Global LC-MS multi-omics for investigating the effects of High Intensity Training (HIT)

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BACKGROUND

Physical activity is generally accepted to improve both health and well-being, and is included in the recommended therapy for many diseases. The metabolic changes induced by physical activity occur both during and after the exercise. The effects of physical activity on metabolism is widely studied, but only partially understood. High Intensity Training (HIT) has gained more popularity over the years, and recent studies have shown that HIT-training reduces the overall mortality significantly [1]. However, the metabolic effects of exercise on a global scale is not fully explored. In order to better understand the widespread effects of exercise, approaches such as global metabolomics and lipidomics can be applied. However, the two omics techniques are rarely combined in the same studies, reducing the possibility to study multiple aspects of the metabolic changes under identical conditions. In this study global LC-MS metabolomics and lipidomics were applied to the same sample set, investigating the effects of HIT. **Figure 1** shows the study design.

STUDY DESIGN

Day	1				2									3
Time	08:55	11:20	15:30	21:00	08:55	09:00	09:20	09:30	09:50	10:20	11:20	15:30	21:00	08:55
DBS Sample	●	●	●	●	●	High Intensity Exercise	●	●	●	●	●	●	●	●
	Fasting until lunch	Controlled equal diet			Fasting until lunch	Exercising while fasting	1 min after exercise	10 min after exercise	30 min after exercise	60 min after exercise	Controlled equal diet as Day 1			Fasting until lunch

Figure 1: Study design including dried blood spot (DBS) sampling time points over a 3 day period. The same DBS samples were analyzed with the metabolomics and lipidomics methods.

METHODS

Metabolomics:
For sample preparation 100 µL of 80% MeOH with 0.1% formic acid was added to one DBS punch (3.2 mm), and then mixed for 45 minutes at 45°C at 700 rpm using a Thermomixer Comfort. Supernatant was analyzed.

Instrumentation: Ultimate 3000 HPLC coupled to a Q Exactive Orbitrap MS, **Figure 2**. For separation of metabolites a Pursuit XR Diphenyl (250 x 2.0 mm, 3.0 µm) column was used. See [2] for more information about the global metabolomics LC-MS method.

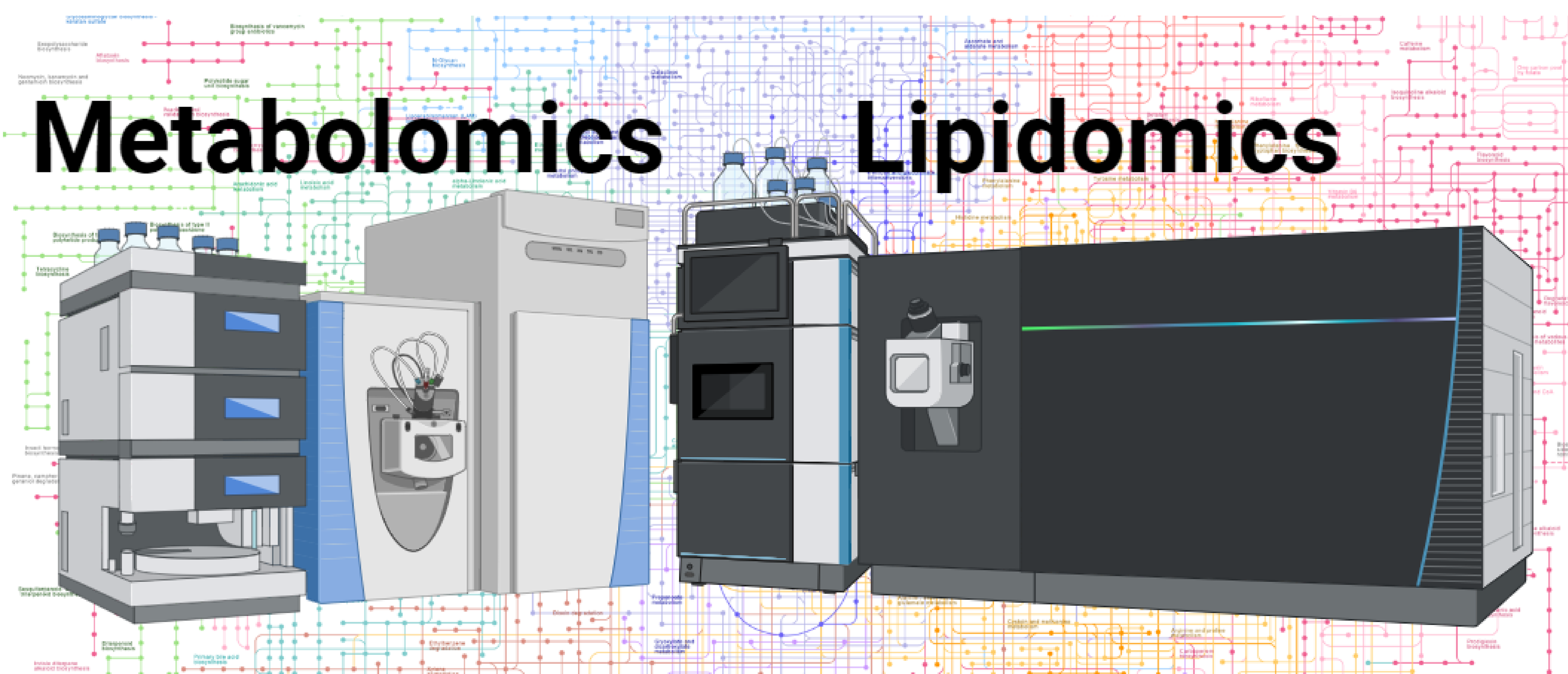

Figure 2: Illustration of Ultimate 3000 HPLC coupled to a Q Exactive Orbitrap MS (left) used in the global metabolomics method and Vanquish Horizon HPLC coupled to a Fusion Orbitrap Tribrid MS (right) used in the global lipidomics method.

Lipidomics:
The extraction process is similar to what is used in the metabolomics sample preparation, except instead of using 80% MeOH we use 100% isopropanol for lipid extraction.

Instrumentation: Vanquish Horizon HPLC coupled to a Fusion Orbitrap Tribrid MS, **Figure 2**. For separation of lipids an Accucore C30 (150 x 2.1 mm, 2.6 µm) column was used. For more information about the global lipidomics LC-MS method please ask Sander Guttorm.

RESULTS

Five volunteers participated in a HIT session and 4 volunteers acted as a control group. Dried blood spot (DBS) samples were collected from the participants at the same time over a 3-day period, **Figure 1**, all participants followed identical diets. **Figure 3** shows the principal component analysis (PCA) plot obtained from the lipidomics analysis.

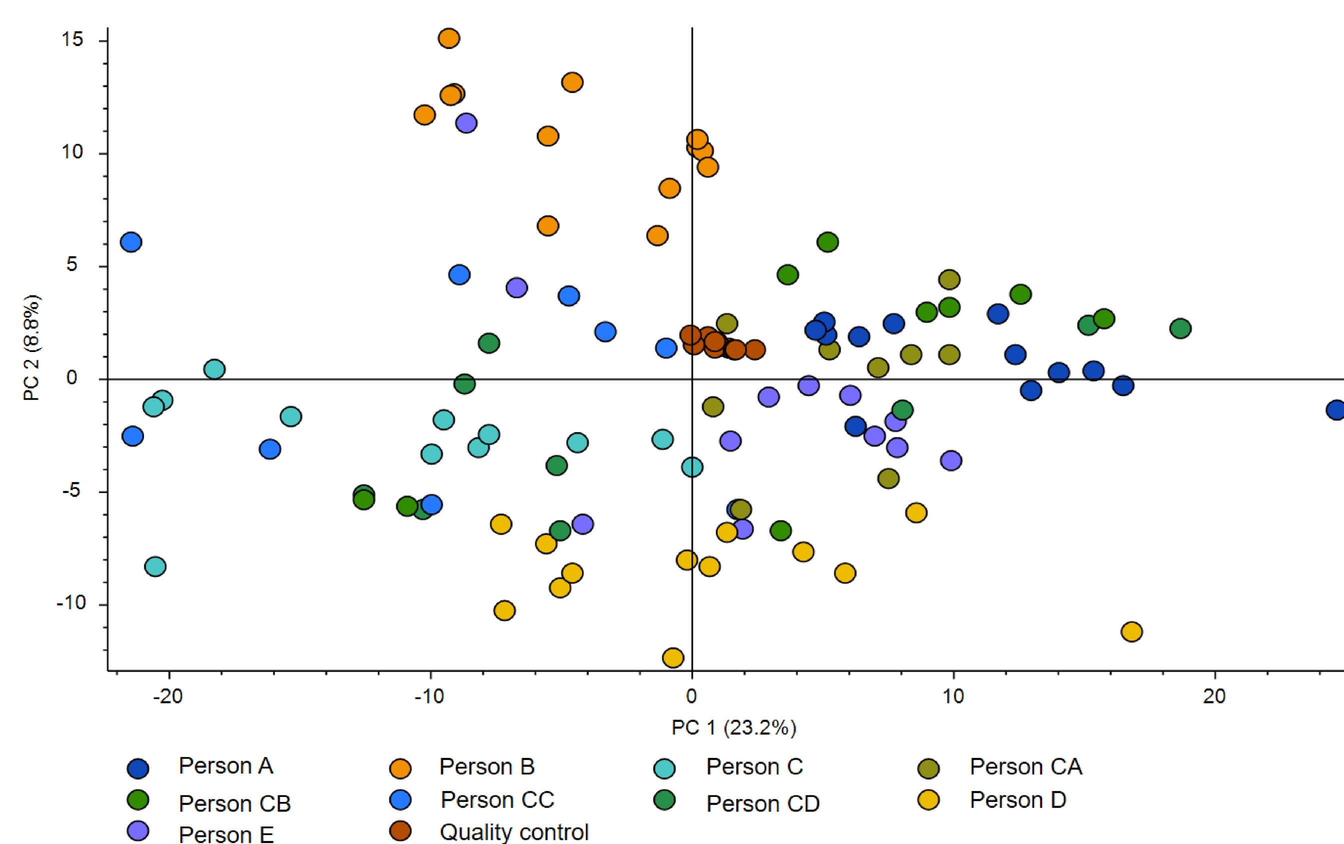

Figure 3: PCA plot of samples analyzed with the global lipidomics method in negative ionization mode. Samples colour coded by participants and quality controls. Person CA, CB, CC and CD were not participating in the HIT session and are therefore the control group.

PCA plot for the collected lipidomes shows some clustering of the different participants, the same trend was seen for the metabolomes. There was no separate clustering of the participants who conducted the exercise and the control group. The quality controls are nicely grouped indicating that the variation observed for the different samples on the PCA level correspond to biological variation and not instrumental variation.

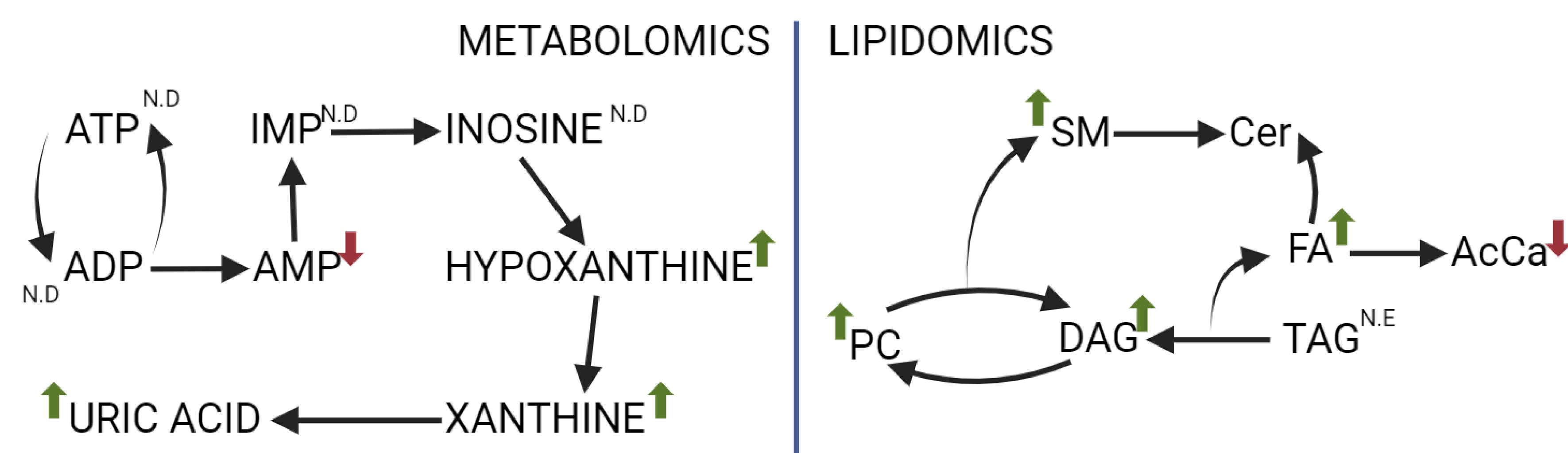

Figure 4: Effects of a single HIT session on single metabolites and lipid classes. N.D: Not detected, N.E: No significant effect from exercising. Phosphatidylcholine (PC), sphingomyelin (SM), ceramide (Cer), diacylglycerol (DAG), triacylglycerol (TAG), fatty acid (FA), acylcarnitine (AcCa).

The global metabolomics approach revealed significant metabolic alterations in energy metabolism, specifically adenine nucleotide catabolism, and hypoxia responses (lactate). The global lipidomics approach, however, could not reveal such responses. Likewise, with the lipidomics approach, significant inflammatory responses and significant alterations in lipolysis were observed that were not detected with the metabolomics approach, **Figure 4**. Two unidentified features were also detected, one with each approach, and were found to be highly significantly associated with HIT, **Figure 5** . For further work these features will be fully identified.

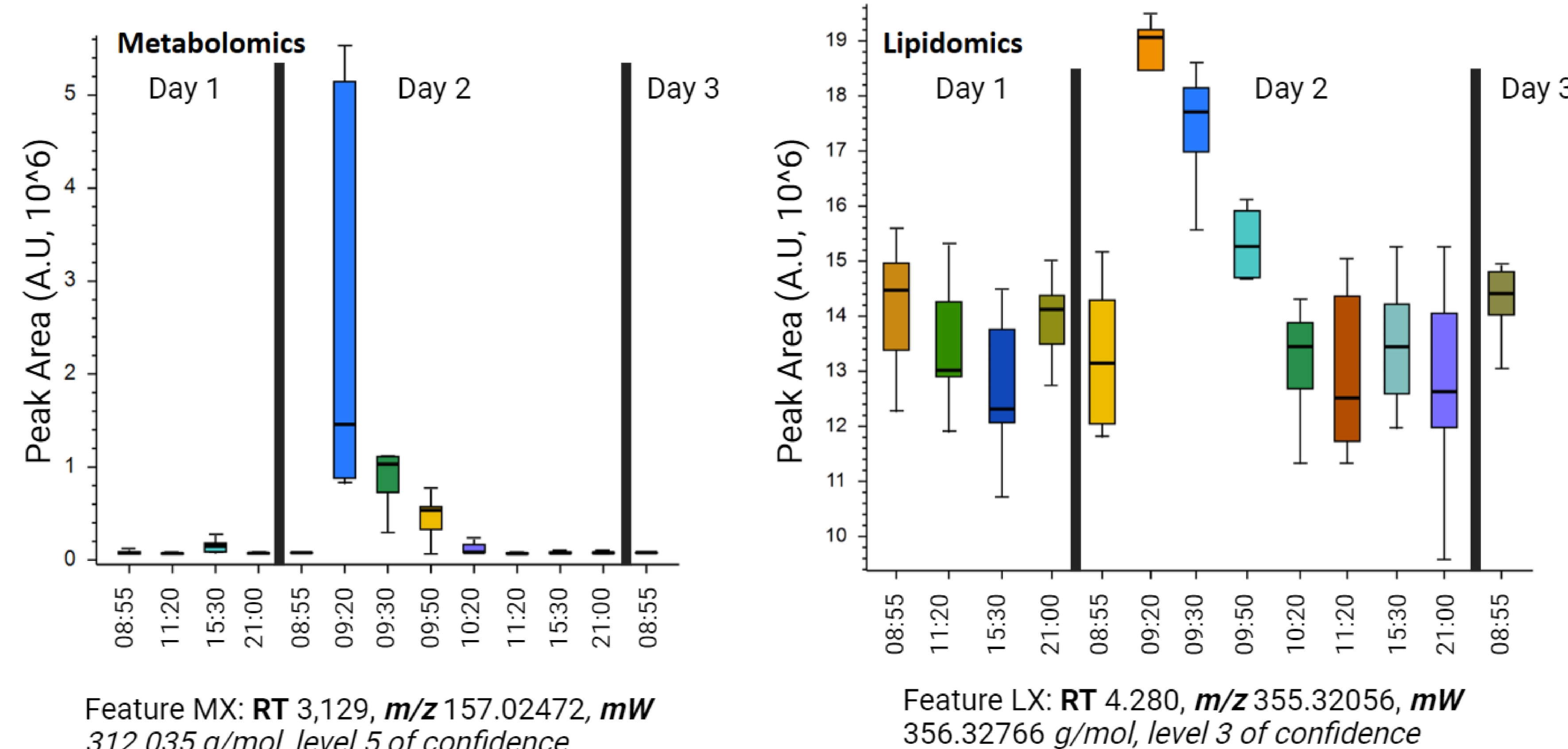

Figure 5: Two unidentified features were found to be highly significantly associated with HIT, one with the metabolomics approach (*m/z* 157.02472, level of confidence 5) and one with the lipidomics approach (C22 lipid, *m/z* 355.32056 with confidence level 3). Peak areas from the 5 participants conducting the high intensity exercise. RT retention time, mW molecular weight.

CONCLUSION

Diversity in chemical properties for the metabolome creates analytical segregation. A combination of global metabolomics and lipidomics therefore provides a wider understanding of how the whole metabolome is affected by an intervention such as a single HIT session.

[1] Stamatakis E., *et al.* Association of wearable device-measured vigorous intermittent lifestyle physical activity with mortality. *Nat Med* **28**, 2521–2529 (2022). https://doi.org/10.1038/s41591-022-02100-x
[2] Skogvold H.B., *et al.* Bridging the Polar and Hydrophobic Metabolome in Single-Run Untargeted Liquid Chromatography-Mass Spectrometry Dried Blood Spot Metabolomics for Clinical Purposes. *J Proteome Res.* 2021;20(8):4010-4021. doi:10.1021/acs.jproteome.1c00326

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