI) liver (a), HFD –WLEX (-ESI) liver (b) and HFD –WLEX (+ESI) adipose tissue (c), based on the UHPLC–TOF/MS lipidomic profile.

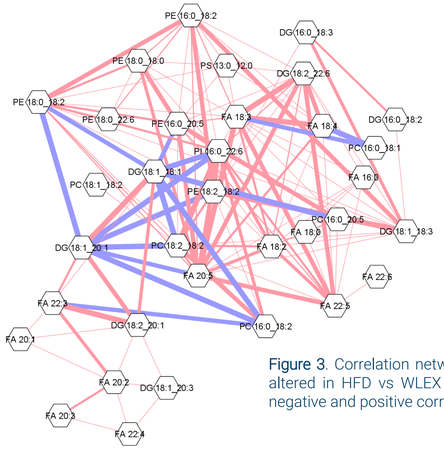


Figure 3. Correlation network (Pearson's r^2 value above 0.9) for lipids differentially altered in HFD vs WLEX groups in liver samples. Purple and pink lines illustrate negative and positive correlation, respectively; thicker lines reflect higher r^2 values.

Summary

- In liver samples strong correlations between PC and PE with same bulk numbers were observed (correlation network Figure 3) indicating dysregulation in biosynthesis of PE and PC.
- PS species were also found to be reduced. This may be due to lower levels of PE and PC species, which the PS synthases 1 and 2 uses as substrates to catalyze formation of PS.
- Fatty acids content was decreased in HFD mice which is mentioned in the literature[1] for FAs 18:2 n-6 and 18:3 n-3 as well.
- n-6/n-3 PUFA ratio is also found significantly induced in liver mostly because of decreased n-3 PUFAs (FA 18:4, FA 20:5, FA 22:5 in HFD mice (log2 Fold change, Figure 2).
- In WLEX mice the majority of DGs that were found up-regulated, included more PUFA with reduction of saturated fatty acids content, which may be caused by an exercise-induced release of dietary fatty acids that were stored in adipose tissue.

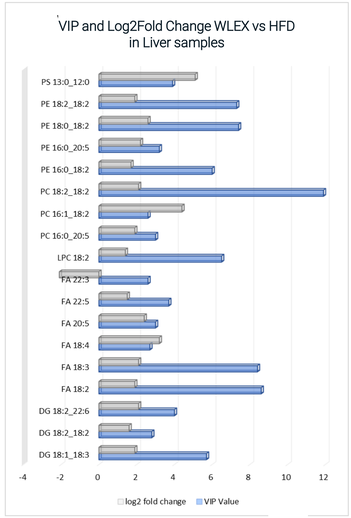


Figure 2. List of identified lipid species in both polarities showing enrichment (positive values) or depletion (negative values) in WLEX livers relative to HFD animals. Only lipids with log2 fold change over ± 1.5 are shown. VIP – variable importance of projection.

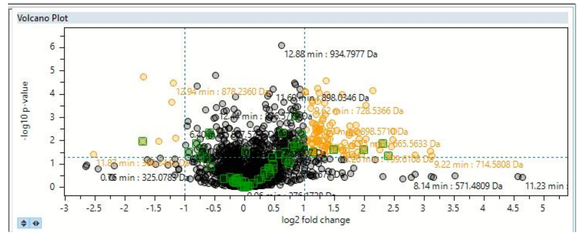


Figure 4. Volcano plot of lipidomics illustrating compounds differing between HFD and WLEX groups in adipose tissue. Each dot represents one compound. The x-axis represents log2 (Fold change), and the y-axis represents -log10 (P-value).

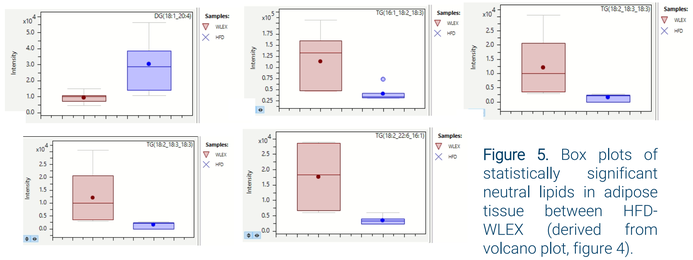


Figure 5. Box plots of statistically significant neutral lipids in adipose tissue between HFD-WLEX (derived from volcano plot, figure 4).

Conclusion

- The results of this preliminary investigation suggest that liver and adipose tissue of HFD-WLEX mice have a distinct lipidomic profile.
- Lipidomic analysis revealed significant alterations in several lipid classes including FA, LPC, LPE, PE, PS, PC and confirmed an already reported reduction of phosphatidylcholine in the developed fatty liver.
- For further investigation, targeted metabolomics analysis of the hydrophilic fraction will be performed to provide more information regarding the correlated pathways.

LC-TIMS-MS

References: 1. Eisinger K et al. doi: 10.1016/j.yexmp.2014.05.002. Epub 2014 May 14. PMID: 24830603.