Application Note

SHOTGUN LIPIDOMICS ANALYSIS

Using the TriVersa NanoMate® LESA® for Lipid Analysis in Biomedical Research

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INTRODUCTION

Infusion based Lipidomics analysis (Shotgun Lipidomics) is an analysis strategy that emerged in the last 15 years and involves the liquid extraction of lipids from samples such as blood, tissue homogenates or dried blood spots. As a next step, the extract will be automatically processed for nano-electrospray nESI followed by targeted or global mass spectrometry detection of lipids in the absence of liquid chromatography (Figure 1).

Shotgun Lipidomic experiments can be designed as relative or absolute quantitative assays utilizing the mass spectrometer intensity data and significant computer processing to investigate changes in e.g free fatty acids by principle component analysis. This approach is suitable for a wide range of biomedical questions of lipid differentiation and lipid flux in the field of metabolic disease, neurological disorders, cancers or eye diseases, to state a few [1].

Here, we highlight select recent publications in the field utilizing the Advion TriVersa NanoMate® for high throughput Shotgun Lipidomics analysis.

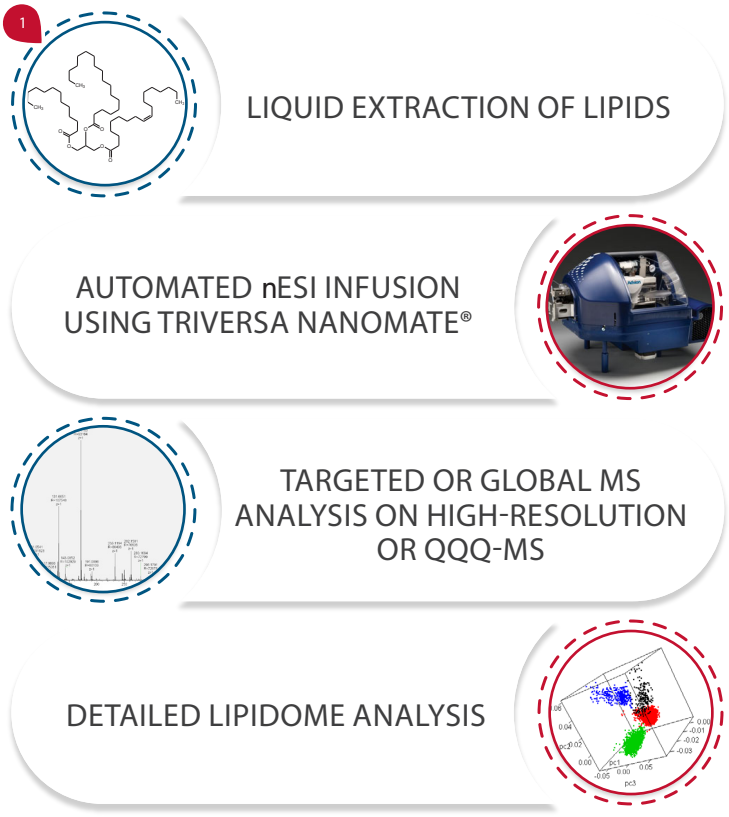

Figure 1: Schematic workflow of an Infusion based Shotgun Lipidomics experiment

RESULTS

In the area of cancer research, Zhang at al. [2] published that certain free fatty acids detected from patient serum samples can be utilized as biomarkers for pancreatic cancer and are distinctly different from biomarkers for pancreatitis or healthy control. In their study, they found a combination of C16:1, C18:3, C18:2, C18:1, C20:4 and C22:6 serum levels provided a predictive assay sensitivity of 92% and a specificity of 99% for the sample set of 361 patients.

In a similar approach to analyze free fatty acids from patient serum via shotgun Lipidomics on a high-resolution FT-MS instrument, the group also found free fatty acids to be valuable biomarkers for lung cancer [3] with the identical panel of C16:1, C18:3, C18:2, C18:1, C20:4 and C22:6 being suitable for early detection of non-small cell lung cancer (NSCLC) compared to healthy control and benign lung disease (BLD) with C16:1 and C18:1 suitable markers for disease progression monitoring. Here, assay sensitivity was slightly reduced to 84 % and specificity was reduced to 89% in a sample group of 597 patients. These findings are raising interesting questions of commonality between cancers, general metabolic impact of cancer and cancer drug influence on fatty acid metabolism.

In the context of metabolic disease, Brown et al. studied the placenta of women with preeclampsia during pregnancy and found evidence for lipid storage in the placenta caused by preeclampsia [4]:

> *... in a comprehensive Lipidomics analysis of human placentae with quantitative comparison between placenta from women with healthy pregnancies and those from women with preeclampsia (PET). This study has directly ascertained ... that placental neutral storage lipid content (TAG and CE) is specifically higher in PET compared to healthy control ... these differences remained after correction for gestational age. [4]*

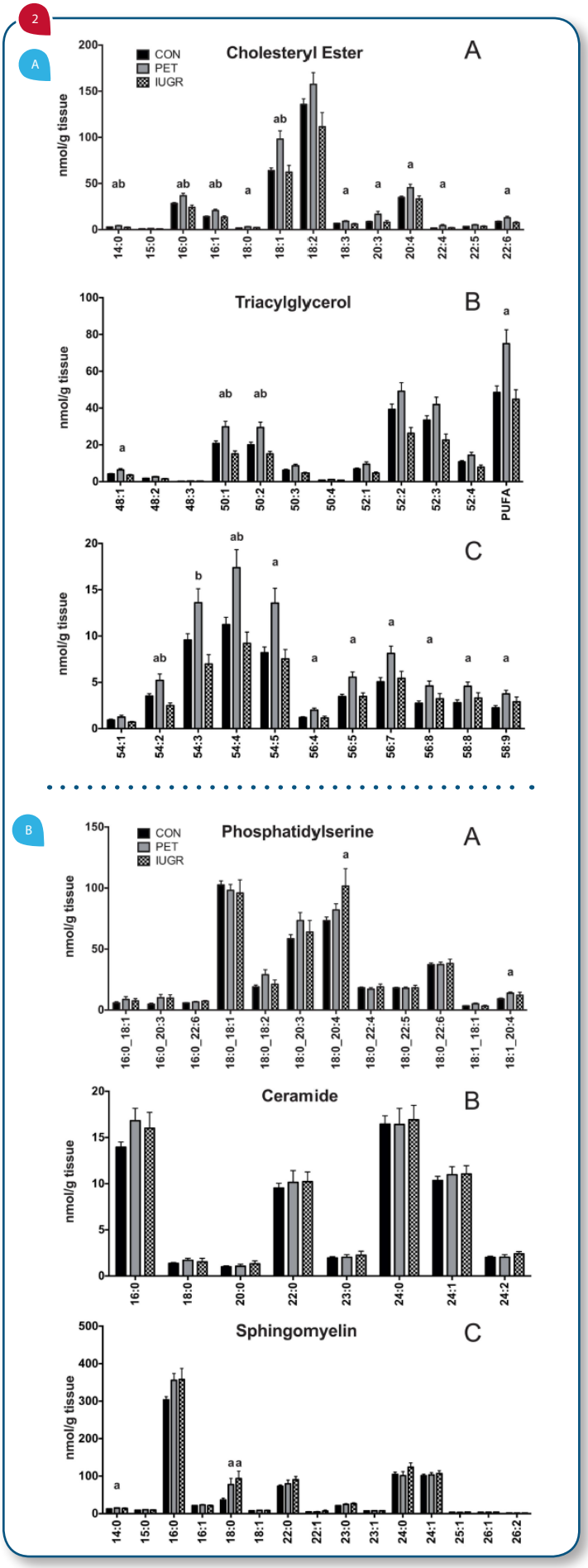

Figure 2:

2a – Storage Lipid Profiles in Placenta.

Quantitative comparison of A) triacylglycerol and B,C) cholesteryl ester molecular lipid profiles in placenta between control (N = 68), PET (N = 23) and IUGR (N = 10). Values are shown as the mean of all measurements ± SEM. a = $p < 0.01$ versus control, b = $p < 0.01$ versus IUGR (Intra Uterine Growth Restriction).

2b– Phosphatidylserine and Sphingolipid Lipid Profiles in Placenta.

Quantitative comparison of A) phosphatidylserine B) ceramide and C) sphingomyelin molecular lipid profiles in placenta between control (N = 68), PET (N = 23) and IUGR (N = 10). Values are shown as the mean of all measurements ± SEM. a = $p < 0.01$ versus control.

RESULTS, CONTINUED

Shotgun Lipidomics with data mining (Kopprasch et al. [5]) found that certain plasma lipids had a strong correlation with standard insulin sensitivity indices such as the homeostasis model of insulin resistance (HOMA-IR), the glucose insulin sensitivity index (GSI) and the insulin sensitivity index (ISI) as established for determination of insulin sensitivity in a group of patients covering normal glucose tolerance, impaired tolerance and newly detected type 2 diabetes mellitus (T2D).

...using sensitive Shotgun Lipidomics, we demonstrate in the present study varying relationships of plasma lipid species to four distinct insulin sensitivity indices. After LASSO (least absolute shrinkage and selection operator) selection, the plasma lipidome explained up to 53% variability of the sensitivity indices. Close association of insulin sensitivity indices with a large number of molecular lipid species reflects the importance of changes in lipid homeostasis in the pathogenesis of T2D [5].

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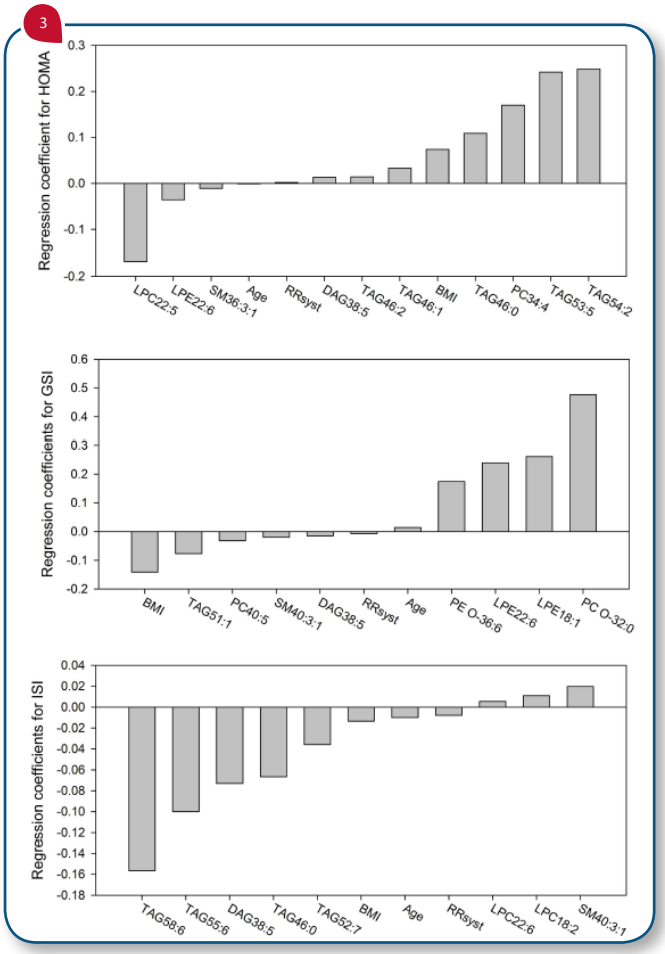

Figure 3: LASSO regression coefficients for three of the insulin sensitivity indices (Top – HOMA, Middle – GSI and Bottom – ISI) show correlation or reverse correlation with specific lipids, suggesting plasma shotgun lipidomic analysis to be a valuable tool in disease progression monitoring as well as a more detailed predictor of insulin resistance than current indices.

RESULTS, CONTINUED

Lipid shotgun analysis from dried blood spots was demonstrated by Koulman et al. [6]. They found DBS sampling to be a viable alternative for blood or plasma analysis – a useful extension of lipidomics for the intended study in infants, since DBS is the only reasonable blood sampling procedure for infants.

... lipid profiles of dried blood spots are an important new tool to study the metabolism of infants... This now gives us the unique opportunity to explore infancy lipidomics and relationships with later anthropometric and metabolic outcomes in large epidemiological study cohorts using samples already gathered, for example during the newborn bloodspot test [6].

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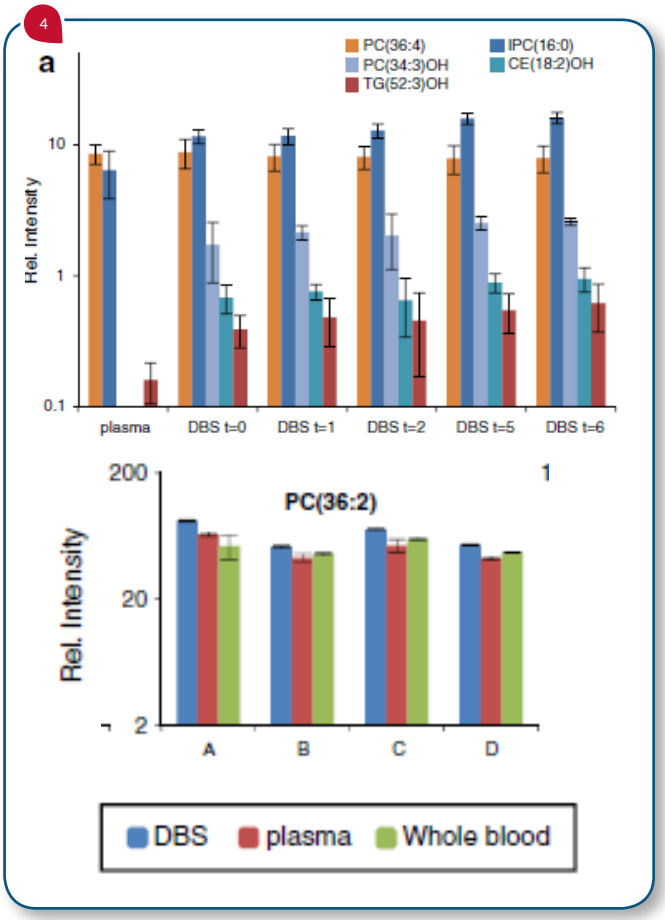

Figure 4: Lipid analysis from DBS cards under accelerated aging shows no significant deterioration over time (t=days at 40 deg C) - A.

Comparison between DBS, plasma and whole blood analysis from four different adults (A to D) also shows no significant disadvantage for DBS card collection (n of 4) - B.

RESULTS, CONTINUED

In a follow up study of the same group [7], they were able to distinguish the blood lipid composition of infants fed with either human milk (HM), formula milk (FM) or various degrees of a combination regime of both human and formula (HM&FM). Measured lipid profiles could predict the amount of FM fed in addition to HM and in more general terms, the three lipids PC(35:2), SM(36:2) and SM(39:1) could in combination be used as robust biomarkers for infant nutrition status.

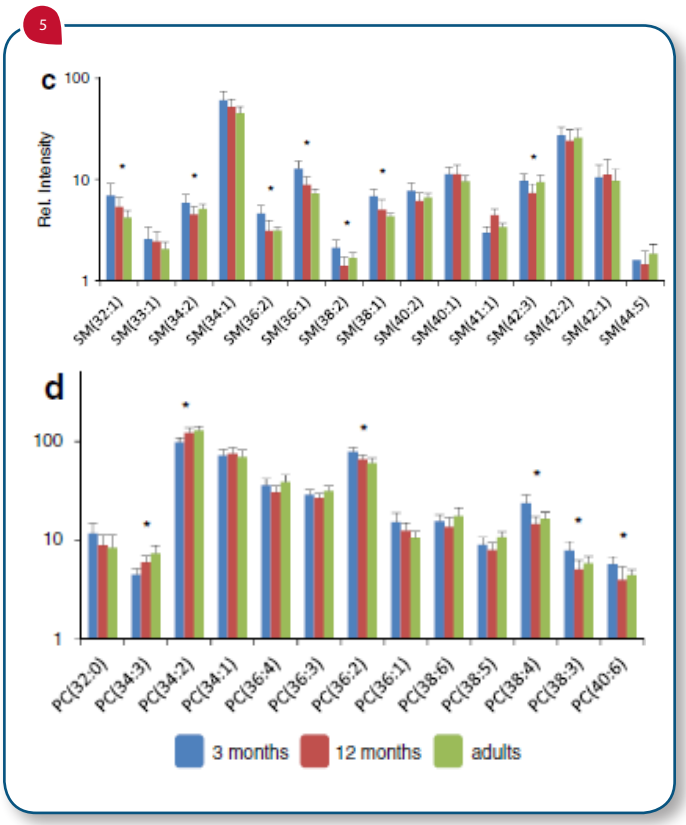

Figure 5: Changes in infant blood lipid composition over time

Application of this DBS blood sampling and Shotgun Lipidomics workflow results in new insights into the infant metabolism and lipid change over time with significant changes in sphingomyelins and phosphatidylcholine lipids [6].

Reprinted under the creative commons licensing from Koulman et al. [6].

CONCLUSION: INFUSION BASED (SHOTGUN) LIPIDOMICS ANALYSIS WITH THE ADVION TRIVERSA NANOMATE®

- No sample carry over due to one-Tip, one-sample one-emitter strategy
- Nano-electrospray (nESI) for unrivaled ionization efficiency
- High-throughput analysis from 96 or 384 sample well plates
- Relative or absolute quantification assays
- Targeted or global lipid profiling

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